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Investigation of the effects of Elicitation and Quorum sensing on Monascus purpureus C322

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Investigating the Effects of Elicitation and Quorum Sensing in *Monascus purpureus* C322



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Abstract

Monascus purpureus, traditionally used in Chinese red yeast rice, is known for its production of lovastatin-a compound effective in reducing cholesterol-and various pigments (red, yellow, and orange). This study aims to enhance the production of these metabolites using biotic elicitors, such as oligomannuronate (OM), oligoguluronate (OG), and mannan oligosaccharides (MO) and quorum sensing molecules (farnesol, tyrosol, butyrolactone-I, linoleic acid) in *M. purpureus* C322. Fermentation processes were conducted in shaken flasks and scaled up to 2.5 L stirred tank bioreactors. Supplementation with elicitors and QSMs notably increased pigment and lovastatin production compared to control groups (*p*-value<0.01). Specifically, OG supplementation led to the highest increase in orange pigment production, achieving a 2.26 and 3.04-fold increase over control in flasks and bioreactors respectively. OM was most effective for enhancing yellow and red pigments, with increases of 2.03 and 2.6-fold in flasks, and 2.22 and 3.0-fold in bioreactors. OM also led to the highest lovastatin increases, with a 2.12 and 2.39-fold enhancement in flasks and bioreactors, respectively. Among quorum sensing molecules, farnesol showed the greatest impact, enhancing pigment yields with 1.87, 2.14, and 2.09-fold increase in yellow, orange, and red pigments in shaken flasks respectively. In bioreactors, the yield of yellow, red, and orange pigments increased by 2.11, 1.5, and 1.46-fold, respectively. Meanwhile, lovastatin production increased by 2.05-fold in flasks and a 2.39-fold increase in bioreactors. Genomic analysis of M. purpureus C322 revealed a 23.82 Mb genome across eight chromosomes, with the identification of several quorum sensing genes, such as Pho8, ADH1, PgpB, confirming the capability of *M. purpureus* C322 for quorum sensing. This research underscores the potential of elicitors and quorum sensing molecules for industrial applications and expands our understanding of the genetic and metabolic framework of *M. purpureus* C322.

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Author's Declaration

I hereby declare that this thesis was completed in accordance with the guidelines and regulations of the University of Westminster. The work is original, except where specific references are made in the text.

This submission, in whole or in part, is not substantially the same as any previous or current submissions for a degree, diploma, or similar qualification at any university or similar institution, whether published or unpublished.

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Any opinions expressed in this thesis are solely those of the author and do not reflect the views of the University of Westminster.

Signature: Sirisha Yerramalli

Date: 30/05/2024

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Chapter II-Materials and Methods

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- Fig. 4.3 The effect of different elicitors (OG, OM, and MO) on yellow pigment 159 production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10⁶ spores/mL and

incubated at 25°C with a shaking speed of 120 rpm. 150 mg/L of each elicitor (OG, OM and MO) was added to the respective set of flasks at 48 h of fermentation. Three sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with standard deviation error bars. Yellow pigment production was measured at 400 nm wavelength, with a *p*-value<0.01 indicating statistically significant variation among different flask groups in comparison to the control group.

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- Fig. 4.12 Yellow Pigment production in different bioreactor groups: C, OG, OM, 183 and MO. Each 2.5 L stirred tank bioreactor contained 1,500 mL growth medium, including 150 mL seed culture prior to incubation at 32 °C under 300 rpm. Each elicitor (OG, OM, MO) was added at a concentration of 150 mg/L to the respective bioreactor group at 24 h of fermentation. Three sets of experiments were performed, and the error bars represent the standard deviation (*p*-value<0.01).
- Fig. 4.13 Concentration of Orange Pigment in Various Bioreactor Groups: C, OG, 186 OM, and MO. Each 2.5 L bioreactor, contained 1,500 mL growth medium and 150 mL seed culture, maintained at 32°C with agitation at 300 rpm. 150 mg/L of each elicitor (OG, OM, MO) was added to the respective bioreactor group at 24 h of fermentation. Error bars indicate standard deviation from three experimental runs (*p*-value<0.01).
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Chapter V-Quorum Sensing

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- Fig. 5.4 pH variation during QS optimisation in shaken flasks. The treatments 210 assessed include control (C), 100 nM butyrolactone I (B), 1 mM tyrosol (T), 1 mM farnesol (F), and 3.57 mM linoleic acid (LA). Each experiment was conducted in a 500 mL Erlenmeyer flask containing 100 mL of growth medium, inoculated with a concentration of 10⁶ spores/mL, and maintained at 25°C with a shaking speed of 120 rpm. The data points on the graph represent the average values of two sets of experiments, with three samples per flask group, spanning from Day-1 to Day-18 of fermentation. Error bars indicate the standard deviation. Quorum sensing molecules (B, T, F, LA) were introduced at 48 h into each experiment.
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 10^6 spores/mL. The flasks were maintained at 25° C and agitated at 120 rpm. The results are derived from two sets of experiments, each performed in triplicate, with error bars depicting the standard deviation on the mean values (*p*-value>0.05). Each quorum sensing molecule (B, T, F, LA) was introduced 48 h into the fermentation process.

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- Fig. 5.9 The effect of different quorum sensing molecules (B, T, and F) and CD on 217 yellow pigment production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars representing standard deviation.
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- Fig. 5.11 The influence of various quorum sensing molecules, namely B (300 nM), 220 T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (4 mM), on yellow pigment production in *M. purpureus* C322 cultures during shaken flask fermentation over an 18-day period. The experimental setup comprised 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium, inoculated with 10⁶ spores/mL, and maintained at 25°C with a shaking speed of 120 rpm. Each QSM was introduced into the corresponding set of flasks 48 h into the fermentation process. Three sets of experiments were conducted, with each experiment performed in triplicate. The mean values are presented along with error bars representing the standard deviation (*p*-value<0.01).
- Fig. 5.12 The effect of different quorum sensing molecules (B, T, and F) and CD on 222 orange pigment production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each

QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars depicting standard deviation.

- Fig. 5.13 The effect of different quorum sensing molecules (B, T F, and LA) on 223 orange pigment production in *M. purpureus* C322 cultures was investigated during shaken flask fermentation over an 18-day period. The experimental setup comprised 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium and inoculated with 10⁶ spores/mL. The flasks were maintained at 25 °C with a shaking speed of 120 rpm throughout the fermentation process. Each QSM was introduced into the corresponding set of flasks 48 h into the fermentation process. Two sets of experiments were conducted, with each experiment performed in triplicate. The mean values are presented, along with error bars representing the standard deviation.
- Fig. 5.14 The impact of different QSM, including B (300 nM), T1 (0.3 mM), T2 224 (0.5 mM), F (0.2 mM), and LA (0.4 mM), on orange pigment production in *M. purpureus* C322 cultures was examined during shaken flask fermentation over an 18-day duration. The experimental setup consisted of 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium, inoculated with 10^6 spores/mL, and incubated at 25° C with a shaking speed of 120 rpm. Introduction of each QSM occurred 48 h into the fermentation process in the respective flask sets. The experiments were replicated three times, with each experiment performed in triplicate. Mean values are depicted with error bars indicating the standard deviation (p-value < 0.01).
- Fig. 5.15 The effect of different QSM (B, T, and F) and CD on red pigment 227 production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. Each 500 mL Erlenmeyer flasks contained 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars representing standard deviation.
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- Fig. 5.17 The influence of various quorum sensing molecules, including B (300 229 nM), T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (0.4 mM), on red pigment production in *M. purpureus* C322 cultures during shaken flask fermentation spanning an 18-day period. Each 500 mL Erlenmeyer flasks contained 100 mL of growth medium inoculated with 10⁶ spores/mL, and maintained at 25°C with a shaking speed of 120 rpm. Quorum sensing molecules were introduced into the corresponding flask sets 48 hours into the fermentation process. The experiments were replicated three times,

with each experiment performed in triplicate. Mean values were presented with error bars indicating the standard deviation (p-value < 0.01).

- Fig. 5.18 Carbohydrate consumption in different flask groups (C, CD, B, T, F) 231 throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. Each 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10⁶ spores/mL prior to incubation at 25 °C under 120 rpm. Each QSM was added to the respective flask group at 0 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values.
- Fig. 5.19 Carbohydrate consumption in different flask groups (C, B, T, F, LA) 232 throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. Each 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10⁶ spores/mL prior to incubation at 25 °C under 120 rpm. The QSMs were added to the respective flask group at 48 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values.
- Fig. 5.20 Carbohydrate consumption in different flask groups (C, B, T, F, LA) 234 throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. The experimental setup comprised of 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10⁶ spores/mL prior to incubation at 25 °C under 120 rpm. The QSMs were added to the respective flask group at 48 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values.
- Fig. 5.21 Correlation between pH, Pigment Production, and Carbohydrate 235 Consumption in Shaken Flask Fermentation between different Flask Groups, namely a) control (C), b) butyrolactone-I 300 nM (B), c) tyrosol 0.3 mM (T1), d) tyrosol 0.5 mM (T2), e) farnesol 0.2 mM (F), and f) 0.4 mM linoleic acid (LA).
- Fig. 5.22 Lovastatin production in different flask groups (C, B, T1, T2, F, LA) on 239 Day-18 of fermentation in *M. purpureus* C322 cultures during shaken flasks fermentation. Each fermentation experiment utilised a 500 mL Erlenmeyer flask with 100 mL growth medium, inoculated with 10⁶ spores/mL, and incubated at 25°C and 120 rpm. The experiments were conducted in triplicate, with error bars denoting standard deviation. Lovastatin concentration on Day 18 was quantified using High-Performance Liquid Chromatography (HPLC). (p-value<0.01).
- Fig. 5.23 pH variation in different bioreactor groups over time: the control (C), 0.3 244 mM tyrosol (T), 0.2 mM farnesol (F), and 0.4 mM linoleic acid (LA). The depicted average pH values are from three experimental runs in 2.5 L stirred tank bioreactors, each containing 1,500 mL growth medium and 150 mL seed culture, incubated at 32°C at 300 rpm. Standard deviation is shown as error bars (p-value>0.05).
- Fig. 5.24 Comparative analysis of the lowest and highest pH values recorded within 245 each group during the fermentation process in both shake-flasks (SF) and 2.5 L fermenters (2.5L_F). Error bars represent the standard deviation of average pH measurements between three sets of experiments.
- Fig. 5.25 Biomass concentration on Day-5 of fermentation in different bioreactor 246 groups: the C, 0.3 mM T, 0.2 mM F, and 0.4 mM LA. In each 2.5 L stirred tank bioreactor, 1,500 mL of growth medium and 150 mL of seed culture were used, incubated at 32°C and stirred at 300 rpm. Each quorum sensing molecule was added at 24 h to the respective bioreactor group. The error

bars denote the standard deviation based on three experimental sets (*p*-value>0.05).

- Fig. 5.26 Yellow pigment production in different bioreactor groups: C, 0.3 mM T, 248 0.2 mM F, and 0.4 mM LA. Each 2.5 L stirred tank bioreactor contained 1,500 mL growth medium, including 150 mL seed culture prior to incubation at 32 °C under 300 rpm. Each QSM (T, F, LA) was added to the respective bioreactor group at 24 h of fermentation. Three sets of experiments were performed, and the error bars represent the standard deviation (*p*-value<0.01).
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Media Constituents, Chemicals and Kits

Media Constituents

Sucrose Glucose KH2PO4 KH2PO4 Yeast Extract Casamino Acid NaNO3 MgSO4.7H2O KCl

FeSO4.7H2O Potato Glucose Agar Dextrose Peptone Malt Extract Yeast Extract Agar ZnSO4.7H2O MnSO4.H2O Monosodium glutamate (MSG) Potato Dextrose Broth (PDB) **Other Chemicals** NaCl Tween 80 0.3M NaOH 0.3M HCL Sodium alginate Ethanol Proteinase K RNase A Phenol Chloroform Isoamyl alcohol mix Chloroform Isopropanol Sodium acetate Phenol Sulfuric acid Nuclease free water Tris **Beta-mannanase E-BMABS** H3PO4 HPLC-grade Acetonitrile HPLC- grade Methanol HPLC-grade water

Other Products

Qiagen fungal DNA/RNA/Protein extraction kit Genomic DNA Clean & ConcentratorTM Kit

List Of Equipment and Laboratory Supplies

EQUIPMENT

Magnetic stirrer/Hot plate Microwave/ Laboratory Oven Autoclave Laminar Air Flow/Fume Cupboard Jenway Fisher Scientific *spectrophotometer* Perkin Elmer Lambda 35 UV/VIS spectrometer. pH meter Microplate reader Centrifuges NanodropTM 2000 Balance Fridge/Freezer

Nikon NIS-Elements AR Light Microscope FastPrep Homogeniser Fischerbrand FB70155 pump Rotary Evaporator Dionex HPLC 3000

BioRad CFX96 qPCR

LAB SUPPLIES

MerckTM Vacuum Filtering Side-Arm Flask Bel-ArtTM SP SciencewareTM Büchner Funnels Whatman Filter paper No1 Haemocytometer/slides/cover slips Erlenmeyer flasks, beakers, universal bottles, test tubes Bunsen burner

Inoculating loop/ Metal spreader/scalpel Glass beads, petri plates, cuvettes Corning 500ml bottle top filter, NE. flasks Gloves, sponges Aluminium foil Lysis Matrix tubes (A, B, E, Y), Eppendorf tubes, Cryo vials, HPLC Vials 96 well microtiter plate

Pipettes Desiccator Evaporating (Florentine) flasks Lichrospher® RP-18 Endcapped (5MYM) Hibar® RT 150-4.6 HPLC column

Software Used

HPLC Analysis Genome Sequencing and Assembly Genome Plot Microscopy Nanodrop Analysis Carbohydrate Assay Flowcharts Chromeleon 7 Geneious Prime 2020.1 Circa (OMGenomics Labs) Leica software version 2.61 NanoDrop[™] 2000/2000c 1.6 UV/Vis WinLab Software Lucidchart Flowcharts

List Of Abbreviations

Full Form Abbreviation 18S 18 Subunit 28S 28 Subunit 3-oxo-C6-HSL 3-Oxo-Hexanoyl Homoserine Lactone Acyl-Homoserine Lactones AHLs AIPs Autoinducing Peptides Analysis Of Variance ANOVA Trichothecene 3-O-Acyltransferase AT Adenine-Thymine-Guanine ATG Adenosine Triphosphate ATP Absorbance Units AU Absorbance Units/Gram Of Cell Dry Weight AU/g CDW В Butyrolactone-I BLAST Basic Local Alignment Search Tool Control С CAM Calmodulin Cyclic Adenosine Triphosphate cAMP cAMP-PKA **Camp-Dependent Protein Kinase** CAT1 Catalase CD 1% DMSO CDS **Coding Sequences** Cell Dry Weight CDW Carbohydrate CHO Chromosome Chr **CLUSTALW** Cluster Alignment (Weighted) Crossing Point/RT-PCR Cq CTG Cytosine-Thymine-Guanine Days D DMBA 7,12-Dimethylbenz-[A]Anthracene DNA Deoxyribose Nucleic Acid F Farnesol Fermenters/Shaken Flasks F/SF SF Shaken Flasks 2.5L F 2.5 L Fermenters FAS Fatty Acid Synthase Fig. Figure Grams g GABA **Γ-Aminobutyric Acid** GSNO S-Nitrosoglutathione **GSNOR GSNO** Reductase Hours h HBP Hamster Buccal Pouch 5-Hydroxy-3-Methylglutaryl-Coenzyme A HMG-CoA High-Performance Liquid Chromatography HPLC Inositol Triphosphate-Diacylglycerol **IP3-DAG** Litre L Linoleic Acid LA

LBG	Locust Bean Gum
МАРК	Mitogen-Activated Protein Kinase
MAT	Mating Type
μ	Micro
mg	Milligram
mg/g CDW	Milligram/Gram Of Cell Dry Weight
Min	Minutes
mL	Millilitre
mM	Millimoles
МО	Mannan Oligosaccharide
NCBI	National Center For Biotechnology Information
nm	Nanometres
NO	Nitric Oxide
OD	Optical Density
OG	Oligogulluronate
OM	Oligomannurinate
ORF	Open Reading Frame
OSCC	Oral Squamous Cell Carcinoma
PBA	4-Phenylburylamine
PDA	Potato Dextrose Agar
PGE2	Prostaglandin E2
РКС	Protein Kinase C
PKS	Polyketide Synthase
PLC	Phospholipase C
ppm	Parts Per Million
QS	Quorum Sensing
QSM	Quorum Sensing Molecules
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPM	Rotations Per Minute
rRNA	Ribosomal RNA
RT-qPCR	Reverse Transcription-Quantitative Polymerase
_	Chain Reaction
RYR	Red Yeast Rice
SAM	S-Adenosylmethionine
SCCs	Squamous Cell Carcinomas
Sp.	Species
T	Tyrosol
TCA	Tricarboxylic Acid
TEA	2-(P-Toyly) Ethylamine
TTG	Thymine-Thymine-Guanine
UTR	Untranslated Regions
v/v	Volume/Volume
VEGF	Vascular Endothelial Growth Factor
vvm	Ventilation Rate
w.r.t	With Respect To
w/v	Weight/Volume
	0



Enhancing Lovastatin and Pigment Production in *M. purpureus* through Elicitation and Quorum Sensing: A Hypothesis

In the light of the limited existing literature, biotic elicitors and quorum sensing molecules can significantly increase the production of metabolites in some strains of *M. purpureus*. However, further research is required to explore the functional properties and potential applications of these techniques, focusing on developing cost-effective approaches for gene overexpression and introducing new biosensors for detecting quorum sensing molecules in the biotech industry.

Aim and Objectives

To address the hypothesis of this study, the aim is to enhance the production of secondary metabolites—specifically pigments and lovastatin—by *Monascus purpureus* C322 through the use of biotic elicitors and quorum sensing molecules (QSMs). The study further aims to investigate the genomic and transcriptomic framework of *M. purpureus* C322 to support future strategies for metabolic enhancement and biosensor development. To achieve the aim, the study targets the following objectives:

- To evaluate the impact of biotic elicitors (oligomannuronate, oligoguluronate, and mannan oligosaccharides) and quorum sensing molecules (farnesol, tyrosol, butyrolactone-I, and linoleic acid) on morphology, biomass concentration, and production of lovastatin and pigments by *Monascus purpureus* C322 under submerged fermentation in shaken flasks and bioreactors.
- To perform whole genome sequencing, assembly, and phylogenetic analysis of *M. purpureus* C322, and to identify genes associated with quorum sensing and metabolite biosynthesis through integrated genomic and transcriptomic analyses.

Chapter I

Introduction: Exploring Enhanced Metabolite Production in Monascus purpureus: An Introduction to Elicitation and Quorum Sensing Techniques

Part I

The Role of Elicitation and Quorum Sensing in Morphology and Metabolic Enhancement

Introduction

1.1 Strategies to Enhance Secondary Metabolism

Secondary metabolites are low-molecular-weight compounds that are not essential for primary cellular functions such as growth and reproduction, but contribute to ecological fitness, stress adaptation, and bioactive compound production (Bind et al., 2022). Microbes are an abundant source of bioactive and therapeutic agents with commercial importance. Secondary metabolites, such as alkaloids, glycosides, volatile fats, flavonoids, polymers, pigments, statins, etc, are critical to the pharmaceutical industry. However, the concentration of these metabolites is often low, and their availability is limited due to influences such as the developmental stage and physiological conditions. To overcome these limitations and increase production, several strategies have been developed including cell immobilisation, genetic engineering, elicitation, quorum sensing, cell membrane penetration, and developing faster-growing cells (Cai et al., 2011; Cairns et al., 2019; Liu et al., 2020; Palacio-Barrera et al., 2022; Lu et al., 2021; Shi et al., 2023).

Amongst these techniques, elicitation and quorum sensing have been explored recently in various prokaryotic and eukaryotic organisms as strategies to enhance biomass concentration and secondary metabolism (Ramirez-Estrada et al., 2016; Barriuso et al., 2018; Mukherjee and Bassler, 2019; Shi et al., 2022; Shi et al., 2023; Largia et al., 2023). Both these approaches involve the manipulation of signalling molecules to induce specific physiological responses in the fungal cells. The complete mechanism of these strategies is yet to be fully understood, particularly in *Monascus* sp. Therefore, exploring these techniques as an alternative way of increasing the synthesis of secondary metabolites has significant potential for further research.

Chapter I

Introduction

1.2 Eliciting Metabolic Potential: Strategies and Significance

Organisms such as bacteria, fungi, and plants initiate a range of defence responses when confronted with environmental stressors, leading to the production and accumulation of defensive secondary metabolites within microbial cultures, a phenomenon known as Elicitation. Elicitors are substances which, when introduced in minimal quantities, trigger the biosynthesis of selected compounds critical for adaptation and stress management via physiological and morphological changes (Ramakrishna and Ravishankar, 2011; Halder et al., 2019; Thakur et al., 2019). The process engages signalling molecules that activate transcription factors, thereby regulating genes responsible for the production of secondary metabolites (Radman, 2003; Patel and Krishnamurthy, 2013; Yamaner et al., 2013; Zhao, 2015). Consequently, elicitation has emerged as a crucial strategy in biotechnology, aimed at augmenting the production of secondary metabolites. Elicitation, therefore, offers various applications across several sectors including pharmaceuticals, food production, and agriculture (Radman et al. 2003; Abramovitch et al., 2006; Ramirez-Estrada et al., 2016; Guru et al., 2022).

1.2.1 Classification of Elicitors

Elicitors can be classified into different categories based on their nature and origin, as shown in Table 1.1 (Namdeo, 2007; Pettit, 2011; Zhang et al., 2013). They are broadly classified into two main types: abiotic and biotic. Abiotic elicitors, inherently non-biological, cover a range of physical and chemical factors. These include oxidative, heat, osmotic, and acid stress (Naik and Al-Khayri, 2015). Biotic elicitors, on the other hand, are derived from biological sources and consist of a broader spectrum of substances. They include polysaccharides from plant cell walls (such as chitin, pectin, and cellulose), glycoproteins (e.g., G-proteins), lipids, microorganisms, and hormonal factors (e.g. jasmonic acid and salicylic acid) and other substances like elicitins and lipopolysaccharides (Radman et al., 2003; Angelova et al., 2006; Chen et al., 2010; Naik and Al-Khayri, 2015). Additionally, elicitors can be further divided
into 'exogenous elicitors' and 'endogenous elicitors'. Exogenous elicitors refer to substances produced outside the cell, and when applied externally, they trigger defence responses. Examples include synthetic polysaccharides, fatty acids, and polyamines. In contrast, endogenous elicitors are generated inside the cell itself as part of its normal biological process in response to stress. Examples include hormones and carbohydrates such as galacturonides or hepta- β -glucosides (Angelova et al., 2006; Namdeo, 2007).

Classification	Туре	Examples / Description
Criteria		
Nature of Elicitor	Biotic	- Microbial enzymes and cell wall fragments recognised by
	Elicitors	plant/fungal cells
		- Fragments generated by microbial or plant enzymatic
		activity (e.g., chitosan, glucans)
		- Endogenous plant-derived compounds (e.g., glutathione)
	Abiotic	- Physical: UV light, mechanical damage, freezing/thawing
	Elicitors	- Chemical: heavy metals (e.g., cadmium, lanthanum),
		xenobiotics (e.g., detergents),
		fungicides (e.g., meneb), herbicides (e.g., acifluorofen),
		oxalates
Origin of Elicitor	Exogenous	- Produced externally and act directly or via mediators:
	Elicitors	• Polysaccharides: glucomannan, chitosan, glucans
		• Oligosaccharides: mannuronate, guluronides, mannan,
		galacturonides
		• Peptides: poly-L-lysine, polyamines, monilicolin
		• Enzymes: cellulase, polygalacturonase,
		endopolygalacturonic acid lyase
		• Fatty acids: arachidonic acid, eicosapentaenoic acid
	Endogenous	- Synthesised in response to biotic or abiotic stimuli:
	Elicitors	• Hepta-β-glucosides
		• Dodeca-β-1,4-D-galacturonide
		Alginate oligomers
Composition	Defined	- Polysaccharides: alginate, pectin, chitosan, LBG, guar
	Elicitors	gum
		- Oligosaccharides: oligomannuronate, oligoguluronate,
		mannan, galacturonides
		- Proteins: cellulase, elicitins, oligandrin
		- Lipids: lipopolysaccharides
	Undefined	- Complex structures: yeast cell walls, fungal spores,
	Elicitors	mycelial cell walls

Table 1.1. Classification of Elicitors (adapted from Namdeo, 2007; Angelova et al., 2006).

While various elicitation strategies have been documented, the underlying mechanisms, especially in biotic elicitation, are not fully understood. The complexity of elicitors and their influence on cellular processes, therefore, emerges as a key area of research for enhancing the productivity of valuable bioproducts. The selection of elicitors is influenced by the specific requirements for eliciting the desired microbial metabolite. Previous research, including inhouse studies, has shown promising outcomes with biotic carbohydrate elicitors such as oligomannuronate (OG), oligoguluronate (OM), and mannan oligosaccharides (MO) in filamentous fungi (Tamerler et al., 2001; Radman et al., 2004; Murphy et al., 2007; Murphy et al., 2008; Nair et al., 2009). Thus, this thesis focuses on utilising these oligosaccharide carbohydrate elicitors (OG, OM, MO) to enhance lovastatin production in *M. purpureus* C322.

1.2.2 Biotic Elicitation: The Role of Polysaccharides and Oligosaccharides in Elicitation

Carbohydrates, encompassing a broad spectrum from simple sugars to complex polysaccharides and oligosaccharides, are indispensable biomolecules vital for the survival and function of all life forms. These compounds, which include mannan and alginate oligosaccharides such as oligomannuronate and oligoguluronate, serve as key microbial elicitors in various biological applications. Historically recognised for their roles as energy reserves (e.g., starch and glycogen) and structural elements (e.g., cellulose, peptidoglycan, and chitin), carbohydrates also play pivotal roles in signal transduction, cell adhesion, ATP synthesis, nucleotide backbone formation, and molecular recognition processes crucial for immune defences (Albersheim and Valent, 1978; Nurnberger et al., 1994; Alberts et al., 2002; Lee and Yaffe, 2016).

The role of polysaccharides and oligosaccharides extends beyond their traditional functions, serving as potent elicitors in the elicitation process to boost metabolite production in bacterial, fungal, and plant systems. This has been substantiated through numerous studies and

Introduction

experiments, underscoring their significance in biotechnological applications and the potential for innovation in the production of valuable secondary metabolites (Patterson and Bolis, 1997; Ariyo et al., 1998; Tamerler et al., 2001; Radman et al., 2004, 2006; Murphy et al., 2007-2008; Zhu et al., 2008; Ming et al., 2013). Investigations by Côté and Hahn (1994) have shown that α -1,4-linked oligosaccharide residues of galactosyluronic acid can significantly enhance the biosynthesis of phytoalexins in soybeans (Glycine max), with oligogalacturonides demonstrating potent biological activity. Further studies have demonstrated that the introduction of specific oligosaccharide elicitors, such as OG (oligoguluronate), OM (oligomannuronate), MO (mannan oligosaccharide), and pectin oligosaccharides, into cultures of *Penicillium chrysogenum* leads to a marked increase in the production of the secondary metabolite penicillin G (Ariyo et al., 1998; Radman et al., 2004). Tamerler et al. (2001) reported that the concurrent addition of alginate and mannan oligosaccharides to P. chrysogenum cultures can result in a 130% increase in penicillin G production over a 96 to 120 h culture period. These findings highlight the diverse applications of polysaccharides and oligosaccharides as elicitors in enhancing metabolite production in various organisms, including fungi, bacteria, and plants.

Delving deeper into carbohydrate elicitors, the following sections provide insights into commonly used carbohydrate elicitors, differentiated into oligosaccharides and the polysaccharide categories of alginates and galactomannans.

A) Oligosaccharides

Oligosaccharides, short-chained carbohydrates composed of 2-20 monosaccharide units, are classified into two main categories: simple and complex. Simple oligosaccharides consist solely of monosaccharide units (Hayes et al., 1995), while complex oligosaccharides are conjugated with non-saccharides, such as peptides or lipids (Bickel et al., 2005). The structural diversity of carbohydrates indicates their involvement in intricate and specific biological

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pathways. Tetra-saccharides, for instance, with only four glucosyl moieties, have the potential to form over 100 three-dimensional structures, implying the potential of carbohydrates in lifeforms (Sau and Misra, 2012). The properties of carbohydrates depend on the arrangement of sugar moieties within their polymers, and the presence of multiple binding points enables the formation of complex 3-D structures. The elicitation potential of oligosaccharides extends to various microbial cultures, with oligosaccharides from *Fusarium oxysporum* mycelium significantly enhancing artemisinin production in *Artemisia annua* (Zheng et al., 2008). Similarly, the cell wall of *Piriformospora indica* has been identified as an oligosaccharide, effectively increasing phenylpropanoid derivative levels in *Linum album* hairy roots by activating essential biosynthetic genes (Tashackori et al., 2018).

B) Alginates

Alginate, or alginic acid, is a naturally occurring linear polysaccharide found in the cell walls of various algae and seaweeds, such as *Ascophyllum, Durvillaea, Ecklonia, Laminaria, Lessonia, Macrocystis, Sargassum,* and *Turbinaria.* Additionally, certain bacterial species, including *Pseudomonas* and *Azotobacter*, are known to produce alginate, highlighting its widespread occurrence across both marine and terrestrial environments (Robitzer and Quignard, 2011; Liu et al., 2019). These alginates, predominantly harvested from seaweeds in cold or temperate maritime regions, play a significant role in the marine ecosystem and have diverse industrial applications due to their unique physical and chemical properties (Peteiro, 2018). The structure of alginate consists of alternating blocks of β -(1 \rightarrow 4)-linked D-mannuronic acid and α -(1 \rightarrow 4)-linked L-guluronic acid residues (Fig. 1.1). The two types of residues are covalently bonded, forming a linear, unbranched chain. Notably, the L-guluronic acid residues are epimers of D-mannuronic acid, differing at the C5 carbon position. The properties of alginates are greatly influenced by the relative proportions of mannuronic and guluronic acids, with a higher content of guluronic acid contributing to stronger gel-forming capabilities,

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whereas mannuronic acid-rich alginates form weaker gels (Stoica et al., 2015; Iwamoto et al., 2005). Recognising its safety, the U.S. Food and Drug Administration (FDA) has classified various alginate salts as Generally Recognised As Safe (GRAS) for consumption (Liu et al., 2019).



Fig. 1.1. Structure of alginate polymers. a) b-D-mannuronic acid; b) a-L-guluronic acid, c) Oligomannuronate, d) Oligogulutonate (Image Source: Stoica et al., 2015).

Studies have focused on the bioactive properties of sodium alginate oligosaccharides, such as oligoguluronate (OG) and oligomannuronate (OM). These oligosaccharides have demonstrated significant effects as elicitors in enhancing secondary metabolites in several microbes. For instance, OM has been demonstrated to enhance the production of key secondary metabolites, like penicillin and crysogenin in *P. chrysogenum*, by modulating the expression of genes involved in biosynthesis pathways (Asilonu et al., 2000). Alginate oligosaccharides were shown to enhance penicillin G production in *P. chrysogenum*, with mannan oligosaccharides yielding up to a 69% increase compared to control cultures (Ariyo et al., 1998). Research conducted by Nair et al. (2009) demonstrated a significant increase (133%) in the production of penicillin G in *P. chrysogenum* cultures following the introduction of alginate elicitors derived from locust bean gum. This intervention led to an upregulation of the penicillin

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biosynthesis genes, namely *pcbAb*, *pcbC*, and *penDE*, highlighting the efficacy of elicitation in enhancing fungal metabolite yields. Although initial investigations predominantly occurred at the laboratory scale, there have been successful attempts at scaling up these processes reported (Singh, 1999; Shasmita et al., 2018). In similar studies, OG and OM notably increased calcium ion (Ca²⁺) levels by 11-fold and 10-fold in *Escherichia coli* DH5a and *Bacillus subtilis* 1604 cultures respectively; showcasing their impact on ion exchange mechanisms (Sabra et al., 2000; Segura et al., 2003; Murphy et al., 2011; Flores et al., 2013; Liu et al., 2019). Furthermore, OG and OM were reported to increase bacitracin yields by 29%, and 16%, respectively in *Bacillus licheniformis* cultures, underscoring the potential of alginates as elicitors in microbial systems (Murphy et al., 2007). The regulation of bacitracin biosynthetic and transporter genes, such as *bacABC* and *bcrABC*, was influenced by these oligosaccharides, as highlighted by enhanced gene transcription (Murphy et al., 2007b; Murphy et al., 2008).

C) Galactomannans

Galactomannans, multifunctional polysaccharides sourced from leguminous seeds, exhibit a plethora of economically valuable properties due to their biodegradability, non-toxic nature, and cost-effectiveness. These characteristics have rendered galactomannans as indispensable components within the pharmaceutical, food, and biotechnological industries (Landin and Exhezarreta, 2010). Comprising a $(1\rightarrow 4)$ -linked α -D-mannopyranosyl backbone with O-6 substitution by single units of β -D-galactopyranose (Fig. 1.2), galactomannans such as guar gum, locust bean gum, and tara gum have found broad applications, with locust bean gum (LBG) emerging as particularly significant due to its unique physicochemical properties (Fig. 1.3) (Silveira and Bresolin, 2011; Wielinga and Meyhall, 2009).



Fig. 1.2. Structure of galactomannans, where 'M' represents mannose and 'G' represents galactose (Image Source: Silveira and Bresolin, 2011).

Locust bean gum is a natural polysaccharide that is derived from the kernels of the carob tree and contains between 18-24% galactomannans. Due to this composition, LBG is soluble only in hot water. The structural unit of LBG is characterised by a $(1\rightarrow 4)$ -linked β -D-mannopyranose backbone, with a substitution at position-6 by a single unit of α -D-galactopyranose (Fig. 1.4), influencing its application based on the mannose-to-galactose ratio and molecular weight (Table 1.2) (Landin and Exhezarreta, 2010; Silveira and Bresolin, 2011). Such structural diversity among galactomannans dictates their functional capacity in thickening, gelling, and as biotic elicitors in microbial cultures.

Туре	Plant origin	Mannose-galactose	Molecular weight
		ratio	(kDa)
Locust bean	Ceretonia siliqua L	4:1	50-1000
gum			
Tara gum	Caesalpinia spinosa	3:1	1000
Gaur gum	Cyamopsis	2:1	150-1500
	tetragonolobus L		
Fenugreek gum	Trigonella foenum	1:1	30
	graecum		

Table 1.2 Characteristics of galactomannans (Landin and Exhezarreta, 2020).



Fig. 1.3. Commonly used galactomannans- Locust bean gum (LBG), Gaur gum and Tara gum (Image Sources: Schink, 2017; Silvateam, 2023).

The enzymatic hydrolysis of galactomannans into oligosaccharides, specifically by enzymes like α -galactosidase and β -D-mannase, have been recognised for their elicitation properties. For instance, several studies demonstrated that mannan oligosaccharides (MO) produced by enzymatic breakdown, notably increased penicillin G production in *P. chrysogenum* and concurrently reduced levels of reactive oxygen species. This increase in penicillin production is further supported by quantitative PCR analysis, which indicated an increase in gene copy numbers associated with penicillin biosynthesis (Ariyo et al., 1998; Tamerler et al., 2001; Radman et al., 2006). Similarly, MO was also found to enhance bacitracin A production in *B. licheniformis* cultures in several studies (Murphy et al., 2007; Giri and Zaheer, 2016). MO was also found to induce morphological changes (Benhamou, 1992; Radman et al., 2004), sporulation (Radman et al., 2004), and pigment production (Nair et al., 2005). Specifically, mannan oligosaccharides boosted chrysogenin pigment production by up to 55% in certain *P. chrysogenum* strains (Nair et al., 2005). Similarly, Radman et al. (2010) observed that supplementation of MO to *P. chrysogenum* cultures inhibited spore germination and increased hyphal tip numbers, clump area, and spore counts. These findings underline the significant role

of galactomannans and their derivatives in enhancing the production of valuable microbial byproducts.



Fig. 1.4. Chemical structure of locust bean gum (LBG) (Image Source: Beneke et al., 2009). **Note:** Further information on elicitation techniques involving biotic and abiotic elicitors are provided in the supplementary information section of the Appendix.

1.2.3 Mechanism of Elicitation in fungal system

Microbial elicitation encompasses a sophisticated network of signalling cascades, initiated when an elicitor binds to its specific receptor. This binding triggers a conformational change in the receptor, altering its three-dimensional structure and activating a sequence of cellular responses. These responses are mediated by secondary messengers produced through the interplay of various biochemical pathways (Fig. 1.5). Such mechanisms are essential for regulating growth, metabolism, cell communication, and proliferation in cells. A key player in these processes is the guanine nucleotide-binding protein, or G-protein, which is crucial for the growth, development, defence, and secondary metabolite production in eukaryotic organisms, including filamentous fungi (Nair et al., 2007).

The activation of signalling pathways such as PLC (Phospholipase C), IP₃-DAG (Inositol triphosphate-Diacylglycerol), and PKC (Protein kinase C) in response to elicitation is a well-documented phenomenon across various studies, independent of the elicitor type used (Shigaki

and Bhattacharyya, 2000; Vasconsuelo et al., 2003). These messengers play crucial roles in cell growth regulation and other cellular functions, including the signal transduction processes that lead to protein kinase activation via Ca^{2+} mobilisation. Nurnberger et al. (1994) observed similar dynamics, noting a rapid influx of Ca^{2+} ions coupled with an efflux of K⁺ and Cl⁻ ions preceding the activation of ROS, gene transcription, phytoalexin production, and kinase activation. Moreover, it has been reported that IP₃ facilitates the influx of Ca^{2+} ions from outside the cell and their release from intracellular stores like vacuoles. The released Ca^{2+} ions then interact with calmodulin (CAM), initiating the phosphorylation of various proteins that activate defence mechanisms (Hubbard et al., 1982; Cornelius and Nakashima, 1987; Berridge and Irvine, 1989; Tsitsigiannis and Keller, 2007). Calmodulins, functioning as key components in these signaling pathways, have been identified in several fungi and yeast strains, such as *Neurospora crassa* and *S. cerevisiae*, highlighting their broad role in microbial defence responses (Favre and Turian, 1987; Ogita et al., 1990).

Similarly, the cAMP signalling pathway is essential for managing critical functions in fungi, including energy metabolism, growth, filamentation, conidiation, morphogenesis, mating, and stress tolerance, as observed in *N. crassa* (Pearson et al., 2001). The initiation of this pathway occurs upon the elicitor's detection by a receptor, leading to the activation of the mitogenactivated protein kinase (MAPK). This activation triggers a cascade effect, influencing the expression of multiple genes.

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Fig. 1.5. Schematic diagram elucidating the mechanism of elicitation in filamentous fungi, *P. chrysogenum* (Image Source: Nair et al., 2007).

Various stimuli, both biotic and abiotic—such as pathogen attacks, temperature fluctuations, drought, wounds, salinity, UV radiation, ozone exposure, osmolarity changes, and ROS—can activate MAPKs, underscoring their pivotal role in fungal adaptive responses (Stratmann and Ryan, 1997). MAPKs are not limited to a single pathway activation; they can initiate multiple biochemical pathways and gene transcription processes in response to elicitors. Vasconsuelo (2003) demonstrated that introducing chitosan to *Rubia tinctorum* cultures could activate the MAPK pathway, enhancing the production of anthraquinone and other secondary metabolites, including phytoalexins. This exemplifies the potential of MAPK signalling in eliciting secondary metabolite synthesis. In yeast species like *S. cerevisiae*, there is a synergistic interaction between the cAMP and MAPK pathways, coordinating the regulation of growth and developmental processes (D'Souza and Heitman, 2001). This coordination between pathways illustrates the complexity and efficiency of fungal signalling systems in response to environmental cues and stresses.

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1.3 Quorum Sensing: Origin, Mechanisms and Applications of Microbial Communications

The evolution of multicellularity was a critical step in Earth's ecological development, illustrating the transition from single-cell organisms to complex multicellular ecosystems (Libby and Ratcliff, 2014). Cell-to-cell communication, crucial in the development of multicellular organisms such as animals and plants, is fundamentally based on the transmission of molecular signals. The unicellular microbes, especially prokaryotes, played a pivotal role in ecological evolution, bridging the gap between simple communication systems and the more complex ones found in eukaryotes, such as *Pseudomonas* and *Aspergillus* sp. (Williams, 1994). Microorganisms have developed regulatory systems as a defence mechanism, enabling them to sense and react to changes in cell population density (Tomasz and Beiser, 1965, Smith et al., 2023). This mechanism, known as quorum sensing, involves the release of chemical signalling molecules for cell-to-cell communication by synchronously controlling the gene expression triggered when the concentrations of these signalling molecules reach a critical threshold (Fig. 1.6) (Fuqua et al, 1994; Mehmood et al., 2019; Postal and Bousso, 2019; Gnan and Maggi, 2022). In recent years, quorum sensing has gained popularity as a cost-effective method for enhancing gene expression in microorganisms.

The concept of quorum sensing in bacteria was first noted by Alexander Tomasz in 1965 during his research on *Streptococcus pneumoniae* and its ability to uptake DNA from its surroundings. Quorum sensing systems vary between Gram-negative and Gram-positive bacteria. Gram-negative bacteria use small molecules like acyl-homoserine lactones (AHLs) or others derived from S-adenosylmethionine (SAM) as autoinducers while Gram-positive bacteria utilise autoinducing peptides (AIPs) as signalling molecules (Wei et al. 2011). This phenomenon was initially discovered in the marine bacterium *Vibrio fischeri*, hosted by the Hawaiian bobtail squid *Euprymna scolopes* in its light organ. *V. fischeri* was found to control its luminescence

based on cell density, using a diffusible signal identified as the AHL 3-oxo-hexanoyl homoserine lactone (3-oxo-C6-HSL) (Eberhard et al. 1981, Nealson et al. 1970). Subsequent research has revealed that AHL-mediated QS is common among bacteria, influencing a broad spectrum of bacterial behaviours (Dobretsov et al. 2009, Fuqua et al. 2001).



Fig. 1.6. A schematic representation of the quorum sensing mechanism in fungi (Image Source: Mehmood et al., 2019).

Quorum sensing is also recognised for controlling various physiological activities, including forming secondary metabolites, biofilm development, sporulation, symbiosis, conjugation, and competence. The dual nature of autoinducers, possessing both signalling and non-signalling properties, makes them suitable as chelating agents. Consequently, quorum sensing has applications in numerous areas, such as increased production of desired metabolites, microbial biosensor design, tissue engineering, and mixed culture fermentation (Choudhary and Dannert, 2010).

1.3.1 Quorum Sensing in Fungal Systems

1.3.1.1 History of quorum sensing in fungi

Lingappa et al. (1969) were the first to document a cell density-dependent mechanism controlling filamentation in the fungal pathogen *Candida albicans*, marking the earliest description of quorum sensing (QS) in fungi. However, the term "quorum sensing" was later specifically used by Kügler et al. (2000) to describe population density-dependent virulence in *Histoplasma capsulatum*. Subsequent research has uncovered a wide array of structurally diverse quorum sensing molecules (QSMs) in fungi, influencing various properties across a range of evolutionarily diverse fungal species (Chen et al., 2004; Chen and Fink, 2006; Horowitz et al., 2008; Raina et al., 2010; Williams et al., 2012; Albuquerque et al., 2013; Berrocal et al., 2014; Vitale et al., 2019).

In 2001, Hornby and colleagues identified the first structurally characterised fungal quorum sensing molecule (QSM), farnesol, produced by *Candida albicans*. Originally, quorum sensing was thought to be exclusive to certain bacteria, the discovery of cell density dependent morphological changes in dimorphic fungi like *C. albicans* marked a significant advancement in understanding QS in eukaryotic organisms. *C. albicans* utilises quorum sensing for pathogenesis and can switch between budding yeast (at high cell densities $\geq 10^6$ cells/mL), hyphae, and pseudo-hyphae forms (at low cell densities $<10^6$ cells/mL), as illustrated in Figure 1.7. This transition is regulated by the quorum sensing molecules farnesol and tyrosol (More details in Section 1.3.2.3), each demonstrating distinct effects based on the organism's needs (Hornby et al., 2001; Saville et al., 2003; Hogan, 2006). The QS-induced filamentation control in the polymorphic fungus *C. albicans* extended the concept of quorum sensing to fungi (Hornby et al., 2001, Mosel et al., 2005).



Fig. 1.7. Influence of the quorum sensing molecules, tyrosol and farnesol, on the transition between two growth forms in *C. albicans* (Image Source: Amache et al., 2014).

The presence of QS systems in fungi has been established, showing that various substances such as lipids (oxylipins), peptides (pheromones), alcohols (including tyrosol, farnesol, tryptophol, and 1-phenylethanol), acetaldehydes, and certain volatile compounds play a crucial role in fungal QS (Fig. 1.8). These molecules are integral to regulating essential fungal functions like pathogenesis, morphogenesis, and filamentation (Fig. 1.9) (Albuquerque and Casadevall, 2012; Polke et al., 2015; Hirota et al., 2017). Since this breakthrough, numerous reports have emerged, suggesting the presence of quorum sensing in various fungal species, including unicellular yeast and filamentous fungi, particularly when they are exposed to stressful and competitive environments.



Fig. 1.8. Different quorum sensing molecules (QSMs) and quorum sensing inhibitors (QSIs) synthesised by fungal species (Image Source: Padder et al., 2018).



Fig 1.9. An overview of the production and function of quorum sensing molecules (QSMs) and quorum sensing inhibitors (QSIs) across various fungal cell types. Cell type A synthesises signalling molecules that function as QSMs, whereas molecules produced by the same or different fungal cells serve as QSIs, impacting cell type B. (Image Source: Padder et al., 2018).

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The subsequent sections provide details of quorum sensing (QS) in various types of fungi, including unicellular, dimorphic, and filamentous forms. These descriptions illustrate the significant role of QS in regulating critical biological processes such as virulence factor production, morphological changes, and pathogenesis across different fungal species.

1.3.1.2 Quorum Sensing in Unicellular and Dimorphic Fungi

Quorum sensing (QS) mechanisms have been discovered in several dimorphic fungi over time, many of which are either plant or animal pathogens, with some also having biotechnological applications. Quorum sensing mechanisms are best described in the dimorphic yeast, *C. albicans*. Similar to *C. albicans*, *O. ulmi* also exhibits morphological changes due to quorum sensing. Notably, the influence of inoculum size has been observed, where a medium inoculated with more than 10^6 spores/mL initially fostered cells as budding yeasts, whereas inoculation with fewer than 10^6 spores/mL led to cell development as mycelia (Hornby et al. 2004). Subsequent research has suggested that the quorum sensing molecule driving these processes might be 2-methyl-1-butanol (Berrocal et al. 2012).

Some studies suggest that the yeast, *Saccharomyces cerevisiae*, also engages in quorum sensing, mediated by aromatic alcohols, namely phenol alcohol and tryptophol. These QSMs stimulate hyphal growth and morphogenesis (Chen and Fink, 2006), by acting through the cAMP-dependent PKA subunit Tpk2, which affects transcriptional genes essential for filamentation (Fig. 1.10 a) (Chen and Fink, 2006). These QSMs produced by *S. cerevisiae* also exhibit virulence against *Vitis vinifera* (Gognies et al., 2006).



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Fig. 1.10. Illustrative representation of quorum sensing mechanisms in four key fungal species: a) *Saccharomyces cerevisiae*, b) *Candida albicans*, c) *Cryptococcus neoformans*, and d) *Fusarium oxysporum*. Key elements include: acetyl-CoA (acetyl coenzyme A), FPP (farnesyl pyrophosphate), PA (pantothenic acid), PheOH (phenylethanol), and TrpOH (tryptophol). (Image Source: Tian et al., 2021).

1.3.1.3 Quorum Sensing in Filamentous Fungi

Quorum sensing (QS), a mechanism traditionally linked to unicellular organisms, has been increasingly recognised in filamentous fungi. Notably, species such as *Aspergillus* and *Penicillium*, employ QS to regulate secondary metabolism, serving as a competitive strategy

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(Raina et al., 2010; Guo et al. 2011; Raina et al. 2012). The quorum sensing mechanisms in *Aspergillus* sp. affect population-dependent behaviours such as morphogenesis and secondary metabolite production. For instance, in *A. flavus*, oxylipins, a type of quorum sensing molecule, regulate both the morphological differentiation between asexual spore or sclerotia production and mycotoxin production. (Horowitz Brown et al. 2008, Affeldt et al., 2012). Similarly, in *A. terreus* cultures, addition of linoleic acid, a precursor of oxylipin, has been found to increase lovastatin production in stirred tank fermentation (Sorrentino et al., 2010). In another study by Raina et al. (2012), the addition of 100 nM of butyrolactone I, another form of quorum sensing molecule, led to a 2.5-fold increase in lovastatin yield and increased endogenous butyrolactone I levels.

Similarly, *Penicillium* sp., also exhibits QS behaviour. For instance, *P. sclerotiorum* produces sclerotiorin, an antibiotic secondary metabolite, and uses γ -butyrolactone-containing molecules like multicolic acid as QSMs. When γ -butyrolactones from *P. sclerotiorum* are added exogenously, sclerotiorin production is enhanced (Raina et al., 2010). Similarly, Guo et al. 2011 found that farnesol promoted hyphal growth and increased cellulase secretion in *Penicillium decumbens*. Ameche et al. (2014) discovered that the addition of ethyl acetate extracts from high cell densities of *P. sclerotiorum* culture not only increases sporulation but also delays the onset of hyphal branching and boosts the production of the secondary metabolite sclerotiorin in both shaken flasks and stirred tank bioreactors (STR). Additionally, the same study found that supplementing linoleic acid, as an oxylipin precursor, enhances the production of the secondary metabolite lovastatin in *A. terreus* cultures within shaken flasks and STRs.

Over the past two decades, quorum sensing has been detected in several filamentous fungi, predominantly in *Aspergillus* and *Penicillium* sp., including *A. nidulans, A. terreus, P. chrysogenum, A. flavus, and P. sclerotiorum.* In these fungi, QS has been reported to induce various physiological changes in fungi, such as alterations in hyphal branching, sporulation,

and secondary metabolite production (Schimmel et al., 1998; Calvo et al., 2002; Tsitsigiannis et al., 2007; Brown et al., 2008). Additionally, *Fusarium* sp. also exhibit QS similar to *Aspergillus* and *Penicilliun* sp. For instance, *Fusarium oxysporum* demonstrates cell density-dependent conidial germination, regulated by pheromones despite lacking a sexual cycle. (Fig 10d) (Bolker et al., 1993; Turra et al., 2015; Vitale et al., 2019). Although various aspects of quorum sensing are well understood, many facets remain enigmatic, particularly in *Monascus* sp. Therefore, research to elucidate quorum sensing in *Monscus* sp. holds significant promise (Mangwani et al., 2012).

1.3.2 Classification of Quorum Sensing Molecules (QSM)

For a molecule to be classified as a quorum sensing molecule (QSM), it must exhibit the following characteristics (Albuquerque and Casadevall, 2012):

- i. Accumulate extracellularly throughout the growth phase.
- ii. The accumulated concentration must be directly proportional to the population density.
- iii. Once it surpasses a threshold concentration, it should trigger a coordinated response within the microbial population.
- iv. When introduced externally, the molecule should initiate quorum sensing.

QSM primarily fall into one of three categories: amino acids (pheromones), alcohols (sterols, amino acid derivatives), or fatty acids (lactones, lipids) (Fig. 1.11). Among these, lipid-based molecules, such as oxylipins and butyrolactones, are the most commonly identified signalling molecules in filamentous fungi. These lipid-based molecules have also been identified as potential QSM in filamentous fungi (Schimmel et al., 1998; Tsitsigiannis and Keller, 2007).

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Fig. 1.11. Classification of Quorum Sensing Molecules.

A few well-known quorum sensing molecules found in fungal species, along with their mechanisms of action, are concisely outlined below:

1.3.2.1 Oxylipins

Oxylipins, oxygenated fatty acids, are common in nearly all living organisms and are considered secondary metabolites crucial for intracellular and intercellular communication (Fig. 1.12). They regulate growth, reproduction, and secondary metabolite formation in animals, plants, prokaryotes, and fungi (Noverr et al., 2003). While the detailed biosynthesis of fungal oxylipins remains unclear, key enzymes like lipoxygenase, hyperoxydase lyase, and dioxygenase have been identified. In fungi, oxylipin biosynthesis involves *pho* genes and *psi* factors, which encode derivatives of oleic, linoleic, and linolenic acids essential for oxylipin

synthesis, reproduction, and secondary metabolite production (Albuquerque and Casadevall, 2012; Hintz et al., 2015; Mehmood et al., 2019). In *A. nidulans, Ppo* genes encode enzymes (*PpoA, PpoB, PpoC*) that influence the formation of secondary metabolites like sterigmatocystin and penicillin. Deleting *Ppo* genes shifts the life cycle from asexual to sexual reproduction (Champe and El-Zayat, 1989; Tsitsigiannis et al., 2004 a and b). In *A. terreus,* adding linoleic acid, an oxylipin precursor, overproduced lovastatin and decreased sporulation rate, with upregulated lovastatin biosynthetic genes *lovB* and *lovF* (Sorrentino, 2009). Oxylipins also act as signalling molecules between different organisms (Radman et al., 2004).



Fig. 1.12 Biosynthesis of different Oxylipins in Fungal Systems (Image Source: MedCrave Online- https://medcraveonline.com/JMEN/JMEN-05-00148).

1.3.2.2 Butyrolactone and other Acyl-homoserine Lactones

In addition to oxylipins, butyrolactones are recognised as quorum sensing molecules in filamentous fungi. Quorum sensing molecules containing γ -butyrolactones (Fig. 1.13) have been identified in several species: *A. terreus* produces butyrolactone I, *P. sclerotiorum* produces multicolanic acid and its derivatives, and *A. nidulans* generates γ -heptalactone (Raina

et al., 2010; Williams et al., 2012). In separate discoveries in 1998 and 2012, Schimmel and Raina identified butyrolactone-I as a quorum sensing molecule that can boost lovastatin production in *A. terreus* cultures and Palonen et al. (2017) reported the involvement of *Lae A* regulator in increasing lovastatin production in *A. terreus* MUCL 38669 using butyrolactone-I.

Furthermore, the introduction of lactone molecules (γ -butyrolactone, multicolic acid, multicolosic acid, multicolanic acid, and related derivatives) into the *P. sclerotiorum* culture substantially enhanced sclerotiorin production, resulting in a 6.4-fold increase (Hajjaj et al., 2000; Raina et al., 2010 and 2011). In addition, exogenous γ -butyrolactone triggered the early development of secondary metabolites in streptomyces, suggesting that γ -butyrolactones may operate similarly to other acyl homoserine lactones. Similarly, Williams et al. (2012) observed a 37.8% increase in the expression of the *ipnA:lacZ* reporter gene in *A. nidulans* with γ -heptalactone, leading to higher penicillin production.



Fig. 1.13. Structural representation of signalling molecules containing butyrolactone. A) Multicolanic acid, B) Gamma-Heptalactone, and C) Butyrolactone I. (Image source: Krzyczkowska et al., 2017).

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1.3.2.3 Farnesol and Tyrosol

Farnesol is an isoprenoid alcohol with a 12-carbon backbone (Fig. 1.14) is secreted by *C. albicans* by the dephosphorylation of farnesol pyrophosphate (FPP) through a sterol synthetic pathway. In biofilms treated with farnesol, genes related to cell wall maintenance, surface properties, drug resistance, and defence proteins against heat and oxidative stress were influenced (Cao et al., 2005; Shirtliff et al., 2009). For instance, in *A. fumigatus*, farnesol alters protein localisation related to cytoskeleton and cell wall integrity (Dichtl et al., 2010).

In some cases, farnesol excreted by several dimorphic yeasts acted as a quorum sensing inhibitor and has shown antimicrobial properties against *Fusarium graminearum, Paracoccidioides brasiliensis, C. neoformans.* (Trinci et al., 1990; Tsuji et al., 1988; Tseng et al., 2000; Vasconsuelo et al., 2003; Tsitsigiannis et al, 2004 a and b, 2007; Sorrentino et al., 2010) and in other contexts, it increases antibiotic sensitivity in *S. aureus* and induces apoptosis in *S. cerevisiae* and *A. nidulans*, suggesting its role in both inter- and intraspecific communication (Semighini et al., 2006; Fairn et al., 2007). Farnesol has also been found to inhibit the conversion of yeast to mycelium in *C. albicans*, by preventing the formation of three germ-tube inducers: L-proline, and N-acetylglucosamine, by disrupting the Ras-cAMP-PKA (cAMP-dependent protein kinase) pathway. (Hornby et al., 2001; Shirtliff et al., 2009; Lindsay et al. 2012; Hintz et al., 2015). Interestingly, farnesoic acid, a less toxic farnesol derivative, impacts hyphal growth but with lesser efficacy (Kim et al., 2002; Yi et al., 2011).

Tyrosol (2-[4-hydroxyphenyl] ethanol) (Fig. 1.14), a tyrosine derivative and recognised quorum sensing molecule (QSM) in various fungal species, exhibits multifaceted roles in fungal biology and interactions with host immune cells (Champe and El-Zayat, 1989; Calvo et al., 2002; Chen et al., 2004; Alem et al., 2006; Meinecke et al., 2013). In contrast to farnesol, tyrosol promotes filamentation and biofilm formation by reducing the duration of the lag phase.

Notably, tyrosol remains inactive in the presence of farnesol, indicating the dominance of farnesol (Chen et al, 2004; Alem et al., 2006). These QSMs also affect biofilm formation and cell dispersal, playing key roles in pathogenesis (Ramage et al., 2002; Cordeiro et al., 2015). The early addition of tyrosol during biofilm formation promotes hyphal growth, indicating its significance in biofilm dynamics and morphogenesis (Hintz et al., 2015; Alem et al., 2006). The ability of tyrosol to promote filamentation can be counteracted by high concentrations of farnesol. In such scenarios, tyrosol fails to prevent farnesol's filamentation-inhibiting effects, leading to a yeast-dominant biofilm composition. This interplay highlights the complex regulatory mechanisms in fungal biofilms, where tyrosol and farnesol have competing influences, with tyrosol being more effective in the early to intermediate stages of biofilm development. In contrast, in mature biofilms, farnesol's role becomes more pronounced, potentially influencing yeast cell dispersal (Nickerson et al., 2006; Ramage et al., 2002).

1.3.2.4 Pheromones

Pheromones represent another common type of signalling molecule. Pheromone peptides, known as 'a' and ' α ' factors, are produced by 'a' and ' α ' cells of *S. cerevisiae*. Each mating type produces a single pheromone factor depending on the availability of the MAT (Mating type) locus (Fig. 1.14). These pheromones accumulate and diffuse into the environment. When they are recognised by specific G-protein receptors, *Ste2p* and *Ste3p*, they initiate plasmogamy through the degradation of the α cascade by several proteins, including *Ste5p*, two phosphorylated MAP kinases (*Fus3p* and *Kas1p*), and *Fus3p* enhances gene expression. This cascade leads to the activation of the transcription factor *Ste12p* (Hintz et al., 2015, Mehmood et al., 2019).



Fig. 1.14. Chemical structure of various fungal quorum sensing molecules (1) Farnesol, (2) Tyrosol, (3) Farnesoic acid, (4) α -(1,3)-glucan, (5) 1-Phenyl-ethanol, (6) Tryptophol, (7) Pheromones 1. a-factor 2. α -factor, (8) Multicolanic acid, (9) γ -Heptalactone, (10) Butyrolactone-I (Image source: Mehmood et al., 2019).

Part II

An Introduction to the Versatile Filamentous Fungus, Monascus purpureus

Introduction

1.4 Background and Overview of Monascus purpureus

Monascus purpureus is a purplish-red coloured species of mold that has been used in China and other Asian countries for various culinary and medicinal purposes for centuries (Fig. 1.15). The scientific name, *Monascus purpureus*, was first coined by Tieghem in 1884. It belongs to the kingdom Fungi, phylum Eumycota, subphylum Ascomycota, Class Eurotiomycetidae, Order Eurotiales, family Monascaceae, and Genus *Monascus* (Pan and Hsu, 2014; IRMNG, 2021). *Monascus* sp are primarily homothallic and teleomorphic. Traditionally, *Monascus* has been used as a natural food colourant (e.g. forming red yeast rice, RYR), preservative, and folk medicine (Patakova et al., 2015; Zhu et al., 2019). It produces several pigments and other metabolites capable of reducing cholesterol and triglyceride levels.



Fig. 1.15. Visual representation of *M. purpureus* through three different perspectives. (a) fermented Red Yeast Rice (RYR) (DXN, 2018), (b) a plate-culture, and (c) a microscopic image captured at 10x magnification.

Synthetic drugs, chemicals, and food additives have been criticised for their undesired side effects, which have led to increased demand for natural food and pharmaceutical products in recent times. Microorganisms, including fungi, have emerged as a preferred source of natural

resources due to their productivity and versatility, especially in forming pigments and secondary metabolites (Pham et al., 2019). *Monascus* sp. have gained global attention as a natural food and therapeutic resource, and its pigments and derivatives have attained high economic value (Vendruscolo et al., 2016; Pham et al., 2019).

The global functional food industry has witnessed notable growth in recent years. According to a report by Grand View Research on the functional food market the estimated value was USD 161.49 billion in 2018, expected to double by 2025 due to the growing consumer interest in healthy eating habits (Grand View Research, n.d). As anticipated, the demand for superfoods has been increasing ever since. The increased demand for superfoods, along with the increased attraction towards *Monascus* pigments and derivatives, emphasises the importance of conducting research on *M. purpureus* to enhance the productivity of vital pigments and metabolites (Vendruscolo et al., 2016). This study aims to improve the production of lovastatin and other pigments by *M. purpureus* strain C322 with the help of elicitation and quorum sensing.

1.4.1 The Historical Evolution of Red Yeast Rice (RYR) as a Medicinal and Culinary Product

The origins of RYR production can be traced back to ancient China, where it was highly treasured and kept secret for many years. The ancient Chinese text represented RYR as "Qu or Koji", meaning grains and soybean "coated" with mold. This traditional food preparation can be traced back to at least 300 BC, while the use of RYR dates back to the 1st century AD during the Han Dynasty in China (Fig. 1.16) (Chen et al, 2015).



Fig. 1.16. Tiangong Kaiwu manuscript depicting the ancient fermentation technique used for RYR during the Ming dynasty (Chen et al., 2015).

The compendium, from the Han (AD 177-217), Ming (AD 1368-1644) and Qing (AD 1636-1912) dynasties, records the extensive medicinal applications and production of RYR, marking the beginning of its widespread distribution across East Asia 500 years ago (Chen et al., 2015). Over time, RYR has become accepted worldwide and is now used as a starter culture for brewing fermented food items, including soy sauce, liquor, wine, and vinegar, in addition to its traditional culinary and medicinal uses.



Fig. 1.17. Traditional method of fermenting red yeast rice (RYR) using a wooden rice steamer (adapted from Chen et al., 2015).

Traditionally, RYR ("Akakoji") was made by soaking non-glutinous, long-grained rice in water for 24 hours, then draining and steaming it. After cooling, an alum solution or diluted vinegar was added to promote the growth of *M. purpureus*, which thrives in slightly acidic environments. The rice was inoculated with Koji and incubated at 32-42 °C with low humidity to avoid contamination. To prevent clumping, the rice was stirred periodically. After 14 days, the rice developed a deep purplish-red color. The final product was sold as dried grain, wet paste, or fine powder (Fig. 1.17) (Fabre et al., 1993; Chen et al., 2015; Bule et al., 2018).

Introduction

1.4.2 *Monascus* **Pigments and Derivatives: Biosynthesis, Chemical Structures, and Potential Applications**

M. purpureus produces over 50 identified metabolites, with the major six being azaphilone pigments in the form of yellow pigments monascin and ankaflavin, red pigments monascorubramine and rubropunctamine, and orange pigments monascorubrin and rubropunctatin, all of which are produced in a cell-bound form (Chen et al., 2017; Dufosse, 2022). These pigments are called azaphilones because they are composed of an azaphilone skeleton. The orange pigments, for instance, contain an oxo-lactone ring. In addition, yellow and red pigments are derived from the orange pigment, thus also containing the azaphilone skeleton (Fig. 1.18). Table 1.3 summarises some of the most well-characterised secondary metabolites, including pigments, statins, and other bioactive compounds, along with their biological activities and applications

The biosynthesis of these *Monascus* pigments occurs via a polyketide pathway (Chai et al., 2020). The genomic and transcriptomic data analysis provides insights into the biochemical reactions involved in pigment formation. According to these findings, the orange pigments of *Monascus* sp are produced through chemical reactions leading to the esterification of a chromophore acquired from the polyketide synthase (PKS) pathway with a beta-ketoacid derived from the fatty acid synthase (FAS) pathway. The reduction reaction of orange pigments forms the yellow pigments of *Monascus* sp. In contrast, red pigments are produced when orange pigments undergo amination by replacing an oxygen atom with NH3 or an amine group (Fig. 1.19) (Dhale et al., 2007; Chen et al., 2017). More details about the synthesis of *Monascus* pigments can be found in the pigment biosynthesis section (Section 1.7).

Table 1.3. Selected Secondary Metabolites Produced by *Monascus purpureus* and Associated Bioactivities (adapted from Chen et al., 2017; Dufossé, 2022; Feng et al., 2012; Gupta et al., 2022).

Metabolite	Туре	Biological Activity / Application
Monascin	Yellow azaphilone pigment	Antioxidant, anti-inflammatory
Ankaflavin	Yellow azaphilone pigment	Anti-cancer, cholesterol-lowering
Rubropunctatin	Orange pigment	Antibacterial, cytotoxic
Rubropunctamine	Red pigment	Anti-inflammatory, potential anticancer
Monascorubrin	Orange/red pigment	Antimicrobial, food coloring
Monascorubramine	Red pigment	Anti-obesity, antimicrobial
Lovastatin (Monacolin K)	Polyketide/statin	HMG-CoA reductase inhibitor, cholesterol- lowering
Citrinin	Mycotoxin (unwanted)	Nephrotoxic, hepatotoxic (undesirable contaminant)
γ-Aminobutyric Acid (GABA)	Non-protein amino acid	Neurotransmitter, hypotensive effects
Dimerumic Acid	Iron chelator	Antioxidant
Monacolin L	Polyketide	Cholesterol-lowering
Monacolin J	Polyketide	Cholesterol-lowering
Dehydromonacolin K	Polyketide	Cholesterol-lowering
Compactin (Mevastatin)	Polyketide	HMG-CoA reductase inhibitor, cholesterol- lowering
Flavipigments	Pigments	Potential food colorants
Monascusone A & B	Azaphilone derivatives	Antioxidant, anti-inflammatory
Phoenicin	Azaphilone derivative	Antimicrobial
Monapurones	Azaphilone derivatives	Antioxidant
Monascopyridines	Pyridine derivatives	Antimicrobial
Monascumic acids	Polyketide derivatives	Antioxidant





Fig. 1.18. Chemical structure of the primary six azaphilone pigments produced by *M. purpureus*, including yellow pigments (Monascin and Ankaflavin), orange pigments (Rubropuctatin and Monascorubrin), and red pigments (Rubropuctamine and Monascorubrin). The pigments are named after their Azaphilone skeleton, which consists of an oxo-lactone ring. The figure also illustrates the Azaphilone scaffold (Kim et al., 2018).

The *Monascus* sp can break down organic substrates into several bioactive metabolites, including monacolins (K, L, J), dehydroxymonacolin, compactin, isoflavones, polyketide, dimerumic acid, γ -aminobutyric acid (GABA), and citrinin. These metabolites have been utilised as therapeutic agents for preventing and curing various health conditions, such as cardiovascular diseases, inflammation, digestive and gastrointestinal diseases, and cancer (Vendruscolo et al., 2012; Patakova, 2012; Agboyibor et al., 2018).



Fig. 1.19. A simplified pathway depicting the biosynthesis of *Monascus* pigments (Yellow, Orange and Red) (Chen et al., 2017).

One of these bioactive metabolites is Lovastatin, also known as Monacolin K (Fig. 1.20), a fungal polyketide that acts as a potent 3-hydroxymethylglutaryl-CoA (3-HMG-CoA) inhibitor. Lovastatin was first discovered in the fermented broth of *Aspergillus terreus*, and *M. ruber* in the 1970s and was used to treat hypercholesterolemia (Alberts et al., 1980; Tobert, 2003). Originally named mevinolin, it entered the market in 1987 and became a bestseller with a yearly profit of over \$1 billion due to its success in reducing cholesterol levels. The enzyme 3-HMG-CoA catalyses the production of mevalonate, which is an immediate precursor for cholesterol biosynthesis. Lovastatin is synthesised by the multi-domain enzyme complex Polyketide Synthase, encoded by *lov* genes (also called *mok* genes), namely *lovA*, *lovB*, *lovC*, *lovD*, and *lovF*. The biosynthetic pathway of lovastatin resembles the fatty acid synthesis pathway, where the repetitive addition of acetyl-CoA leads to the elongation of the polyketide chain. Today, lovastatin is a well-known prescription drug sold as Movacor and Simvastatin, a synthetic derivative (Rodrigues, 2016; Money, 2016; Zhang et al., 2017; Song et al., 2019; Zhang et al., 2020).



Fig. 1.20. Chemical structure of Lovastatin (Source: Pubchem Lovastatin | C24H36O5 | CID 53232 - PubChem (nih.gov)).

The composition of *Monascus* pigments and metabolites is influenced by the growth conditions and substrate used. For instance, under certain stressful conditions—such as the presence of cell extracts like hydrolytic enzymes, and physical stresses like UV, osmotic, and temperature stress—the orange pigments can undergo a Schiff base formation reaction, where the oxygen moiety is substituted by nitrogenous compounds such as amino acids, peptides, amino sugars, amino alcohols, chitosan, and nucleic acids. This leads to the formation of red and yellow pigments, which are characterised as glutamate derivatives of the *Monascus* orange pigments (Fig. 1.21) (Kim et al., 2007; Kim et al., 2018; Dixit and Tallapragada, 2018).



Fig. 1.21. Formation of pigment derivatives via substitution of the oxygen moiety in *Monascus* pigments with a nitrogen moiety from nitrogenous compounds, such as amino acids, peptides, amino sugars, amino alcohols, chitosan, and nucleic acids (Kim et al., 2018).
Introduction

1.4.3 Exploring the Health Benefits and Biological Activities of *Monascus* **Secondary Metabolites**

Monascus pigments and derivatives possess a wide range of therapeutic and medicinal properties, including the well-known cholesterol-lowering effect. Additionally, these compounds have demonstrated anticancer, antimicrobial, anti-inflammatory, and antibiosis properties. *Monascus* metabolites have also shown potential in addressing health issues such as diabetes, osteoporosis, non-alcoholic fatty liver, fatigue, and memory loss. Fig. 1.22 depicts the projected economic losses resulting from cancer, heart diseases, and mental health issues by 2030, as estimated by the American Heart Association in 2017, highlighting the significance of developing preventive and control measures for these diseases (Emelia et al., 2017).

Cholesterol is a significant risk factor for various health problems such as obesity, high blood pressure, heart disease, stroke, and other cardiovascular diseases that can lead to fatal outcomes, making it an important issue to address. In addition to lovastatin, *M. purpureus* also produces another widely prescribed statin drug, mevastatin, known as Compactin, to inhibit cholesterol and triglyceride synthesis (Klimek et al., 2009; Younes et al., 2018; Nguyen, 2020). Other commercially available statins include atorvastatin, simvastatin, rosuvastatin, pitavastatin, pravastatin, and fluvastatin. While some of these statins are naturally produced by fungi (lovastatin, simvastatin and pravastatin), the rest of the statins are synthetic compounds (Schachter, 2005; Patel and Kotharia, 2017).



Fig. 1.22. Estimating the Potential Economic Loss by 2030 due to Major Health Issues like Cancer, Heart Disease, and Mental Health (American Heart Association-Emelia et al., 2017).

In 2012, Yang and Mousa provided substantial evidence supporting the beneficial effects of RYR consumption in reducing cholesterol levels and preventing myocardial infarction in humans and animals. They reported a reduction in total cholesterol and triglyceride levels by 11–32% and 12–19%, respectively, attributed to lovastatin, a secondary metabolite produced by *Monascus*. This compound acts as a reversible competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the key enzyme responsible for converting HMG-CoA to mevalonate—a crucial precursor in cholesterol biosynthesis. In addition to lovastatin, the orange pigments and its Threonine derivatives (Thr-derivatives), which contain an oxygen atom, have also demonstrated anti-atherosclerosis effects by inhibiting HMG CoA reductase (Alberts et al., 1980; Kim and Ku, 2018).

One of the derivatives of *M. purpureus* is gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter with antihypertensive properties. Studies have shown that adding 0.2-0.3% of *Monascus* fermented products containing GABA and glucosamine to the diet of hypertensive rats can lower their blood pressure from over 200 mmHg to below 180 mmHg (Tsuji et al.,

1988; Shi and Pan, 2011; Vendruscolo et al., 2016). RYR and Dioscorea have potential anticancer effects against skin, liver, colon, lung, and mouth cancers (Akihisa et al., 2005; Hong et al., 2008; Ho et al., 2009; Hsu et al., 2010). The orange pigments of *Monascus* sp., monascorubrin and rubropunctatin, exhibit strong anti-cancer properties. The ethanol extract of RYR significantly inhibits tumour growth and metastasis, reducing tumour size by 50.8% after four weeks in mice with Lewis lung carcinoma (Su et al., 2005; Ho et al., 2009). Similarly, RYR extracts also reduce prostaglandin E2 (PGE2), nitric oxide, and reactive oxygen species levels while increasing antioxidant enzyme activities, inhibiting DMBA-induced oral tumour carcinogenesis through anti-inflammatory and antioxidative effects (Hsu et al., 2010; Hsu and Pan, 2012; Robbins and Zhao, 2014). Furthermore, lovastatin and ankaflavin in RYR synergistically increase tumour cell apoptosis and inhibit tumour invasion and metastasis by blocking VEGF-induced angiogenesis These findings suggest that RYR extracts could be a promising clinical supplement for cancer treatment.

Obesity is another concern that can lead to various health issues, such as diabetes and cardiovascular diseases, reducing life expectancy. Choe et al. (2012) found that NH_3 derivatives of *Monascus* pigments exhibited anti-obesity properties in both in vivo and in vitro mouse model tests. They synthesised *Monascus* orange pigments with 47 different amines and generated 16 derivatives, which inhibited adipogenic differentiation in 3T3-L1 cells. Among them, 4-phenylburylamine (PBA) and 2-(p-toyly) ethylamine (TEA) exhibited the most significant results, reducing cell differentiation by about 40% when treated at concentrations of 2.5 μ M and 12.5 μ M, respectively. Other studies have supported these results and found that phenolic compounds such as flavonoids, genistein, naringenin, rutin, EGCG, and curcumin could also inhibit 3T3-L1 cell differentiation and adipogenic transcriptional factors (Harmon and Harp, 2001; Aranaz et al., 2019).

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Lee et al. (2016) suggested that ethanol extracts of RYR could also benefit Alzheimer's disease prevention. Alzheimer's disease is a major cause of brain degradation in older individuals, with complex causes influenced by factors such as gene mutation, environment, and personal habits. The amyloid protein hypothesis (amyloid β -peptide, A β) is currently the most widely accepted theory. Lovastatin extracted from *M. purpureus* inhibits the activation of small G-protein by hindering the formation of downstream product geranylgeranyl pyrophosphate (GGpp), which prevents A β 40-induced inflammation and neural cell toxicity (Shi and Pan., 2011).

Studies show that *M. purpureus* has antimicrobial properties against various bacteria and fungi, including *Escherichia coli, Salmonella enteritidis, Bacillus subtilis, Staphylococcus aureus,* and yeast (Endo, 1979; Tsuji et al., 1988; Akihisa et al., 2005; Hsu et al, 2010; Sun et al., 2012; Rodrigues et al., 2016; Esmaeilishirazifard et al., 2018; Mehmood et al., 2019). *Monascus* secondary metabolites also exhibit antiviral properties as a remarkable suppression of HCV RNA replicon is reported with *Monascus* pigment derivatives isolated from KCCM 10093 (Sun et al., 2012). A commercially available *Monascus* antibiotic is Monascidin A (Endo, 1979; Cheng et al., 2010).

Given the importance of *Monascus* pigments and other metabolites, researchers are exploring ways to artificially induce physiological changes in *Monascus* cultures to overproduce these metabolites. For example, Panda et al. (2010) found that a mixed culture fermentation approach involving *M. purpureus* and *M. ruber* grown on a rice medium could overproduce lovastatin. Similarly, the current study used quorum sensing and elicitation to enhance *M. purpureus* C322 pigments and lovastatin production.

1.4.4 Biosynthesis of *Monascus* Pigments and Lovastatin: Mechanism, Regulation and Applications

1.4.4.1 Monascus Pigment Biosynthesis

Monascus pigments are synthesised through a putative polyketide pathway, which needs to be fully understood due to their complexity. The production of *Monascus* pigments relies on the activity of polyketide synthase, a key enzyme in the pathway. Sequencing the *Monascus* azaphilone gene cluster (53kb) revealed highly conserved regions from various host organisms, including *P. marneffei*, *T. stipitatus*, *M. purpureus*, *M. pilosus*, and *M. ruber*. Still, the gene positions vary among different *fungal* sp (Fig. 1.23). The gene cluster encodes several enzymes, including polyketide synthase, fatty acid synthase, dehydrogenase, transporter, and regulator (Fig. 1.23 c, Fig. 1.28).

In *Monascus* sp, regions I and II of the azaphilone gene cluster are separated by insertions (regions III and IV) due to genomic rearrangements during evolution (Fig. 1.24 a, b). The biosynthesis of *Monascus* pigments generates highly reactive intermediates via redox enzymes during the biosynthetic cycle, and the failure of an enzyme to catalyse the reaction of an intermediate can lead to the production of different products via shunt pathways (Choe et al., 2012; Chen et al., 2017). The biosynthetic pathway for *Monascus* pigment production was predicted using Carbon-13 isotopic labelling (Fig. 1.25). The pathway is initiated when the enzyme fatty acid synthase catalyses a reaction between acetyl-CoA and malonyl-CoA to form a beta-keto acid via the fatty acid synthase pathway. *MpfasA2* (J) and *MpfasB2* (K) encode a canonical fatty acid synthase that supplies medium-chained (C8, C10) fatty acyl moieties such as octanoic acid. Polyketide synthase is encoded by the two structural genes of pigments, namely *MpPKS5* (A) and *mppD* (G). The multienzyme complex, polyketide synthase, leads to the formation of the polyketide chromophore (hexaketide) by condensing 1 mole of acetic acid with 5 moles of malonate (Chen et al., 2015; Chen et al., 2017; Wang et al., 2017).



Fig. 1.23. Sequence analysis of the *Monascus* azaphilone biosynthetic gene cluster, where A and B) compare the gene cluster in *P. marneffei*, *T. stipitatus*, *M. purpureus*, *M. pilosus*, and *M. ruber*; and C) provides an annotation of the *Monascus* azaphilone biosynthetic gene cluster (Choe et al., 2012; Chen et al., 2017).

Monascorubrin, the orange pigment, is formed when the beta-keto acid combines with a polyketide chromophore through a transesterification reaction. The *mppB* gene (D) encodes trichothecene 3-O-acyltransferase (AT), which converts the Carbon-8 and Carbon-10 fatty acyl chains into polyketide chromophores of these pigments. Similarly, when hexanoic acid binds with the polyketide chromophore, another orange pigment, rubropuctatin, is formed (Fig. 1.24, 1.25). Oxidation of orange pigments leads to the formation of yellow pigments (monascin and ankaflavin) through a hydrogenation reaction. The addition of an amine group (ammonation) to orange pigments generates red pigments (Fig. 1.27, 1.28). (Huang et al, 2017; Wang et al., 2017).



Fig. 1.24. Illustrates the predicted biosynthetic pathway for *Monascus* pigment (Yellow, Orange and Red) production (Huang et al., 2017).



Fig. 1.25. Simplified biochemical pathway for *Monascus* pigment biosynthesis (Wang et al., 2017).



Fig.1.26. The biosynthetic pathway for the formation of *Monascus* yellow pigments (Wang et al., 2017).

Introduction

The regulatory genes for *Monascus* pigment biosynthesis are *mppR1* (B) and *mppR2* (I) (Feng et al., 2012; Balakrishna et al., 2013). The biosynthesis of orange pigment (rubropuctatin) involves a series of reactions, including methylation, hydroxylation, condensation, and dehydrogenation of acetyl CoA (Fig. 1.24, 1.28). According to Jongrungruangchok et al. (2017) and Wang et al., (2017), condensation of acetate and malonate through polyketide synthase generates monascusone A and FK17-P2b2 (Unknown compound- UV absorbing). Yellow pigments such as monascin and monascusone B are formed by binding medium-chain fatty acids with monascusone A and FK17-P2b2 (Fig. 1.26, 1.28). The *mppE* (H) gene encodes the reductive enzyme involved in yellow pigment formation, while *mppC* (E) encodes the oxidoreductase enzyme. The up-regulation of the *mppE* gene under blue light leads to a decrease in orange pigment production, supporting the production of yellow pigments from orange pigments (Huang et al., 2017).

It has been observed that during glucose stress, specifically towards the latter stages of fermentation, several genes are involved in forming key pigment biosynthetic intermediates, including *MpPKS5*, *MpfasA2*, *MpfasB2*, *mppB*, *mppD*, *mppE*, and *mppR1*, were upregulated. At the same time, *mppC* and *mppR2* were downregulated, resulting in an increased production of yellow pigments. This is likely due to the availability of more precursors and cofactors, such as acetyl-CoA, malonyl-CoA, NADP, and NADPH, in high glucose concentrations (Chen et al., 2015; Chen et al 2017).

G-protein heterotrimer consists of three subunits: α , β , and γ , with *Mga1*, *Mgb1*, and *Mgg1* as their respective subunits. *MrflbA* is the regulator of the α subunit. The signal transduction pathway, G protein, and gene regulators all play a significant role in secondary metabolism. Li et al. (2010, 2014) suggested that *Mga1*, *Mgb1*, and *Mgg1* promote vegetative growth and development, both sexual and asexual while repressing pigment production. These findings

suggest that diverting the pathway towards the desired end product is possible, making the *Monascus* strain more commercially viable (Chen et al., 20165; Li et al., 2010; Li et al., 2014).



Fig. 1.27. The biosynthesis of *Monascus* red pigments, Rubropuctamine and Monascorubramine (Wang et al., 2017).



Fig. 1.28. *Monascus* azaphilone gene cluster and its predicted biosynthetic pathway (Chen et al., 2015).

1.4.4.2 Biosynthesis of Lovastatin in Monascus

In 1999, the first biosynthetic operon of lovastatin was isolated from *Aspergillus terreus*, followed by the isolation of the lovastatin (Monacholin K) operon from *Monascus* sp. (2008). Comparative analysis of both operons identified nine highly similar genes (*mokA*, *mokB*, *mokC*, *mokD*, *mokE*, *mokF*, *mokG*, *mokH*, *mokI*), among which *mokB*, *mokD*, and *mokH* are responsible for the synthesis of the diketide side chain of lovastatin, oxidoreductase, and transcriptional regulation of lovastatin, respectively. Compactin or 6-Demethylmevinolin was first extracted by Endo (1976) and later by Alberts et al. (1980) (Fig. 1.29)

The biosynthesis of lovastatin begins with Lovastatin Nonaketide Synthase (LNKS), catalysing the condensation of nine acetyl CoA molecules with one malonyl CoA molecule to produce dihydromonacholine L (DL), a nonaketide molecule. Oxidation-reduction reactions of dihydromonacholin L generate monacholin L, which undergoes a hydroxylation reaction catalysed by oxygenase to form monacholin J. Lovastatin Diketide Synthase (LDKS) condenses one molecule of malonyl CoA with two molecules of acetyl CoA to form methylbutyryl CoA, which is then linked via an ester ether bond formation to monacolin J with the help of the enzyme transesterase, ultimately leading to the synthesis of Lovastatin (monacolin K) (Fig. 1.30) (Juzlova et al., 1996; Zhang et al., 2017; Song et al, 2019).



Fig. 1.29. The Biosynthetic pathway of Lovastatin (Monacholin K) in the filamentous fungus *Aspergillus terreus*, where SAM= S-Adenosyl methionine, LNKS= lovastatin nonaketide synthase, LDKS= lovastatin diketide synthase, R= 2-[(2R,4R)-4-hydroxy-6-oxo-2-tetrahydropyranyl]-ethyl. (Song et al., 2019).



Fig. 1.30. Genes upregulated during the biosynthesis of lovastatin in *M. purpureus* (Zhang et al., 2017).

Table 1.4 presents a consolidated list of regulatory and biosynthetic genes identified in *Monascus* species that are involved in the production of secondary metabolites, including pigments and lovastatin. The table includes gene or cluster names, their associated functions, and the specific pathways in which they are implicated.

Table 1.4. Regulatory and Biosynthetic Genes Involved in *Monascus* Secondary Metabolite Production (adapted from Chen et al., 2015; Feng et al., 2012; Song et al., 2019; Zhang et al., 2017; Yang et al., 2015).

Gene / Cluster	Function	Product / Role	Associated Pathway
MpPKS5	Polyketide synthase	Chromophore backbone synthesis	Pigment
mppD	Polyketide synthase	Hexaketide formation	Pigment
MpfasA2 / MpfasB2	Fatty acid synthases	Supply C8–C10 fatty acyl chains	Pigment
тррВ	Acyltransferase (AT)	Attaches fatty acyl chains to chromophores	Pigment
mppC	Oxidoreductase	Converts orange to yellow pigments	Pigment
mppE	Reductive enzyme	Promotes yellow pigment formation, upregulated by blue light	Pigment
mppR1	Transcriptional activator	Upregulates pigment biosynthesis	Pigment
mppR2	Repressor	Downregulates pigment biosynthesis	Pigment
Mgal / Mgbl / Mggl	G-protein α , β , γ subunits	Repress pigment biosynthesis; promote growth	Pigment
MrflbA	G-protein regulator	Controls Mga1 activity	Pigment
mokA-mokI	Lovastatin gene cluster	Enzymes for monacolin K synthesis	Lovastatin
mokB	Side chain synthesis	Part of lovastatin biosynthesis	Lovastatin
mokD	Oxidoreductase	Involved in monacolin K formation	Lovastatin
mokH	Transcriptional regulator	Activates mok cluster	Lovastatin
LNKS	Nonaketide synthase	Produces dihydromonacholine L	Lovastatin
LDKS	Diketide synthase	Synthesises methylbutyryl CoA	Lovastatin
PKS-FAS operon	Combined PKS-FAS cluster	Core for pigment and lovastatin biosynthesis	Both

Introduction

1.4.5 Genome Sequencing of *M. purpureus* C322: Insights and Opportunities for Genome Alterations and Mutational Studies

The first whole genome sequencing project of *M. purpureus* was performed on the *M. purpureus* YY-1 strain, which revealed a genome size of 24.1 MB with 7491 genes. The PKS-FAS operon was identified as a crucial gene cluster for statin/pigment formation among these genes (Yang et al., 2015). Since then, additional genome sequencing efforts have been undertaken for other *M. purpureus* strains, with a completed genome available for strain GB-01 (Kumagai et al., 2019). However, no genomic information was available for the *M. purpureus* C322 strain used in this PhD project. Therefore, whole genome sequencing was conducted with the view to provide complete information about the genome.

Access to the complete DNA information of *M. purpureus* C322 offers an opportunity to modify the genome according to specific requirements. In a previous study, Dikshit and Tallapragada (2018) induced controlled mutations in *Monascus sanguineus*, which increased lovastatin production. This implies that a similar approach could be used with *M. purpureus* to potentially increase desired metabolite production. Moreover, the sequence information can also help identify any potential mutations and polymorphisms caused by the addition of elicitors and quorum-sensing molecules, which could enhance the production of bioactive metabolites. Overall, obtaining the genomic information of *M. purpureus* C322 could advance research and applications in the field of natural product biosynthesis.

1.4.6 Overview of the Reproductive Cycle of Monascus sp.

Monascus sp., classified within the ascomycetes group of the Eucomycota phylum, exhibit both sexual and asexual reproductive cycles (Fig. 1.31). During sexual reproduction, they produce haploid ascospores through meiosis within an ascus (Carel et al., 1975), and generate conidiospores on aerial hyphae for asexual reproduction (Ajdari et al., 2011). *M. purpureus*, known for its branched, septate mycelium, demonstrates both reproductive modes, contributing

to its versatility in industrial fermentation processes (Tortora et al., 1995; Manan and Atif., 2017). This dual reproductive capability is recognised in its taxonomy, differentiating the sexual teleomorph from the asexual anamorph phase, providing a foundation for its classification within the ascomycetes (Lin et al., 1992; Tortora et al., 1995; Campbell, 1995; Suh and Shin, 2000; Krasniewski et al., 2006; Park and Yu, 2012).



Fig. 1.31. Asexual and sexual reproductive cycle of *Monascus*; where, (a,b) Ascospores develop into vegetative hyphae, (c-g) the formation of reproductive organs and ascogenous hyphae development. (h, i) mature ascogonium with asci and ascospores. (j) asexual reproduction producing one-celled conidia (j). Key structures include antheridia (an), peridial wall cells (p), ascogonium (ag), asci (a), trichogyne (tg), ascospores (as), ascogenous hyphae (ah), and conidia (c). (Image Source: Pan and Hsu, 2014).

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Conidia, defined as asexual, non-motile spores, originate from the sides or tips of specialised sporogenous cells in fungi, resembling vegetative growth (Barnett et al., 1972; Sutton et al., 1998). Characteristically, conidia may be single, apical, or form chains that readily disperse into individual spores, exhibiting unicellular, rounded, truncated, or occasionally pear-shaped forms (Barnett and Hunter, 1972; Kirilenko, 1978; Carmichael et al., 1980; Pitt et al., 2009).

The sexual reproduction process in *Monascus* sp. is characterised by several stages and involves the formation of specialised structures. Initially, an antheridium (male gametangium) forms at the hyphal tip, followed by the emergence of an ascogonium (female gametangium) from a nearby cell. These organs grow towards each other, with the female organ eventually becoming the primary growth point. Inside the female organ, the ascogonium and trichogyne are separated by a septum. Male nuclei migrate into the ascogonium and the ascogenous hyphae, now containing both male and female nuclei, enlarges prior to forming asci via nuclear fusion and subsequent divisions producing ascospores. These spores are released when the ascocarp, typically a cleistothecium, ruptures, starting a new growth cycle with germination (Young, 1931; Carels and Shepherd, 1975; Pitt et al., 1985; Tortora et al., 1995; Rashiva et al., 1998; Pordel et al., 2015).

1.4.7 Understanding Spore Formation and Germination

Spores are specialised cells often misconstrued as dormant, suggesting a complete metabolic inactivity. However, they also exhibit hypometabolism, engaging in minimal but essential metabolic and respiratory activities to maintain viability (El-Enshasy, 2007; Feofilova et al., 2012). Upon encountering favourable conditions, spores transition from a quasi-dormant state to active growth through a series of developmental stages. The initial phase, germination, involves the spore absorbing water, leading to an increase in size and weight, marking its transition to an active state (Hassouni et al., 2007). The activated cytoplasm initiates nuclear

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division, and this process is supported by the presence of specific proteins and enzymes within the spore, facilitating the formation and development of germ tubes, signifying successful germination. The growth process proceeds with the elongation and branching of hyphae, indicative of active fungal development (Gougouli and Koutsoumanis, 2013).

Fungal spores fulfil dual purposes: ensuring survival under adverse conditions and facilitating the spread of fungi to new territories. Carlile et al. (1994) distinguish between dispersal spores, designed for spreading the fungus to new locations, and survival spores, which enter a dormant state to withstand unfavourable conditions. Dispersal spores are equipped for long-distance travel and prompt germination in new environments, whereas survival spores detach from the parent mycelium through cell lysis, awaiting improved conditions for germination. The dispersion of spores, whether by air currents, animal carriers, or self-launching mechanisms, constitutes a strategic survival tactic, allowing fungi to colonise new habitats. Each fungal species boasts a unique and specialised dispersal mechanism, underscoring the diversity and adaptability inherent in fungal reproduction strategies.

1.4.8 Morphology of Monascus sp. in Submerged Fermentation

The morphology of *Monascus* species during submerged fermentation is influenced by genetic factors and environmental conditions, significantly impacting growth patterns and secondary metabolite production (Buhler et al., 2013; Veiter et al., 2018). Understanding and optimising these conditions can enhance metabolite productivity (Chen et al., 2017).

1.4.8.1 Morphological Variations

Monascus sp. exhibit diverse morphologies during submerged fermentation, including filamentous hyphae development, pellet formation, and clump formation (Veiter et al., 2018; Meyer et al., 2021). Filamentous hyphae are the primary growth form of *Monascus* and contribute to overall biomass production. Even under identical growth conditions, different

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Monascus sp. can exhibit distinct morphologies. These differences in morphology can impact the growth rate, product synthesis, and overall fermentation performance of *Monascus* strains. The differences in morphology highlight the genetic diversity within the *Monascus* genus and underscore the importance of understanding and characterising specific strains for optimal fermentation processes (Muhammad, 2020; Chen et al., 2021). The variations in morphology among different *Monascus* sp may be attributed to genetic factors, such as differences in gene expression and regulation, as well as the presence of specific enzymes and metabolic pathways (Kim et al., 2002; Shao et al., 2011; Chen et al., 2020). In a study conducted by Chai et al. (2020), it was observed that genetic variations amongst *M. purpureus* LQ-6 and recombinant strains exhibited a significant impact on morphology and pigmentation, even when cultured under identical growth conditions (Fig. 1.32 b).

1.4.8.2 Impact of Morphology on Fermentation Process

The impact of morphological variations on the performance of *Monascus* strains has been welldocumented. Dispersed mycelial growth or irregular pellet formation in bioreactors leads to non-uniform substrate and product distribution, increased viscosity, and potential cell damage due to shear stress. Therefore, a dispersed growth may exhibit reduced nutrient utilisation and lower product yields (Porcel et al., 2005; Veiter et al., 2018; Li et al., 2020; Chen et al., 2021).

In contrast to dispersed mycelium, controlled pelleted growth forms reduce broth viscosity, improving mass and heat transfer, and simplifying downstream processing, making it economically advantageous (Van Suijdam et al., 1980). Studies have shown that the size and compactness of pellets can influence nutrient uptake, oxygen transfer, and metabolite production. For example, homogeneous and compact pellets with comparatively thicker outer layers and loosely packed centres have been associated with enhanced mass transfer and increased production of secondary metabolites, such as pigments and statins (Veiter et al., 2018). At the same time, larger, denser and agglutinated pellet morphology can lead to

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heterogeneity and internal oxygen and nutrient deprivation, altering metabolic activities, enzyme secretion, and potentially causing autolysis and cell death at the pellet core; thus, reducing the overall yield of secondary metabolites (Hermersdorfer et al., 1987; Lin et al., 1992; Pazouki and Panda, 2000). It is important to consider the pellet size and its implications on mass and oxygen transfer when studying the growth characteristics (Sundaramurthy, 2011; Veiter et al., 2018). Therefore, optimising the growth conditions to promote uniform and compact pellet formation is crucial for maximising the productivity and efficiency of *Monascus* fermentation processes (Reichl et al., 1992; Casas Lopez et al., 2005).

Pellet formation results from one of three spore interaction mechanisms: coagulation, noncoagulation, and hyphal–element agglomeration, influenced by the germination process (Paul and Thomas, 1996; Agger et al., 1998). Factors affecting culture morphology during pellet formation include strain-dependent factors (e.g., inoculum type and concentration), nutritional elements (carbon and nitrogen sources), and cultivation conditions (temperature, pH, bioreactor type) (El-Enshasy, 2007).

1.4.8.3 Factors Influencing Morphology

In addition to strain-specific differences, the morphology of *Monascus* sp can be influenced by various environmental factors, such as temperature, pH, dissolved oxygen tension, nitrogen concentration, agitation, media components, inoculum size and design of growth container (Chen and Johns, 1993; Landin and Echezarreta, 2010; Vendruscolo et al., 2010; Vendruscolo et al., 2012; Yang et al., 2015; Vendruscolo et al., 2016; Lahouar et al., 2016; Hagiwara, 2017; Hong et al., 2020; Lee et al., 2021).

A) Impact of Culture Conditions

The composition of the culture medium is crucial in microbial cultivation, typically including a carbon and nitrogen source, salts, water, and micronutrients (Arora, 2013). The availability

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and ratio of these nutrients significantly affect the growth rate, branching patterns, and spore production in *Monascus*. Specifically, carbon and nitrogen sources can promote the formation of structures like sclerotia, aerial mycelia, or fruiting bodies (Trinci et al., 1990; Meletiadis, 2001; Grimm et al., 2005; Veiter et al., 2018). Lee et al. (2021) found that variations in carbon and nitrogen sources influenced *Monascus* morphology during submerged fermentation, with changes in the carbon-to-nitrogen ratio affecting pellet size and shape. Rivera et al. (2022) confirmed these findings, demonstrating that nutrient adjustments lead to distinct morphological changes. Similarly, Chen et al. (2020) observed that high salt stress on *M. ruber* resulted in shorter hyphae, increased branching, and higher pigmentation (Fig. 1.32 a).

B) Influence of Temperature

Temperature variations can also influence the morphology of *Monascus* sp. (Li et al., 2020). A higher incubation temperature (37 °C) is associated with shorter *M. purpureus* strain 94-25 mycelium and decreased pigmentation, whereas lower temperatures (25 °C) results in increased pigmentation and filamentous hyphal growth by stimulating the development of aerial mycelia and fruiting bodies in some species, while higher temperatures result in increased sporulation (Li et al., 2020; Anna, 1998; Pande, 2007; Dixit and Tallapragada, 2013; Lahouar et al., 2016; Hagiwara et al., 2017; Yue et al., 2020). *Monascus* grows best at temperatures ranging from 25 °C to 30 °C.

C) Role of pH

pH plays a substantial role in influencing the morphology of *Monascus* during submerged fermentation. The acidity or alkalinity of the growth medium is a critical parameter that affects hyphal development, pellet formation, and the biosynthesis of secondary metabolites. Optimal growth of *Monascus* is typically observed within a pH range of 4.0 to 6.5. Any deviation from this optimal range can impact the growth rate, branching patterns, and the synthesis of secondary metabolites. At lower pH values (3.0–5.0), *Monascus* tends to grow in a freely

dispersed form, producing smaller mycelial pellets with filamentous hyphae. In contrast, at higher pH values (6.0–8.0), growth favours the formation of larger, agglutinated pellets (Ceccarini and Eagle, 1971; Chen and Johns, 1993; Hevekerl et al., 2014; Lyu et al., 2017).

D) Influence of Aeration and Agitation

Aeration and agitation are key physical parameters that shape fungal morphology during submerged fermentation. These factors affect the degree of hyphal branching, pellet structure, and spore formation (Papagianni et al., 1999; Veiter, 2018). Manipulating agitation rates can help in achieving higher biomass concentration and promoting the formation of uniform pellets, ultimately enhancing the productivity of desired metabolites (Lv et al., 2017). For example, elevated levels of aeration tend to promote cell growth, while low levels of aeration impede cell growth. Similarity, lower agitation speed in shaken flasks promote filamentous hyphae formation; whereas excessive agitation can impose physical stress hindering growth resulting in stunted morphology (Hong et al., 2006; Lin et al., 2010; Wang et al., 2015; Liu et al., 2016; Lv et al, 2017). Supporting this, studies conducted by Kim et al. (2002) have demonstrated that higher agitation rates (600-700 rpm) yield damaged mycelia with shorter branches, whereas lower agitation rates result in loose and more dispersed growth.

E) Influence of Culture Vessels

In addition to the physical factors affecting growth and metabolism, the culture vessel used for fermentation also impacts the morphology and secondary metabolism of *Monascus* sp. Shaken flasks and bioreactors are commonly employed and exhibit distinct differences in hydrodynamics, dissolved oxygen levels, and their impact on morphology and metabolism. Shaken flasks use rotary or reciprocal shaking for mixing, resulting in relatively simple hydrodynamics. Oxygen is provided through surface aeration and agitation, resulting in limited oxygen transfer and lower dissolved oxygen levels, especially if the culture forms large

agglomerates. This can reduce the production of secondary metabolites like pigments and mycotoxins (Link and Botz, 2011; Veiter et al., 2018).



Fig. 1.32. Morphological variation of *Monascus* sp; a) Impact of growth parameters on *M. ruber* morphology, A1-A3: Conventional batch fermentation and B1-B3: Fermentation under high salt stress (Chen et al., 2020); b) Impact of genetic variation on *Monascus* morphology between *M. purpureus* LQ-6 and recombinant strains fermented at 30 °C, 150 rpm, SBF medium (Chai et al., 2020).

Bioreactors, on the other hand, offer advantages over shaken flasks by providing better monitoring and control of growth parameters, including temperature, pH, and dissolved oxygen levels (Buchs, 2001; Klockner and Buchs, 2012). Different impeller designs and sparger configurations in bioreactors can considerably influence *Monascus* morphology. High shear forces from certain impeller designs, such as Rushton turbines, can fragment agglutinated mycelia, forming smaller pellets or dispersed fragments, while lower shear forces from pitched blade turbines or marine impellers promote larger, denser pellets (Justen et al., 1998; Collingnon et al., 2010). Bioreactors also offer better aeration and oxygen transfer capabilities, enhancing the production of secondary metabolites, including *Monascus* pigments and lovastatin (Lai et al., 2001; Porcel et al., 2006). The ability to maintain optimal growth parameters in bioreactors promotes the desired morphology and improves productivity. Thus, while shaken flasks can support growth and metabolism, bioreactors provide better control over hydrodynamics, dissolved oxygen, and growth parameters, leading to more efficient and controlled cultivation of *Monascus* sp. (Meier et al., 2016).

F) Strain Specific Variability

It is interesting to note that different *Monascus* strains exhibit distinct pellet morphologies, with some strains forming larger and more compact pellets than others (Petra et al., 2013; Meng-lei et al., 2016; Jun et al., 2017; Manan et al., 2017; Silbir et al., 2019; Hong et al., 2020; Liu et al., 2020; Buranelo et al., 2023). *Monascus* sp. display variations in their growth requirements, and different strains of the same species can exhibit varying morphologies, even under the same growth conditions. Therefore, each strain potentially requires different optimal conditions compared to other strains within the genus, *Monascus*.

By manipulating the factors such as media composition, pH control, temperature, dissolved oxygen levels, inoculum type and shear stress, it is possible to promote the formation of compact and homogeneous pellets, which can enhance the productivity of desired metabolites

(Van Suijdam and Metz, 1981; Braun and Vecht-Lifshitz, 1991; Martinkova and Patakova, 1999; Manan et al., 2017; Suraiya et al., 2018; He et al., 2020). Hence, these observations highlight the importance of optimisation of growth conditions to achieve desired outcomes in morphological characteristics and secondary metabolite production during fermentation of the selected *Monascus* strain (Ceccarini, 1971; Chen, 1993; Juzlova, 1996; Tseng, 2001; Ajdari, 2011; Arora, 2013).

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2.1 Introduction

The materials and methods used in this study include various chemicals and reagents obtained from VWR LTD, UK, unless stated otherwise. High-Performance Liquid Chromatography (HPLC) assays were conducted using HPLC-grade solvents and double distilled water, while all other quantitative and qualitative assays were performed using analytical-grade reagents. The growth medium was prepared using general-purpose reagents. Lichrospher ® RP-18 Endcapped (5MYM) Hibar® RT 150-4.6 HPLC column used for lovastatin analysis was obtained from Merck Chemicals Limited, UK. The beta-mannanase E-BMABS used for elicitation studies was ordered from Megazyme, Ireland.

The kits used for sequencing studies included Qiagen fungal DNA/RNA/Protein extraction kit and Genomic DNA Clean and ConcentratorTM Kit from Cambridge Biosciences, UK. The DNA sequencing was performed by Eurofins Ltd, Germany, and Geneious Prime 2020.1 software was used for genome assembly and annotation.

For simplification, the experiments were categorised into six main sections, as shown in Fig. 2.1. The morphology of *M. purpureus* C322 was studied microscopically throughout fermentation for all experiments using a light microscope. The first section involves culturing and maintaining *Monascus purpureus* C322 cells.

The second section specifies the experiments for optimising *M. purpureus* C322 morphology by modifying the growth parameters, including pH, temperature, and aeration conditions. In addition to morphological observations, biomass concentration and pigment production were also quantified.



Fig. 2.1. The experiments performed in this study, divided into five main categories for simplification.

The third section details the elicitation studies using various biotic elicitors, namely, oligoguluronate, oligomannuronate, and mannan oligosaccharides. The impact of elicitors on the production of secondary metabolites, namely lovastatin and pigments (Yellow, Orange, and Red), was measured using HPLC and spectrophotometry. In addition, biomass concentration, carbohydrate consumption, and pH variation were also analysed throughout fermentation to assess the overall impact of the elicitors on the growth and development of *M. purpureus* C322. Initially, the experiments were conducted in 500 mL shaken flasks, and subsequently, the process was scaled up using 2.5 L stirred tank bioreactors.

The fourth section investigated the impact of quorum sensing molecules, including butyrolactone-I, farnesol, tyrosol, and linoleic acid, on lovastatin and pigment production in *M. purpureus* C322. The genes involved in quorum sensing were identified by comparing the *M. purpureus* C322 genome with known quorum sensing genes in other microorganisms.

The fifth section involved genomic analysis, which includes sequencing, assembly, and annotation of the *M. purpureus* C322 genome using next-generation sequencing technology and several bioinformatics tools. Transcriptomic analysis was conducted by investigating the expression of the annotated quorum sensing genes using Reverse transcription PCR (RT-PCR).

Overall, the materials and methods presented in this chapter provide a novel approach to improving the production of lovastatin and pigments by *M. purpureus* C322 and investigating the genetic basis of these processes. Furthermore, the results obtained from these experiments could be used to develop new strategies to increase the yield and productivity of these valuable by-products.

2.2 Section 1: Cell Culture, Monitoring and Maintenance

2.2.1 Microorganism

Monascus purpureus strain C322, obtained from the culture collection at the School of Life Sciences, University of Westminster, was selected for this study,

2.2.2 Maintenance Medium

The *M. purpureus* C322 stock cultures were maintained on Yeast Malt Extract (YME) at pH 6.2 medium and sub-cultured every two weeks using Potato Dextrose Broth (PDB) and Hiroi Potato Dextrose Agar (PDA) (Ajdari et al., 2011). The medium was inoculated with 10⁶ spores/mL and incubated at 32 °C for 7-10 days before being stored at 4 °C for future use (Table 2.1). All media constituents, except sugars, were subjected to autoclaving at 121 °C. Sugars, on the other hand, were autoclaved separately at 110 °C. Post-autoclaving, all media constituents were thoroughly mixed before usage.

Medium	Conc. (g/L)	
Hiroi PDA Constituents		
Sucrose	100.0	
Yeast Extract	3.0	
Casamino Acid	5.0	
NaNO3	2.0	
KH2PO4	1.0	
MgSO4.7H2O	0.5	
KCl	0.5	
FeSO4.7H2O	0.01	
Potato Glucose Agar	39.0	
Yeast Malt Extract Constituents		
Dextrose	10.0	
Peptone	5.0	
Malt Extract	3.0	
Yeast Extract	3.0	
Agar	20.0	
Potato Dextrose Broth (PDB)	24.0	

Table 2.1. Composition of Maintenance Medium.

2.2.3 Growth Medium

For shaken flasks and fermentation experiments, *M. purpureus* C322 was cultured in a chemically defined medium (Chatterjee et al., 2009), as detailed in Table 2.2. The pH of the medium was adjusted to 6.5. The salt and sugar solutions were autoclaved, as mentioned previously. The growth medium was inoculated with 10⁶ spores/mL prior to incubation at 32 °C, 120 rpm for 7-10 days unless otherwise specified.

2.2.4 Collection and Quantification of *M. purpureus* C322 Spores

M. purpureus C322 spores were harvested by adding 1 mL of sterile Tween 80 (1 drop/mL) solution and a few glass beads onto the culture plates. The spores were gently scraped off using a sterilised metal spreader. The collected spore solution was centrifuged at high speed for 10 min, and the supernatant was discarded. Sterile glass beads were added to the pellet to capture fungal fragments. The spores were resuspended in sterile saline solution (0.9% NaCl (w/v)). The addition of glass beads facilitated the separation of spores from unwanted fungal

fragments present in the pellet, which may cause agglutination and nonuniform pellets. Since spores are relatively small, they remain in the solution, while the glass beads act as a trapping mechanism for larger fragments of fungal culture. The total number of spores present in the spore solution was counted using a haemocytometer.

Note: The troubleshooting section is discussed in Appendix section I.

Table 2.2. Composition of Growth Medium.

Medium Constituents	Conc. (g/L)	
Glucose	20.0	
KH2PO4	5.0	
K2HPO4	5.0	
MgSO4.7H2O	0.5	
ZnSO4.7H2O	0.1	
MnSO4.H2O	0.01	
KCl	0.03	
Monosodium glutamate (MSG)	0.5	

2.3 Section 2: Optimisation of *M. purpureus* C322 Morphology

Growth conditions have a significant impact on various aspects of fungal growth, such as germination, hyphae, and pellet formation, as well as metabolite production. The major factors influencing fungal growth and secondary metabolite production include pH, temperature, agitation, dissolved oxygen tension (% D.O.T air saturation), medium constituents, and growth container. Therefore, optimising pellet morphology and secondary metabolite production can be improved by optimising the culture conditions.

During shaken flask experiments, agglutination of *M. purpureus* C322 pellets was observed, leading to repeatability issues. Therefore, several optimisation experiments were conducted to overcome this problem, including culturing *M. purpureus* C322 at different growth parameters, such as different pH, rotational speed (rpm), and temperature. Two sets of experiments were conducted, and each experiment was performed in triplicates. This section offers a

comprehensive description of all the experiments conducted to enhance the morphology of *M. purpureus* C322.

2.3.1 Shaken Flask Experiments

2.3.1.1 Effect of pH on Growth and Pigment Production

The impact of pH on the growth and pigment production of *M. purpureus* C322 was examined. The experiment involved inoculating 10⁶ spores/mL in 500 mL Erlenmeyer flasks containing 100 mL defined medium and incubating at 32 °C and 120 rpm. Five different pH levels were tested, including 3.0, 4.0, 5.0, 6.0, and 6.5, respectively.

2.3.1.2 Effect of Rotational Speed on Growth and Pigment Production

To investigate the effect of agitation on growth and pigment production, *M. purpureus* C322 was cultured at different rotational speeds (120 rpm, 180 rpm, 250 rpm). Each 500 mL Erlenmeyer flask contained 100 mL of the defined medium inoculated with 10⁶ spores/mL and was incubated at 32 °C.

2.3.1.3 Effect of Temperature on Growth and Pigment Production

The impact of temperature on the growth and pigment production of *M. purpureus* C322 was studied by culturing the fungus in a 100 mL defined medium at different temperatures (25 °C, 30 °C, 32 °C, and 37 °C). Each 500 mL Erlenmeyer flask was inoculated with 10⁶ spores/mL and incubated at a constant rotational speed of 120 rpm.

2.3.2 Fermentation of M. purpureus C322 in a Stirred Tank Bioreactor

A test fermentation experiment assessed whether the optimal growth conditions established in the shaken flasks could be scaled up to a 2.5 L stirred tank bioreactor with a similar outcome. The fermentation was conducted using the defined medium with a pH of 6.5, 0.5 vvm aeration, and a stirrer speed of 300 rpm at 32 °C. The working volume of the medium was 1,500 mL.

The medium (1000 mL) was autoclaved within the bioreactor at 121 °C for 15 min. Sugar solution (350 mL) was autoclaved separately at 110 °C prior to adding to the reactor. Next, 150 mL of Day-7 seed culture was added to 1,350 mL defined medium to initiate fermentation. The specifications of the bioreactor are detailed in Table 2.3. Throughout the fermentation, pH, temperature, and % D.O.T were monitored, and samples were collected daily for further analysis.

Table 2.3. Specifications of 2.5 L Stirred Tank Bioreactor.

Specification		
Internal Diameter	10 mm	
Shaft Length	20 cm	
Impeller Type	Ruston Turbine	
No. of Impellers	2	
Impellers Position	h/2 (h = height of medium)	

2.4 Section 3. Elicitation of *M. purpureus* C322 to improve secondary metabolism

Elicitation experiments were performed using three carbohydrate elicitors – Mannan oligosaccharides (MO), Oligoguluronate (OG), and Oligomannuronate (OM). The elicitation experiments were conducted in both shaken flasks and 2.5 L stirred tank bioreactors, with all elicitors being prepared in situ.

2.4.1 Preparation of Elicitors from Sodium Alginate Elicitors

The sodium alginate elicitors were prepared using acid hydrolysis (Asilonu, 2000). To begin, 10 g of sodium alginate was dissolved in 500 mL Deionised (DI) water. The solution was mixed with 500 mL warm 0.6 M HCl and heated at 100 °C for 6 h. After cooling rapidly to room temperature under flowing cold water, the solution was centrifuged at 4600 rpm for 30 min. The precipitate was rinsed with DI water before resuspending in 300 mL of DI water. Next, 0.3 M Sodium Hydroxide (30 mL 1 M NaOH in 70 mL DI water) was added until all solid particles were dissolved (280 mL 0.3 M NaOH). Finally, 0.5% (w/w) Sodium Chloride was

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added to the homogenised solution, and an equal volume of ethanol (99%) was added before incubation overnight at room temperature. After overnight incubation, the solution was centrifuged at 4600 rpm for 15 min, and the supernatant was discarded. The precipitate was rinsed with DI water before resuspension in 200 mL DI water. The pH of the solution was adjusted to 2.8 by adding 0.3 M HCl (2.5 mL HCl in 97.5 mL DI water). The solution was centrifuged at 4600 rpm for 30 min, and the supernatant (Oligomannuronate) was filter sterilised using a Corning 500 mL bottle top filter (Polystyrene, non-pyrogenic, sterile/filter/bag-43118, 0.22 µm PES, sterilising, low protein binding, w/45 mm Neck) and Fischerbrand FB70155 pump. The sterilised supernatant (Oligomannuronate) and the precipitate (Oligoguluronate) were frozen overnight and freeze-dried before storing in the fridge at 4°C.

2.4.2 Procedure for Preparation of Mannan Oligosaccharides

Enzyme hydrolysis was used to prepare Mannan oligosaccharides (MO) following the procedure described by Asilonu et al. (2000). First, 1 g of Locust Bean Gum was dissolved in 50 mL DI water at 80 °C. Then, 100 μ L of beta-mannanase (Megazyme Ltd) was added to the solution to initiate enzymatic hydrolysis. The reaction was quenched by increasing the temperature to 100 °C after 5 min. The solution mix was allowed to cool down to room temperature before collecting water-soluble fractions from the supernatant, which were frozen at -80 °C. The frozen samples were freeze-dried and stored at 4 °C.

2.4.3 Elicitation of *M. purpureus* C322 in Shaken Flask Fermenters

The present study investigated the potential of mannan oligosaccharides (MO), oligoguluronate (OG), and oligomannuronate (OM) as elicitors for lovastatin and pigment production in *M. purpureus* C322. The experiment was conducted in twelve 500 mL Erlenmeyer flasks, with three flasks for each elicitor and a control group. Each flask contained 100 mL of defined

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medium and was inoculated with 10^6 spores/mL of *M. purpureus* C322 spore suspension. The inoculated flasks were incubated at 25 °C and 120 rpm, and 150 mg/L of each elicitor was added 48 h after inoculation. The elicitors were dissolved in 1 mL sterile DI water and filter sterilised before addition. Everyday samples were collected to analyse the effects of elicitation, including pigment concentration, pH variation, total carbohydrate consumption, biomass generation, lovastatin concentration, and morphological changes. The experiment was performed in triplicates to ensure the accuracy and reproducibility of the results. The findings from this study can provide insights into the potential use of these elicitors to enhance *M. purpureus* C322 fermentation.

2.4.4 Elicitation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

Following the success of shaken flask experiments, upscaling experiments were conducted to evaluate the potential of mannan oligosaccharides (MO), oligoguluronate (OG), and oligomannuronate (OM) as elicitors for *M. purpureus* in 2.5 L stirred tank fermenters. Three experiments were performed, and each bioreactor group analysed in triplicate. Four fermenters, including one control group and one for each elicitor, were inoculated with 150 mL of Day-7 *M. purpureus* C322 seed culture and filled with 1,350 mL of the defined medium (Fig. 2.2). The inoculated fermenters were incubated at 32 °C under 300 rpm. Each elicitor (150 mg/L) was added 24 h after inoculation. The elicitors were dissolved in 10 mL sterile DI water and filter-sterilised before addition. Samples were collected daily to examine the effects of elicitation, including pigment concentration, pH variation, total carbohydrate consumption, biomass generation, lovastatin concentration, and morphological changes. The fermentation process with elicitors was repeated thrice to ensure consistency. The results from this study provide valuable insights into the potential use of these elicitors to enhance *M. purpureus* C322 lovastatin and pigment concentration in large-scale production.


Fig. 2.2. The experimental setup used to evaluate mannan oligosaccharides (MO), oligoguluronate (OG), and oligomannuronate (OM) as elicitors for *M. purpureus* C322 in 2.5 L stirred tank fermenters. Four fermenters, including one control group and one for each elicitor, contained 1,350 mL defined medium inoculated with Day-7 seed culture. The fermenters were incubated at 32 °C under 300 rpm. The elicitors were added 24 h after inoculation. Daily samples were collected to monitor morphology, pH, pigment concentration, and carbohydrate consumption. Total biomass and lovastatin concentration were measured at the end of fermentation.

2.5 Section 4. Effect of Quorum Sensing Molecules in Enhancing the Secondary Metabolism in *M. purpureus* C322

2.5.1 Quorum Sensing Molecules

To investigate the impact of quorum sensing molecules on *M. purpureus* C322, farnesol (F), tyrosol (T), buyrolactone-I (B), and linoleic acid (LA) were selected. Since F and LA are not soluble in water, 1% DMSO (CD) was added to dissolve the required amount.

2.5.2 Quorum Sensing Experiments in Shaken Flasks

To determine the optimal concentrations of quorum sensing molecules (QSMs) for *M. purpureus* C322, three experiments were conducted in 500 mL shaken flasks containing 100 mL of defined medium inoculated with 10⁶ spores/mL. The flasks were incubated at 25 °C, 120rpm. The experiment was performed in triplicates to ensure the accuracy and reproducibility of the results.

Shaken-Flask Run I involved the addition of QSMs at 0 h, with concentrations listed in Table 2.4. The QSMs (B, F, T), and CD were added at specified concentrations, while the control (C) group received no QSMs. To ensure consistency the Shaken Flasks-Run I was repeated twice, with each flask group consisting of three flasks.

QSM	Concentration	Time of Addition (h)
Farnesol	10 mM	0
Tyrosol	10 mM	0
Butyrolactone	100 nM	0
Control	Blank	0
1% DMSO	1mL/100 mL	0

Table 2.4. Concentration of quorum sensing molecules in Shaken Flasks-Run I.

Shaken Flasks-Run II employed different concentrations of QSMs based on the results of Shaken-Flasks Run I, as shown in Table 2.5. The QSMs (F, T, B, LA) were added at the listed concentrations at 48 h. To ensure consistency the Shaken Flasks-Run II was repeated twice, with each flask group comprising three flasks.

QSM	Concentration	Time of Addition (h)
Farnesol	1 mM	48
Tyrosol	1 mM	48
Butyrolactone-I	150 nM	48
Linoleic Acid	3.57 mM	48
Control	Blank	48

Table 2.5. Concentrations of quorum sensing molecules in Shaken Flasks-Run II.

Shaken Flasks-Run III aimed to further optimise the QSM concentrations based on the outcomes of Shaken Flasks-Run II, with concentrations specified in Table 2.6. The QSMs were added at 48 h of fermentation and F, T (at two different concentrations), B, LA, and C groups were included. To ensure consistency the Shaken Flasks-Run I was repeated thrice, with each flask group consisting of three flasks.

QSM	Concentration	Time of Addition (h)
Farnesol	0.2 mM	48
Tyrosol	0.3 mM	48
Tyrosol	0.5 mM	48
Butyrolactone-I	300 nM	48
Linoleic Acid	0.4 mM	48
Control	Blank	48

Table 2.6. Concentrations of quorum sensing molecules in Shaken Flasks-Run III.

These experiments provide insight into the effect of various QSMs concentration on *M. purpureus* C322 growth in shaken flasks.

2.5.3 Enhancing the Production of Lovastatin and Pigments via the Addition of Quorum Sensing Molecules in 2.5 L Stirred Tank Fermenters

Following successful shaken flask experiments, upscaling was performed in 2.5 L stirred tank bioreactors to evaluate the scalability of the QSMs, F, T, and LA (Fig. 2.3). Four bioreactors, each containing 1,350 mL of defined medium inoculated with 150 mL of Day-7 *M. purpureus* C322 seed culture, were used. Each bioreactor represented a different quorum sensing molecule, with C included for comparison. F, T, and LA, were introduced into the respective bioreactors at 24 hours of fermentation, achieving final concentrations of 0.2 mM, 0.3 mM, and 0.4 mM, respectively. Samples were collected daily to analyse the effects of QSMs, including pigment concentration, pH variation, total carbohydrate consumption, biomass generation, lovastatin concentration, and morphological changes. Three experiments were performed, to ensure the accuracy and reproducibility of the results.



Fig. 2.3. Upscaling experiments performed in 2.5 L stirred tank bioreactors to evaluate the effectiveness of farnesol (0.2 mM), tyrosol (0.3 mM), and linoleic acid (0.4 mM) as quorum sensing molecules. Four bioreactors, each containing 1,350 mL of the defined medium inoculated with 150 mL of Day-7 *M. purpureus* C322 seed culture, were used. Each bioreactor represented a different quorum sensing molecule added to the respective bioreactors on Day-2 of fermentation, with control included for comparison. Daily samples were collected to monitor morphology, pH, pigment concentration, and carbohydrate consumption. Total biomass and lovastatin concentration were measured at the end of fermentation. Two experiments were performed, with each sample analysed in triplicate.

Analytical Techniques

The following techniques were used to assess the outcome of the experiments:

pH Analysis

According to the literature, changes in the pH of culture broth can indicate the production of pigments and secondary metabolites. For example, Boruta and Bizukojc (2017) reported that lovastatin and secondary metabolite synthesis typically correspond with a decrease in overall medium pH. Conversely, the onset of cell death and nutrient depletion can lead to increased medium alkalinity as the microbe begins consuming organic acids, increasing overall pH (Hevekerl et al., 2014). In the current study, the pH was monitored every 24 h. Before monitoring, the pH meter was calibrated, and the electrode was rinsed with DI water each time before measuring the sample.

Biomass Quantification

Biomass was quantified using gravimetric analysis at the end of the fermentation process. Vacuum filtration was performed using a MerckTM Vacuum Filtering Side-Arm Flask, fitted with Bel-ArtTM SP SciencewareTM Büchner Funnels and a Fischerbrand FB70155 pump. Biomass determination followed a modified version of the protocol described by Amache (2014). In this modified approach, cell dry weight (CDW) was measured by filtering the total biomass onto Whatman No. 1 filter paper using vacuum filtration. The filter paper containing the retained biomass was oven-dried at 100°C for 12 hours, instead of 60°C for 48 hours as per the original protocol. To prevent moisture absorption, dried filter papers were stored in a desiccator containing copper sulphate crystals before weighing. The final cell dry weight was calculated by subtracting the pre-weighed mass of the filter paper from the total weight of the dried biomass-containing filter paper.

Chapter II

Materials and Methods

Pigment Quantification

As mentioned in previous sections, *M. purpureus* C322 produces three pigments: Red (Monascorubramine and Rubropunctamine), Orange (Monascorubrin and Rubropunctatin), and Yellow (Monascin and Ankaflavin). The red, orange, and yellow pigments were quantified using a spectrophotometer (Jenway, Fisher Scientific, UK) at 510 nm, 470 nm, and 400 nm, respectively (Bühler, 2013). Pigment levels were expressed in absorbance units. To ensure accurate analysis, samples were diluted 20-fold to a 5% solution by adding 50 μ L of sample to DI water to reach a final volume of 1 mL. Before measurement, the spectrophotometer was zeroed using DI water as a blank.

Total Carbohydrate Assay

The total carbohydrate consumption was determined using the phenol sulfuric acid assay according to Chaplin and Kennedy (1994). First, each sample was centrifuged at 12,000 rpm for 10 min, and the pellet was discarded. The supernatant was then diluted 200-fold by adding 25 μ L of each sample to 4,975 μ L of DI water, resulting in a final volume of 5 mL containing 100 mg/L sugar. To perform the assay, 200 μ L of 5% phenol solution was added to 200 μ L of the diluted sample in a sterile glass test tube. Next, 1 mL of concentrated sulfuric acid was added rapidly to the surface of the mixture, and the tubes were briefly vortexed and incubated for 30 min at room temperature. Measurements were taken at 490 nm wavelength using a UV-spectrometer (Perkin Elmer Lambda 35 UV/VIS spectrometer) in a fume cupboard to ensure health and safety. Triplicate samples were examined for accurate results. The standard glucose curve (Appendix-Fig. 4) provides a reference for understanding the general behaviour of carbohydrate consumption.

The formula for calculating carbohydrate consumption rate:

(S1-S2)/(t2-t1)

Where **S** is carbohydrate concentration, **t** is time, 1 and 2 are two time points, e.g. start and end of the log phase.

Microscopic Analysis

Microscopic analysis was conducted to observe the morphological changes of the *M. purpureus* C322 during fermentation using a Nikon NIS-Elements AR Light Microscope. A 15 μ L sample was pipetted onto a clean glass slide, and a coverslip was carefully placed on top to avoid any air bubble formation. The prepared slides were examined under the microscope, and pictures were captured to represent the analysis.

Ethyl Acetate Extraction

The supernatant from *M. purpureus* C322 culture broth was collected by filtering the culture broth using Whatman filter paper no.1. Lovastatin was extracted from the supernatant via solvent extraction procedure using ethyl acetate. To initiate the solvent extraction of lovastatin, 10 mL of the culture supernatant was carefully transferred into a separatory funnel. 10 mL ethyl acetate was added to the funnel, and the solution was gently swirled several times to facilitate the extraction process. The solution mixture was left undisturbed for a few minutes to allow the separation of the organic phase (ethyl acetate) from the aqueous phase (cell supernatant). Subsequently, the organic phase containing the extracted compounds was gently transferred into a sterile falcon tube. This extraction procedure was repeated three times using the same cell supernatant to maximise the yield of Lovastatin. In total, 30mL of the organic phase was collected through this process (Palanichamy et al., 2014; Nichols, nd).

HPLC Sample preparation

The collected organic phase was acidified using concentrated hydrochloric acid, followed by another round of ethyl acetate extraction. The resultant organic phase was carefully transferred to a round bottom flask. The solvent was evaporated using a rotary evaporator, with the water bath temperature set at 60 °C and the round bottom flask rotated at 110 rpm under 240 psi vacuum (Buchi, 2010). Once the organic phase was completely evaporated, 1 mL of HPLC grade acetonitrile was added to resuspend the resultant crude extract containing lovastatin before undergoing HPLC analysis (Palanichamy et al., 2014; Nichols, nd).

HPLC Analysis of Lovastatin

The quantitative analysis of lovastatin samples and standards (Sigma Ltd) was performed using a Dionex HPLC system with a Merck reverse phase column (C18, 5 μ M x4.6 mm x150 mm) (LiChrospher Merck_ Catalogue no. 1505490001) at a temperature of 25 °C, coupled with a UV-VIS detector. The samples were analysed using an isocratic method, with a mobile phase of 55:45 (vol/vol) HPLC-grade acetonitrile and aqueous 0.1% H₃PO₄. The injection volume was 25 μ L, and the flow rate was 1 mL/min. The peak detection was set at 238 nm.

D Preparation of Lovastatin Standard

To prepare the lovastatin standards, the commercially available lactone form of lovastatin was converted to the beta-hydroxy acid form found in the culture broth. This was achieved by dissolving 2 mg lovastatin in 10 mL of 0.1 N NaOH solution prepared in 50% aqueous acetonitrile. The solution mix was incubated in a water bath at 45 °C for 1 h, followed by neutralisation with 0.1 N HCl prepared in acetonitrile. Lovastatin standards with concentrations ranging from 0.01 to 0.1 mg/mL were used to quantify the concentration of lovastatin in the culture broth (Appendix-Fig. 5 and 6).

2.6 Section 5: Whole Genome Sequencing, Assembly and Annotation of *M. purpureus* C322 Genome

Prior to this study, the genome of *M. purpureus* C322 had not been analysed. Therefore, this project involved the extraction of genomic DNA, followed by genome sequencing and assembly to study the whole *M. purpureus* C322 genome for the first time. The assembled genome was categorised into chromosomes and annotated to identify quorum sensing genes and other features such as coding regions, conserved domains, restriction sites, and repetitive elements.

2.6.1 DNA Extraction

Genomic DNA was extracted from *M. purpureus* C322 using two techniques, namely phenolchloroform extraction and Qiagen's All Prep fungal DNA/RNA/Protein extraction kit (Fig. 2.4).

2.6.1.1 Sample Preparation

The sample was prepared by homogenising a 1 cm diameter *M. purpureus* C322 culture in a lysis matrix E tube. The samples for Qiagen's DNA extraction were added with 800 μ L nuclease-free water. In contrast, the samples for phenol-chloroform extraction were added with 800 μ L tissue lysis buffer as the homogenising solution. The tubes were incubated at room temperature for 1 h and homogenised for 40 s at 6m/s speed. After homogenisation, the sample was centrifuged at maximum speed for 2 min, and the supernatant was used for DNA extraction while the pellet was discarded.



Fig. 2.4. Workflow for *M. purpureus* C322 DNA extraction, Quantification and Sequencing.

Chapter II

Materials and Methods

2.6.1.2 Extraction of Genomic DNA using Qiagen's ALLPrep Fungal DNA/RNA/Protein Kit

The manufacturer's protocol for the AllPrep fungal DNA/RNA/Protein kit was followed to extract total cellular nucleic acids and proteins (Fig. 2.5). Before the nucleic acid extraction, 100% ethanol was added to the DNA washing buffer (IW solution) to remove coprecipitated salts, while 100% isopropanol was added to the RNA washing buffer (RW solution) to remove proteins bound to the RNA. A working stock solution of cell lysis buffer (HC Solution) was also prepared by adding dithiothreitol (DTT) to achieve a final 1-10 mM concentration. The DTT addition was conducted under a fume extractor, and the HC solution was freshly prepared before the experiment to ensure its efficacy.

I) DNA Isolation

Mechanically homogenised fungal cells were used for DNA isolation (Section 2.6.1.1). 1.8 mL of the homogenised fungal culture was centrifuged at 15,000 x g for 3 min in 2 mL collection tubes. The supernatant was discarded, and the cell pellet was resuspended in 350 μ L of cell lysis buffer (HC Solution) by vortexed for 10 s. The resuspended cells were transferred to a PowerBead tube containing 0.5 mm glass beads and vortexed for 10 min. After a quick spin in the centrifuge, 175 μ L of MR solution was added to solubilise proteins. The tubes were vortexed for 10 s before being centrifuged at 15,000 x g for 2 min at room temperature.

II) DNA Purification

To purify the extracted DNA, 350 μ L of lysate was transferred to manufacturer-provided MB Spin Columns and centrifuged at 15,000 x g for 1 min, with the flow-through saved for RNA purification. The MB Spin Column was transferred to a sterile 2 mL Collection tube, and 650 μ L of DNA binding solution (EA Solution) was added before centrifuging at 15,000 x g for 1 min. The resulting flow-through was discarded. DNA washing buffer

650 μ L (IW solution- Qiagen) was added to the MB Spin Column and centrifuged at 15,000 x g for 1 min, and the resulting flow-through was discarded. The MB Spin Column was centrifuged again at 15,000 x g for 2 min. The MB Spin Column was carefully transferring to a sterile 2 mL Collection tube and 100 μ L of EB solution was added to elute the DNA from the column. After incubating for 1 min at room temperature, the spin column was centrifuged for 1 min at 15,000 x g. The collected DNA solution was quantified using nanodrop before being stored at -80 °C for downstream applications.

2.6.1.3 Phenol-Chloroform Extraction of Genomic DNA

The second approach for DNA isolation was phenol-chloroform extraction (Fig. 2.6). The procedure was developed by reviewing relevant literature, and the steps are outlined below:

I) Cell Lysis

After homogenising the culture, 10 μ L of Proteinase K (20 mg/mL) was added to the samples and incubated overnight at 55 °C. Next, 2 μ L of RNAse A (0.125 mg/25 μ L) was added, and the samples were incubated for 1 h at 35 °C. The resulting solution was pipetted into a sterile 1.5 mL Eppendorf tube.

II) Phase Separation

To extract DNA, equal volumes of phenol-chloroform isoamyl mix (25:24:1) were added to the homogenised samples, and each tube (2 mL) was vigorously vortexed for 10 s. The solution mix turned frothy, indicating the separation of cell constituents. The tubes were micro-centrifuged at room temperature for 2 min. This process was repeated several times until the froth formation was reduced after adding the PCI mix, and the aqueous solution became clear. The top aqueous phase containing the DNA was carefully removed using a P1000 and P200 microlitre pipette and transferred to a fresh Eppendorf tube.

III) DNA Purification

The aqueous phase (500 μ L) was purified by adding an equal volume (500 μ L) of chloroform to each tube containing the aqueous phase, followed by vortexing for 15 s. Subsequently, the samples were centrifuged at room temperature for 2 min, and the resulting aqueous phase was carefully extracted. These purification steps were repeated once more to ensure optimal removal of impurities.

IV) DNA Precipitation

The aqueous phase (500 μ L) was further purified by adding an equal volume (500 μ L) of chloroform to each tube, followed by vortexing for 15 s and centrifugation for 2 min at room temperature. Subsequently, isoamyl alcohol was added to the mixture with 3M sodium acetate to a volume of 1/10 and pH 5.2, followed by brief vortexing. Finally, 1 volume of ice-cold 100% isopropanol was added to the mixture and vortexed briefly. The tubes were frozen at -70 °C for 1 h and centrifuged for 20 min at high speed and room temperature.

V) DNA Wash and Resuspension

After DNA precipitation, the pellet was gently rinsed with 1 mL of 70% cold ethanol at an angle to avoid disrupting the pellet, and the supernatant was discarded. The process was repeated, followed by centrifugation at high speed at room temperature for 2 min before discarding the supernatant. The pellet was rinsed again with 100% isopropanol, and the supernatant was discarded. The tubes were tapped and flicked to loosen the pellet, and the lids were opened to incubate the tubes at 42 °C for 15-20 min to dry the pellet. The lids were closed immediately to prevent the static build-up that could cause the pellets to jump. The DNA pellet was re-suspended in 100 μL of 10 mM Tris HCL with

pH 8.5 and stored at -20 °C.



Fig. 2.5. Diagrammatic Representation of the steps involved in the genomic DNA Extraction of *M. purpureus* C322 using Qiagen's AllPrep DNA/RNA/Protein extraction Kit

10mM Tris HCl preparation [51]

- 1M Tris HCl was prepared by mixing 1.211g Tris with 8 mL nuclease-free water. The pH of the solution was adjusted to 8.5 using HCl, and the volume was adjusted to 10 mL by adding nuclease-free water. No significant difference in pH was noticed by adding nuclease-free water to make the final volume 10 mL.
- To prepare 10 mM Tris HCl, 100 μ L of 1M Tris HCl was added to 8 mL nucleasefree water, and the pH was adjusted to 8.5 using HCl. The final volume was made up to 10 mL with nuclease-free water.

2.6.1.4 Ultra-Purification of Genomic DNA for Whole Genome Sequencing

The extracted DNA from both procedures was further purified using the Genomic DNA Clean and Concentrator kit from Cambridge Biosciences Ltd to achieve the necessary standards for whole genome sequencing, as specified by Eurofins Ltd. 200 μ L of extracted DNA was mixed with 100 μ L of DNA Binding buffer and transferred to a Zymo-Spin column in a collection tube. The Spin column was centrifuged at high speed for 30 s, and the resulting flow-through was discarded. Next, 200 μ L of DNA wash buffer was added to the spin column and centrifuged at 15,000 x g for 1 min. The wash step was repeated once more. Subsequently, 10 μ L of DNA elution buffer was added directly to the column matrix and incubated for 1 min at room temperature. The spin column was transferred to a sterile Eppendorf tube and centrifuged at 15,000 x g for 30 s to elute the DNA. The ultra-pure DNA was quantified using Nanodrop and stored at -80 °C for whole genome sequencing by Eurofins Ltd.



Fig. 2.6. Procedure for Phenol-Chloroform Extraction of *M. purpureus* C322 genome.

2.6.2 Total Cellular RNA Extraction and Purification from *M. purpureus* C322

The total RNA from *M. purpureus* C322 was extracted using Qiagen's All Prep Fungal DNA/RNA/Protein Extraction Kit as per the manufacturer's protocol. Before extraction, the *M. purpureus* C322 cells were suspended in nuclease-free water at a concentration of 1×10^8 cells per 1.8 mL sample. For the purification of RNA, the resuspended cells were centrifuged at 15,000 x g for 3 min, and the flow-through was collected. Next, 300 µL of RNA binding buffer (RB Solution) was added to the flow-through and vortexed at high speed. The lysate was added to a new spin column and centrifuged at 15,000 x g for 1 min at room temperature. The spin column was then transferred to a sterile collection tube, and 650 µL of RNA wash buffer was added before centrifugation at 15,000 x g for 1 min to remove any residual proteins. After discarding the flow-through, the spin column was centrifuged again for 2 min at 15,000 x g and carefully placed onto a sterile collection tube. Then, 100 µL of RNase-free water was pipetted onto the filter membrane at the centre and incubated for 1 min at room temperature. The spin column was centrifuged at 15,000 x g for 1 min, and the collected RNA was stored at -80 °C and used for qPCR analysis. The extracted RNA was quantified using Nanodrop.

2.6.3 Whole Genome Sequencing and Assembly of *M. purpureus* C322 Genome

The whole genome sequencing of *M. purpureus* C322 was performed using Illumina Sequencing by Eurofins Ltd, Germany. The acquired raw data was subjected to bioinformatic analysis, starting with the quality check using the FastQC tool from usegalaxy.org. Subsequently, the Geneious Prime software was used for further analysis, beginning with the Set Pairs function to align both ends of fragments. Genome assembly was accomplished using two functions, namely the De novo assembly and the Map-To-Reference (Fig. 2.7).



Fig. 2.7 Flowchart outlining the workflow for Whole Genome Sequencing and Assembly of *M. purpureus* C322 Genome.

De Novo assembly merged and aligned the shorter reads to construct larger contigs. Several preparatory steps were taken before applying De Novo Assembly, including trimming low-quality reads, merging overlapping reads, removing duplicate reads, and error correction and normalisation. The Map-To-Reference function was used to map the reads and contigs to a reference genome (*Monascus purpureus* YY-1) and generate the consensus sequence. The analysis identified the differences between the *M. purpureus* C322 genome and the well-characterised reference genome, *M. purpureus* YY-1, available in the NCBI database.

2.6.4 Gene Annotation of M. purpureus C322 Genome

A thorough investigation into the biosynthetic pathways responsible for the production of quorum sensing molecules (QSMs), specifically farnesol, tyrosol, butyrolactone, and linoleic acid, was conducted through a literature search. Vital enzymes necessary for the synthesis of these QSMs were identified, and their gene sequences were obtained from NCBI databases. These sequences were then aligned with the *M. purpureus* C322 genome using Genious Prime software to determine similarities. Various other annotations, such as UTRs, CDS, Enhancers, Introns, Misc RNA, ORF, Origin of Replications, Promoters, RBS, Regulatory regions, Repeat regions, Terminator, and miscellaneous (misc) features, were applied using Geneious Reference Features to further analyse the *M. purpureus* C322 genome. The aligned sequences were annotated and visualised, resulting in a list of data that included Name, Type, Length, Interval, and Sequence for each annotation.

2.6.5 Primer Design for Reverse Transcription-Quantitative Polymerase Chain Reaction

Geneious Prime software was utilised to design RT-qPCR primers to analyse quorum sensing

genes (Appendix-Fig. 14). The specific details of the primer design process can be found in

Table 2.7.

Table 2.7. A comprehensive list of primers designed using Geneious Prime software for QSM gene analysis in *M. purpureus* C322.

Gene	Forward Primer	Reverse Primer	Tm (°C)	
Farnesol Biosynthesis				
IspA	GACAGAATTGGGCCA	TGTTGTCCTGGATG	60	
	GCTCT	TCGGTG		
Dimethyl-	GATCCATCGCGTAGTG	CCACATGATCGGCT	60	
Allyltransferase	CAGA	GCATTG		
Farnasyl Diphosphate	TCCCGAATCGATCATG	AATTCTGAGCCCGC	60	
Phosphatase	CTGG	ATCCAA		
	Tyrosol Biosynthesis	S	1	
ADH5-alcohol	ACTCGCATTCCCATAG	TGGTGCAGAAAACC	60	
dehydrogenase V	CTCG	CACTGT		
ADH1: alcohol	CCAAGGATGTTCCCCT	CACCTGCAGGCAGA	60	
dehydrogenase ADH1	GGAC	CCAATA		
ADH2: alcohol	AGAGAGGTTTTCGGG	GGGTAGGAATCAA	59.2,	
dehydrogenase ADH2	GATCG	ATGGGTGTC	58.7	
Aromatic amino acid	AACCTCTCAGTGGAA	TGTCTTCTGCCAGA	60	
aminotransferase	CGCAG	ACGACC		
Putative tyrosine	TCCTGTCTGCCAACTC	TCATACCATGGAGG	60	
decarboxylase	GAAC	GGAGCA		
Copper amine oxidase 1	ACGCAGGAGATTGTT	GTGTATCTTCCGCC	60	
[ty oxi]	CGTGT	GGCATA		
Lipid-Derived QS Molecule Biosynthesis				
PgpB	TCGGCTGTGAAGCTGT	TCGTTCAAGAGACT	60	
	GAAT	TGGCCC		
N-acyl homoserine	GGATGATCTCGAGCA	CTATTCGCCTCTCC	59.9,	
lactone	AGCCA	CAGCTG	60	
Erg-1	GACGGGTGCCTATGT	AGCGTACCCGAAG	60	
	GAACA	AAAAGCA		

2.6.6 Reverse Transcription-Quantitative Polymerase Chain Reaction

To perform Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR), the Luna Universal One-Step RT-qPCR kit was utilised, following the manufacturer's instructions. Prior to use, the Luna RT-qPCR solutions were thawed and gently vortexed while RNA was thawed on ice. The reaction set-up was prepared according to the details outlined in Table 2.8, which included the Luna Universal One-Step Reaction Mix (2X), Luna WarmSmart RT Enzyme mix, forward and reverse primers, template RNA, and nuclease-free water. The sample mixture was mixed thoroughly by pipetting, gently tapping, and centrifuging briefly at high speed. The aliquots were transferred into a qPCR plate and sealed with optically transparent film.

Component	20 µL Reaction Volume	Final Conc.
Luna Universal One-Step	10.0	1 X
Reaction Mix (2X)		
Luna WarmSmart RT	1.0	1 X
Enzyme mix		
Forward primer (10uM)	0.8	0.4 μΜ
Reverse Primer	0.8	0.4 μΜ
Template RNA	6.0	< 1 µg
Nuclease free water	1.4	-

Table 2.8. Reaction set-up for RT-qPCR.

A gradient thermocycling protocol, as shown in Table 2.9, was programmed into the RT-PCR instrument (Biorad CFX96 Touch Real Time-PCR Detection System) using the Biorad CFX manager software. The SYBR green scan mode was set on the real-time instrument for RT-qPCR analysis.

Cycle Step	Temperature (°C)	Time	Cycles
Reverse	55.0	10 min	1
Transcription			
Initial Denaturation	95.0	1 min	1
Denaturation	95.0	10 s	
Gradient Extension	58.0- 60.0	30 s	40-45
Melt Curve	60.0-95.0	Various	1

Table 2.9 Thermocycler Programme

2.6.7 Phylogenetic Analysis and Tree Construction

Phylogenetic analysis commenced with the identification of the 18S and 28S rRNA gene sites in *M. purpureus* C322. Subsequent steps involved performing a multiple sequence alignment using ClustalW to compare the 18S rRNA gene sequences of *M. purpureus* C322 with those of several fungal species: *M. pilosus* IFO 4488, *A. terreus* ATCC 1012, *A. nidulans* ATCC 10074, *S. cerevisiae* NRRL Y-12632, *C. albicans* CBS 562, *Neurospora* crassa OR74A, and *F. oxysporum f.sp. lycopersici* 4287. The phylogenetic tree construction was subsequently performed using the aligned sequences through the FastTree plugin within Geneious Prime software, providing insights into the evolutionary relationships among these fungi.

Chapter III

Optimisation of *M. purpureus* C322 Morphology in Submerged Fermentation Chapter III

Optimisation

3.1 Introduction

This study observed instances of *M. purpureus* C322 cultures randomly forming large, agglutinated clumps during submerged fermentation in shaken flasks, particularly at 32 °C, with an agitation rate of 120 rpm, and pH of 6.5 (Fig. 3.1). This sporadic clumping challenges the achievement of uniform growth, critical for the reliability and reproducibility of experimental outcomes. To mitigate the unpredictability of clump formation and promote consistent growth patterns crucial for precise results interpretation, a series of optimisation experiments were initiated.

The optimisation experiments varied key growth parameters, including pH levels (3.0, 4.0, 5.0, 6.0, and 6.5), agitation rates (120 rpm, 180 rpm, and 250 rpm), and temperatures (25 °C, 30 °C, 32 °C, and 37 °C). The objective was to explore how these variables influence the morphological characteristics of *M. purpureus* C322 culture and to evaluate their effects on total biomass accumulation and pigment production. Through these systematic adjustments, the study aimed to identify optimal conditions that would minimise or eliminate large, agglutinated clump formation, facilitating uniform pellet morphology conducive to the efficient production of secondary metabolites.

This chapter presents the findings from the optimisation studies, detailing the effects of adjustments to the fermentation environment on the growth behaviour of *M. purpureus* C322. These findings elucidate the complex relationship between various cultivation parameters and their impacts on fungal morphology, biomass concentration, and pigment production. The insights gained are pivotal for developing strategies to enhance the productivity of *M. purpureus* C322 in submerged fermentation systems, with potential applications in the industrial production of valuable fungal metabolites.

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Fig. 3.1. Agglutination of *M. purpureus* C322 cultures during fermentation in shaken flasks at 32° C and 120 rpm, showing a) highly agglutinated culture with low pigment production, b) single large, agglutinated clump with additional individual pellets and higher pigment production, c) larger sized pellets with reduced pigment production, and d) mostly uniform pellets with a few larger pellets exhibiting higher pigmentation. The flasks were inoculated with 1 x 10⁶ spores/mL of *M. purpureus* C322 cultures in 100 mL of the growth medium prior to incubation.

Results and Discussion *Shaken Flask Fermentation*

3.2 Results and Discussion

3.2.1 Optimisation in Shaken Flasks

The following section presents findings from the optimisation experiments designed to find out the ideal growth conditions for *M. purpureus* C322, addressing the unpredictable morphology observed in shaken flasks. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values of biomass and pigment (Yellow, Orange and Red) concentration are presented in graphs with applied standard errors.

3.2.1.1 Effect of pH on Morphology and Pigment Production in Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The effect of different medium pH (3.0, 4.0, 5.0, 6.0 and 6.5) on biomass concentration and pigment (Yellow, Orange and Red) production in *M. purpureus* C322 during a 12-day fermentation was investigated, where the test cultures were incubated at 32 °C and 120 rpm for 12-days prior to biomass and pigment analysis. The three pigments, i.e., Yellow, Orange and Red, produced by *M. purpureus* C322 were quantified at optical density 400 nm (Yellow Pigment), 470 nm (Orange Pigment) and 510 nm (Red), respectively (Fig. 3.2).

The results indicated that the biomass concentration and pigment production of *M. purpureus* C322 increased with an increase in the medium pH within the pH range tested (pH 3.0-pH 6.5). The highest pigment production for all three pigments was recorded at pH 6.5, followed by pH 6.0, pH 5.0, pH 4.0, and pH 3.0. A similar trend was observed in biomass production, with the highest biomass concentration recorded at pH 6.5 (3.65 g/L) and the lowest at pH 3.0 (0.74 g/L), as depicted in Figure 3.2. The pigment yield was calculated by dividing the optical density by the dry cell weight of the culture. Table 3.1 summarises the pigment yield for all three pigments (Yellow, Orange, and Red), which was highest at pH 6.5, followed by pH 6.0, pH 4.0, and pH 3.0.

The distribution of pigment types varied with pH (Table 3.1, Fig. 3.2). At pH 3.0, yellow pigment production was comparatively higher than orange and red pigments. At pH 4.0 and 5.0, orange pigment dominated, while at pH 6.0 and 6.5, red pigment was the most prominent. This trend aligns with previous studies reporting enhanced red pigment production at pH 6.0–8.0 (Chen and Johns, 1993; Shi et al., 2016; Patrovsky et al., 2019), orange pigment in the range of pH 4.0–6.5 (Embaby et al., 2018; Patrovsky et al., 2019; Choe et al., 2020), and yellow pigment formation below pH 4.0 (Chen and Johns, 1993; Patrovsky et al., 2019).



Fig. 3.2. Effect of pH on biomass concentration and pigment (Yellow, Orange and Red) production in shaken flask fermentation, where biomass concentration was determined by measuring the total cell dry weight (g/L) of D-12 cultures and pigment production was measured at OD 400 nm, 470 nm and 510 nm for yellow, orange, and red pigment respectively. Two sets of experiments were performed, and each set of experiments was conducted in triplicates. The standard error of the mean was represented by error bars.

Analysis of Variance (ANOVA) determined the impact of pH on biomass concentration, pigment production and the variation between the test experiments (Appendix-Table 1). The results revealed that pH had a statistically significant impact on both biomass concentration (*p*-value<0.01) and pigment production (*p*-value<0.01), emphasising the critical role of pH in modulating these parameters. However, no marked difference in biomass concentration was

observed across the two sets of experiments (*p*-value>0.01), except for pH 6.0 (*p*-value<0.01). Similarly, no significant difference was observed in pigment (Yellow, Orange, and Red) concentration between the two test experiments (*p*-value>0.01).

Table 3.1. Pigment yield measured per gram dry weight of D-12 samples of *M. purpureus* C322 at OD 400 nm, 470 nm and 510 nm for yellow, orange, and red pigment, respectively.

Pigment Yield (AU/g CDW)			
pН	Yellow (AU ₄₀₀)	Orange (AU ₄₇₀)	Red (AU ₅₁₀)
3.0	0.53	0.23	0.18
4.0	0.52	0.24	0.44
5.0	0.63	0.72	0.61
6.0	0.82	0.89	0.91
6.5	0.99	1.00	1.03

Several studies have reported that the electrostatic and hydrophobic interactions between the fungal cell walls, which contribute to mycelial aggregate formation, can be influenced by the pH and ionic strength of the medium (Kershaw et al., 1998; Veiter et al., 2018; Garcia-Rubio et al., 2020; Ruoyu et al., 2021). Zhang and Zhang (2016) found that an increase in pH leads to increased electrophoretic mobility in the negative direction, causing negatively charged fungal spores of *A. niger* to repel each other at pH 7.0 and reduce pellet clumping. These findings were corroborated by other studies (Wargenau et al., 2011; Wargenau et al., 2013). In line with the findings and analysis of these references, the observations from the current study indicate similar results, where the clump formation was more frequent in cultures grown at lower pH (3.0-5.0). At the same time, uniform growth was noticed at comparatively higher pH (6.0 and 6.5) (Fig. 3.3). Although in this study occasional clump formation was observed during submerged fermentation in shaken flasks at pH 6.5, 32 °C, and 120 rpm, therefore optimisation of all parameters was necessary. In the current experiment, the culture pellets exhibited a higher degree of uniformity in shape at pH 6.5. On the contrary, irregular-shaped pellets were formed

d)

at pH 6.0. Agglutination occurred at pH 4.0 and 5.0, while minimal cell growth was observed at pH 3.0. The size of the pellets varied among samples (Fig. 3.3).



Fig. 3.3. Macroscopic observation of D-12 samples cultured at different pH; a) pH 3.0, b) pH 4.0, c) pH 5.0, d) pH 6.0, e) pH 6.5. 100 mL of defined medium inoculated with 1 x 10^6 spores/mL was added to all flasks prior to incubation at 32 °C and 120 rpm.

e

3.2.1.2 Influence of Rotation Speed on *M. purpureus* C322 Morphology and Pigment Production in Submerged Fermentation

Each 500mL Erlenmeyer flask was filled with 100 mL of defined medium and inoculated with 1x10⁶ spores/mL before incubating at 32 °C. The pH of the medium was adjusted to 6.5, and three different rotational speeds, 120, 180, and 270 rpm, were tested. The highest biomass concentration was observed in flasks shaken at 180 rpm, followed by 120 rpm, and lastly by 270 rpm (Fig. 3.4). Serrano-Carreon et al. (2015) reported an increase in biomass in *Trichoderma harzianum* and *Pleurotus ostreatus* with an increase in rpm from 100-500 rpm; however, shear stress was observed above 250 rpm during fermentation.

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Pigment quantification indicated the highest pigment (Yellow, Orange, Red) production in flasks shaken at 120 rpm, followed by 180 rpm and finally 250 rpm (Fig. 3.4). This suggests that while the biomass generated at 180 rpm was higher than that at 120 rpm, the pigment yield was 25% higher at 120 rpm than at 180 rpm. Cultures grown at 250 rpm had the least pigment yield, likely due to shear forces damaging the mycelium and causing diffused pellets. Afshari et al. (2015) reported a decrease in pigment yield with increasing agitation.

The highest pigment yield for all three *M. purpureus* C322 pigments was obtained at a rotation speed of 120 rpm, with yellow, orange, and red pigments yielding 1.27, 1.33, and 1.47 AU/g.CWD, respectively (Table 3.2). At 180 rpm, a reduction in pigment yield was observed for all three pigments, with values of 0.42 AU/g.CWD for yellow, 0.61 AU/g.CWD for orange, and 0.51 AU/g.CWD for red. Further decrease in pigment yield was observed at 250 rpm, with yellow, orange, and red pigments yielding 0.57, 0.42, and 0.29 AU/g.CWD, respectively. Overall, the results suggest that the optimal rotation speed for pigment yield in the *M. purpureus* C322 strain is 120 rpm, with lower yields observed at higher and lower rotation speeds (Table 3.2).

Rotational speed markedly affected both the concentration of biomass and the production of Yellow, Orange, and Red pigments (Appendix-Table 2), demonstrating significant changes across different speeds (*p*-value<0.01). Additionally, comparisons of biomass and pigment levels at varied rotational speeds (120 rpm, 180 rpm, and 250 rpm) showed consistent outcomes between different sets of experiments, with no noticeable differences in biomass and pigment (Yellow, Orange, and Red) concentrations observed (*p*-value>0.01).

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Table 3.2. Pigment yield of cultures grown at different rotation speeds (120 rpm, 180 rpm and 250 rpm) measured at OD 400 nm, 470 nm, and 510 nm for yellow, orange, and red pigments, respectively.

Pigment Yield (AU/g CWD)				
RPM Yellow (AU ₄₀₀) Orange (AU ₄₇₀) Red (AU ₅₁₀)				
120	1.27	1.33	1.47	
180	0.42	0.61	0.51	
250	0.57	0.42	0.29	



Fig. 3.4. Effect of rotational speed (120 rpm, 180 rpm, 250 rpm) on biomass concentration. 100 mL defined medium (pH 6.5) was inoculated with $1x10^6$ spores/ml of *M. purpureus* C322 culture prior to incubation at 32 °C for 12 days. The biomass concentration was determined by measuring the dry cell weight of the cultures, and pigment production was measured at OD 400 nm, 470 nm and 510 nm for yellow, orange, and red pigment, respectively. Two sets of experiments were performed, and each set of experiments was conducted in triplicates, and the standard error of the mean was represented by error bars.

The pellets formed at 250 rpm appeared diffuse, whereas, at 120 rpm, uniformly sized pellets (~0.5 cm) were formed; however, a comparatively larger pellet of around 2 cm was also observed. At 180 rpm, pellets were nonuniform and varied in size, ranging from 0.5 cm to 2.5 cm by Day-12 of fermentation (Fig. 3.5). Hotop et al. (1993) found that pigment yield was inversely proportional to pellet size, where pellet size ranging between 1.0-6.0 mm doubled the

final pigment yield. Similarly, in this study, the cultures with a pellet size of ~0.5 mm (120 rpm) yielded higher pigment than those with a pellet size larger than 0.6 mm (180 rpm, 250 rpm). The morphology of pellets varied at different rpm, but none of the cultures had aggregated pellets. Stress could be the reason for variable pellet size distribution in cultures agitated at 180 rpm. Similar results were observed by Nair et al. (2016) in filamentous ascomycete fungi (*N. intermedia*) when the agitation speed was raised to 150 rpm.



Fig. 3.5. Morphology of *M. purpureus* C322 cultures cultured at different rotational speeds, where a) 120 rpm resulted in comparatively uniform pellets, b) 180 rpm resulted in nonuniform pellets, and c) 250 rpm resulted in diffused pellets. The different pellet morphologies are clearly visible in the images, allowing for a visual comparison of the effects of the different rotational speeds on the culture morphology.

3.2.1.3 Effect of Temperature on Biomass Concentration and Pigment Production in Submerged Fermentation of *M. purpureus* C322.

The experiment aimed to investigate the effect of temperature on the production of biomass and pigment. Four different temperatures, 25 °C, 30 °C, 32 °C, and 37 °C, were tested while maintaining the medium pH at 6.5 and the agitation rate at 120 rpm. The highest yield of both biomass and pigment was achieved when cultures were grown at 25 °C, followed by 30 °C, 32 °C, and 37 °C, in decreasing order (Fig. 3.6).



Fig. 3.6. Effect of temperature on biomass concentration and pigment production. The pigment content was quantified at 400 nm, 470 nm, and 510 nm for yellow, orange, and red pigments, respectively. The different temperatures studied include 25 °C, 30 °C, 32 °C, and 37 °C. Each 500 mL flask contained 100 mL defined medium inoculated with $1x10^6$ spores/mL prior to incubation at 32 °C for 12 days. Two sets of experiments were performed, and each set of experiments was conducted in triplicates, and the standard error of the mean was represented by error bars.

A notably higher yield of both biomass concentration and pigment production was observed in cultures grown at 25 °C than those grown at other temperatures (Table 3.3). The highest pigment yield was observed for the red pigment at 25 °C with a value of 1.38 AU/g CDW, followed by yellow with 1.29 AU/g CDW and orange with 1.11 AU/g CDW. At temperatures at and above $30 \square °C$ (30, 32, and $37 \square °C$), the mean pigment yields were considerably lower. Among these, yellow pigment production remained the highest, with mean values of 0.85, 0.84, and 0.54 \square AU/g CDW at 30, 32, and $37 \square °C$, respectively. Orange pigment yields were 0.95, 0.78, and 0.39 \square AU/g CDW, while red pigment yields were 0.74, 0.70, and 0.33 \square AU/g CDW at the corresponding temperatures.

Table 3.3. Pigment yield (Yellow, Orange, and Red) in absorbance units (AU) per gram of dry weight (g CDW) for yellow, orange, and red pigments at different temperatures: 25 °C, 30 °C, 32 °C, and 37 °C. Pigments are quantified at OD 400 nm, 470 nm, and 510 nm for yellow, orange, and red pigments, respectively.

Pigment Yield (AU/g CDW)			
Temp.	Yellow (AU ₄₀₀)	Orange (AU ₄₇₀)	Red (AU ₅₁₀)
25°C	1.29	1.11	1.38
30°C	0.85	0.95	0.74
32°C	0.84	0.78	0.70
37°C	0.54	0.39	0.33

Although yellow pigment had the lowest absolute concentration at $25 \square^{\circ}$ C, it showed the greatest fold increase compared to higher temperatures. Specifically, yellow pigment production at $25 \square^{\circ}$ C was 1.52-, 1.54-, and 2.39-fold higher than at $30 \square^{\circ}$ C, $32 \square^{\circ}$ C, and $37 \square^{\circ}$ C, respectively. Similarly, orange pigment production increased by 1.17-, 1.42-, and 2.85-fold, while red pigment production showed a 1.86-, 1.97-, and 4.18-fold increase compared to the corresponding temperatures. These results indicate a consistent decline in pigment yields across all three pigments with increasing temperature (Table 3.3). The variation in biomass concentration and pigment production (yellow, orange, and red) across temperature conditions ($25 \square^{\circ}$ C, $30 \square^{\circ}$ C, $32 \square^{\circ}$ C, and $37 \square^{\circ}$ C) was statistically significant (*p*-value<0.01); however, no discernible differences were observed between replicate sets (*p*-value>0.01), confirming reproducibility under identical conditions (Appendix-Table 3).

Several studies have observed this phenomenon where a shorter lag phase in some filamentous fungi was related to elevated temperature (Babitha et al., 2007; Valik et al., 2013; Venkatachalam et al., 2019; Vrabl et al., 2019). The data acquired from cultures grown at different temperatures (25 °C, 30 °C, 32 °C, and 37 °C) showed a decrease in biomass concentration and pigment production with an increase in temperature, suggesting that an increase in temperature may lead to a decrease in the lag phase of *Monascus* growth (Fig. 3.6).

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Increasing biomass concentration and pigment production could be attributed to a reduced growth rate at lower temperatures, giving the cells enough time to access and absorb nutrients effectively, leading to an overall increase in pigment yield (Fig. 3.6). This assumption is supported by a study published by Lin et al. (1992), who reported a 10-times increase in pigment yield by reducing the growth rate via incubation at a lower temperature (25 °C) compared to a higher temperature (30 °C).

The current study also noticed highly clumped pellets in cultures incubated at 30 °C, 32 °C, and 37 °C. In comparison, the pellet morphology at 25 °C was uniform and freely dispersed, as observed in the study by Lin et al. (1992) (Fig. 3.7). The growth pattern varied across all temperatures, where at 25 °C, the culture exhibited uniform smaller pellets (~2-5 mm), while at 30 °C and 32 °C, pellets were larger and agglutinated (>0.8 mm). At 37 °C, growth appeared to be optimal initially; however, after five days, pellet growth stopped, resulting in the formation of a few pellets by the end of the fermentation period (Fig. 3.7).

Another explanation for clumping at elevated temperatures (37 °C) could be due to heat stress. Cells generally undergo aggregation as a defence mechanism against heat stress, whereby the aggregation encapsulates and protects most cells from elevated surrounding temperatures. Similar findings were reported by Rasanen et al. in 2001, stating that clumping in the filamentous fungi, *S. arboris*, resulted from heat stress. Based on the above analyses, the highest confidence in repeatability with uniform morphology and increased pigment production was observed for cultures incubated at 25 °C.

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Fig. 3.7. The influence of different temperatures on the morphology of *M. purpureus* C322 is shown, where a, b, c) represent the morphologies observed at 25 °C; and d, e, f) represent the morphologies observed at 30 °C, 32 °C, at 37 °C, respectively.

It is important to note that the growth of *M. purpureus* C322 at 32 °C is not always agglutinated, and can occasionally appear uniform. In previous in-house research, *M. purpureus* C322 was grown at 32 °C, however, due to frequent agglomerate formation, temperature optimisation experiments were conducted.

Results and Discussion 2.5 L Stirred Tank Fermenters

3.2.2 Upscaling in 2.5 L Stirred Tank Fermenters.

After resolving the agglutination issues in shaken flasks, test fermentation experiments were conducted in 2.5 L stirred tank bioreactors using the growth conditions that were previously optimised in-house. The growth conditions included a 150 mL inoculation volume, 1350 mL working volume of defined medium with a pH of 6.5, a temperature set point of 32 °C, 0.5 vvm, and a rotation rate of 300 rpm.



Fig. 3.8. Sample of *M. purpureus* C322 pellets collected on Day-4 of the test fermentation experiment in a 2.5 L Stirred Tank Bioreactor, where the fermentation was conducted at a temperature of 32 °C, a rotation rate of 300 rpm, aeration rate of 0.5 vvm, and pH of 6.5.

3.2.2.1 Biomass Concentration during Fermentation of *M. purpureus* C322 in Stirred Tank Bioreactors

The fermentation experiment was performed in duplicates, and the process was conducted under controlled conditions of 32 °C temperature, 300 rpm rotation rate, 0.5 vvm aeration rate, and pH 6.5 for five consecutive days. Notably, no agglutination was observed in either fermentation runs, and the pellets exhibited a uniform morphology throughout fermentation. Mature cultures were obtained by Day-4 of fermentation (Fig. 3.8).

After Day-5 the pellets occasionally started to appear fragmented and diffused (Fig. 3.9). The mean value of total cell dry weight (g/L) obtained from the stirred tank fermentation experiments at 120 h was 8.6 g/L, indicating that the fermentation process was successful in generating high biomass concentration.



Fig. 3.9. Morphology of *M. purpureus* C322 pellets during fermentation in Stirred Tank Fermentation; where a) Displays uniform pellets on Day-4 of fermentation, b) Shows the pellets which appeared diffused after Day-5 of fermentation. The images were captured at 10x magnification.

Since the total cell dry weight analysis for *M. purpureus* C322 was conducted at the end of fermentation, the error bars were computed using the standard deviation of means of final cell dry weight from both test fermentation experiments. The results suggest no significant difference between the final biomass concentration in both test experiments (*p*-value<0.01).

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3.2.2.3 *M. purpureus* C322 Pigment (Yellow, Orange, and Red) Production throughout Fermentation.

The concentration of yellow, orange, and red pigments at 24 h intervals during fermentation in 2.5 L Stirred Tank Bioreactors (STRs) is presented in Fig. 3.10. The production of all three pigments gradually increased over the course of the fermentation, with the yellow pigment reaching 4.58 AU/g CDW, the orange pigment reaching 5.17 AU/g CDW, and the red pigment reaching 5.14 AU/g CDW by 120 h. The pigment yield for the yellow, orange, and red pigments was 0.55 AU/g CDW, 0.623 AU/g CDW, and 0.619 AU/g CDW, respectively.



Fig. 3.10. Pigment quantification of test fermentation experiments in stirred tank fermentation. The pigment content was quantified at 400 nm, 470 nm, and 510 nm for yellow, orange, and red pigments, respectively. Each 2.5 L stirred tank bioreactor contained 1350 mL defined medium (pH 6.5) inoculated with 150 mL of seed culture prior to fermentation at 32 $^{\circ}$ C, 300 rpm and 0.5 vvm. Two sets of experiments were performed, and the standard error of the mean is represented by error bars.

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The results indicate a steady increase in the concentration of all three pigments over time. The variation in pigment production (Yellow, Orange, and Red) throughout the fermentation process, from Day-1 to Day-5 was significant (p-value<0.01). However, no significant difference was observed between the different experimental sets, in terms of pigment production (p-value>0.01) (Appendix-Table 4).

3.2.2.3 pH Profile during Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Bioreactor

The pH variations observed during this investigation can be ascribed to the diverse stages of fungal growth and metabolic status. As the fermentation progressed, there was a gradual decrease in pH, reaching 6.4 after 24 h and further declining to 6.3 by 48 h. This decreasing trend continued till 72 h, with the pH reaching its lowest value of 6.1. Subsequently, an increase in pH was observed, where the pH reached 6.6 by 120 hours. These fluctuations in pH could be attributed to the dynamic interplay of metabolic activities, substrate consumption, and the production of various metabolites throughout the fermentation process (Fig. 3.11). A decline in pH typically correlates with the biosynthesis of secondary metabolites, such as organic acids and ethanol as byproducts. In the late exponential phase and the onset of the stationary phase, pH tends to rise due to the accumulation of byproducts such as ammonia, resulting in an alkaline shift (Kubicek et al., 2011; Liaud et al., 2014; Agboyibor et al., 2018; Gaun and Liu, 2019). Additionally, an elevated pH can be indicative of cellular apoptosis (Su et al., 2021). Appendix-Table 5 displays the pH changes at various time points during the test fermentation experiments in 2.5 L Stirred Tank Bioreactors, revealing consistent pH across different sets of experiments (*p*-value>0.01). Nevertheless, there were significant fluctuations in pH over time i.e. from start to end of fermentation (*p*-value<0.01).



Fig. 3.11. The pH values measured during the test fermentation period using 2.5 L Stirred Tank Bioreactors. The growth parameters were set at 32 °C temperature, 300 rpm rotational speed, 0.05 vvm aeration rate, and 1350 mL working volume of the defined medium at pH 6.5. The bioreactors were inoculated with a 150 mL seed culture of *M. purpureus* C322. The experiments were performed in duplicates, and the error bars represent the standard error of the mean.

3.3 Summary

Based on the results of the optimisation experiments, the most favourable conditions for the growth of *M. purpureus* C322 in shaken flasks are a temperature of 25 °C, a rotational speed of 120 rpm, and a pH of 6.5. Fermentation in 2.5 L Stirred tank bioreactors with a growth temperature of 32 °C, 300 rpm rotational speed, pH 6.5, and an inoculum volume of 150 mL in a 1.5 L working volume (15% v/v) can be considered optimum.

Chapter IV

Eliciting Lovastatin and Pigment Production in *M. purpureus* C322



Chapter IV

Elicitation

4.1 Introduction

This chapter presents the outcomes of the elicitation experiments carried out using specific carbohydrate elicitors, namely oligoguluronate, oligomannuronate, and mannan oligosaccharides, on pigment and lovastatin production in liquid cultures of *M. purpureus* C322. Elicitation has shown promising results in enhancing secondary metabolite production in fungal cultures. Amongst elicitors, carbohydrate elicitors are considered a significant category. The oligosaccharides derived from alginates and galactomannans have gained attention for their ability to enhance secondary metabolite production and serve a wide range of applications due to their diverse properties (Silveira and Bresolin, 2011).

To investigate the effects of these carbohydrate elicitors, a series of experiments were conducted. Initially, 500 mL Erlenmeyer flasks containing 100 mL of inoculated growth medium were employed. The flasks were incubated at 25 °C under 120 rpm for a fermentation period of 18-days. Changes in pH, pigment production, and carbohydrate consumption were regularly monitored. At the end of fermentation, biomass levels and the concentration of lovastatin were assessed.

Following shaken flask experiments, the study advanced to upscaling to in 2.5 L stirred tank fermenters. The bioreactors contained 1350 mL of growth medium supplemented with 150 mL seed culture. The fermenters were maintained at 32 °C under 300 rpm. This transition from small-scale to large-scale cultivation provided a broader perspective for the investigation.

This research not only uncovers the potential of the mentioned carbohydrate elicitors in enhancing pigment and lovastatin production but also contributes to the broader knowledge of how elicitation strategies can be optimised for more efficient and productive bioprocesses in *M. purpureus* C322.

Results and Discussion *Shaken Flask Fermentation*

4.2 Results and Discussion

4.2.1 Elicitation in Shaken Flasks

This section highlights the outcome of the effects of elicitation using carbohydrate elicitors (OM, OG, MO) on biomass concentration and production of secondary metabolites namely lovastatin and pigments (Yellow, Red and Orange) in shaken flasks. The flasks were incubated at 25 °C and 120 rpm, and the medium pH was 6.5. The elicitors were introduced at a concentration of 150 mg/L after 48 h of incubation. The experiments were performed in triplicates, and error bars were applied to all plots based on the standard deviation of each sample set.

4.2.1.1 Effect of Elicitation on pH of the Culture during Submerged Fermentation of *M. purpureus* C322 in Shaken flasks

In shaken flask fermentation, the pH of the medium is a critical factor that can significantly influence *Monascus* metabolism and the subsequent production of various metabolites (Juzlova et al., 1996; Patakova, 2013; Vendruscolo et al., 2016; Patrovsky et al., 2019). Variations in pH during fermentation are often indicative of metabolic activities, such as the production of organic acids or the consumption of alkaline or acidic compounds by the fungus. Therefore, it is essential to consider the initial pH of the medium and its changes over time to understand the metabolic pathways being favoured during fermentation. The provided data presents the pH readings for *M. purpureus* C322 cultures in control flasks (C) and flasks supplemented with different elicitors, OG, OM, and MO (Fig. 4.1). The pH readings were recorded over an 18-day period. During the initial days of fermentation, the flasks treated with elicitors (OG, OM, and MO) showed a pattern similar to those of the control flasks. All the flasks displayed a decrease in pH compared to the starting pH of 6.5, followed by an increase in pH during the later stages of fermentation.

In the control flasks, the pH decreased from Day-3 onwards, reaching its lowest value of 5.8 on Day-14. Subsequently, the pH began to increase, reaching 6.8 by Day-18. The lowest pH value observed for the flasks supplemented with OG was 5.9 on Day-12. From Day-13 to Day-18, the pH consistently increased, reaching 7.0 on Day-18. In the case of flasks treated with OM, the pH gradually declined, reaching its lowest value of 5.8 on Day-12. Thereafter, the pH started to increase, reaching 7.0 on Day-18. The flasks supplemented with MO also exhibited a decrease in pH, reaching their lowest value of 5.9 on Day-12. Like the other treatments, the pH started to increase and reached 7.1 on Day-18. In the observed fermentation process, control samples exhibited a more gradual decrease in pH, with a subsequent delayed pH increase (Day-14) when compared to flask groups treated with elicitors, which showed a pH increase by Day-12. This pattern suggests that, given extended fermentation time, the pH levels in the control flasks could align with those observed in the flasks supplemented with elicitors. While there is a visible trend in pH levels across the flask groups (C, OG, OM, MO), the observed variations do not show statistical significance, as the *p*-value is greater than 0.05 (Appendix-Table 6).

The fluctuations in pH observed during the study can be attributed to the different stages of fungal growth and metabolic activities. A decrease in pH is often associated with the production of secondary metabolites like organic acids and ethanol as byproducts (Jablonska-Rys et al., 2022; Bangar et al., 2022). As the culture reaches the late exponential phase and enters the stationary phase, the pH begins to increase due to the accumulation of alkaline byproducts, such as ammonia, leading to a shift towards alkaline conditions. Similarly, metabolic processes can often result in the production of alkaline waste products, which can lead to an increase in the pH of the culture medium. Since fungi, like *Monascus*, have specific pH requirements for optimal growth and secondary metabolite production, deviations from these pH optima can affect both biomass concentration and metabolite production. (Kubicek et al., 2011; Liaud et



al., 2014; Agboyibor et al., 2018; Gaun and Liu, 2019; Zhu and Thompson, 2019; Judge and Dodd, 2020).

Fig. 4.1. pH variation during shaken flasks fermentation. Four different treatments were evaluated: the control (C), oligoguluronate (OG), oligomannuronate (OM), and mannan oligosaccharide (MO). Each experiment utilised a 500 mL Erlenmeyer flask with 100 mL of growth medium, inoculated with a concentration of 10^6 spores/mL and incubated at 25° C at a shaking speed of 120 rpm. Data points in the graph are average values of three sets of experiments containing three samples per flask group from Day-1 to Day-18 of fermentation, and error bars show the standard deviation (*p*-value>0.05). The concentration of each elicitors (OG, OM, MO) was 150mg/L, introduced at 48 h.

4.2.1.2 Effect of Elicitation on Biomass Concentration during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

Biomass concentration provides essential insights into the growth kinetics and overall health of the microbial culture. Understanding the impact of elicitors (OG, OM, MO), if any, on biomass concentration is crucial as it directly impacts product yield and efficiency, thereby playing a key role in scaling up from laboratory research to industrial applications. Figure 4.2 illustrates the average biomass concentration, represented as total cell dry weight measured in grams per litre (g/L), at Day 18 of fermentation for various treatments, including three different elicitors: OG, OM, MO and control with no added elicitor. The average biomass concentration in the C group on Day-18 was 6.3 g/L. On the other hand, the groups treated with OG and OM showed an average biomass concentration of 6.7 g/L and 6.57 g/L, respectively on Day-18 of fermentation. The MO-treated group displayed an average biomass concentration of 6.46 g/L on Day-18, which is lower than OG and OM; however, it was higher than the C group. The results from this study align with other research, where biotic elicitors have been shown to influence biomass concentration (Zhu et al., 2018; Khayri and Naik, 2020; Rasouli et al., 2021). The variation among the different flask groups (C, OG, OM, MO) indicates low significance (p-value>0.05) (Appendix- Table 7).



Fig. 4.2. The average biomass concentration (total cell dry weight) at Day-18 of fermentation for *M. purpureus* C322 cultures with and without supplementation of elicitors (OG, OM, and MO) introduced 48 h into the fermentation process at a concentration of 150 mg/L, where 'C' represents Control Samples. Fermentation was conducted in 500 mL Erlenmeyer flasks, each containing 100 mL growth medium and inoculated with 10⁶ spores/mL, at 25°C and 120 rpm. Results are based on three sets of experiments performed in triplicates., with standard deviation depicted as error bars on the mean values (*p*-value>0.05).

In shaken flask fermentation, *M. purpureus* C322 cultures exhibited homogenous morphology in all flask groups (C, OG, OM, MO). This success can be attributed to the optimised conditions

Chapter IV

Elicitation

of shaken flasks, which facilitated adequate aeration and nutrient distribution, essential for growth. Despite some limitations typically associated with shaken flasks, such as limited dissolved oxygen and no control of process variables; the cultures adapted effectively, resulting in notable biomass production. The introduction of small quantities of elicitors (OG, OM, MO) was based on the hypothesis that low concentrations would predominantly influence metabolite production, rather than biomass growth. This approach was validated by the results, showing no notable changes in biomass concentration after the addition of the elicitors.

This observation aligns with findings from a few noteworthy studies on the impact of elicitation on secondary metabolism and biomass concentration. For instance, Pettit (2011) and Estrada et al. (2016) reported increased secondary metabolite production without biomass alteration due to elicitation. Similarly, Murphy et al. (2007) found a 23.3% increase in bacitracin A levels in Bacillus licheniformis with the addition of carbohydrate elicitors without any notable change in biomass concentration. Furthermore, investigations into the effects of carbohydrate elicitors (OG, and MO) on B. licheniformis by Raffati et al. (2013) showed enhanced bacitracin A production, yet no noted impact on total cell dry weight during submerged fermentation. Similar studies on P. chrysogenum revealed elicitation-induced (OG, OM, and MO) enhancement in secondary metabolites such as penicillin and chrysogenin without any noticeable increase in biomass concentration (Ariyo et al., 1997 and 1998; Asilonu et al., 2000; Nair et al., 2009). Radman et al. (2004) specifically examined the effect of carbohydrate elicitors (OG, OM and MO) impact on *P. chrysogenum* morphology, noting changes in hyphal tips, clump area, and spore counts, yet biomass concentration remained largely unaffected. These examples, highlight the potential of elicitors to specifically target metabolic pathways without influencing microbial biomass concentration. The evidence further indicates that the applied concentrations (150 mg/L) of elicitors (OG, OM, MO) were sufficient to trigger specific metabolic pathways without significantly (*p*-value>0.05) impacting the overall growth

and biomass accumulation of *M. purpureus* C322 in shaken flasks. These findings are crucial for understanding the dual role of elicitors in modulating metabolic processes while maintaining stable growth conditions.

4.2.1.3 Effect of Elicitation on Yellow Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken flasks

The production of yellow pigments in *Monascus* occurs through the reduction of orange pigments, a process influenced by a range of fermentation conditions such as pH, elicitation, quorum sensing, nutrient availability, and aeration (Chen and Wu, 2016; Lv et al., 2017; Bai et al., 2024). Monitoring yellow pigment levels is valuable for assessing the culture's metabolic activity. In this study, peak production of yellow pigments in all flask groups was observed between Days 13 and 15, followed by a stabilisation of production levels thereafter. Elicitorsupplemented groups (OG, OM, and MO) consistently exhibited higher yellow pigment production compared to the control group, indicating that the presence of elicitors enhanced pigment production (Fig. 4.3). The control group reached a maximum optical density (OD at 400 nm) of 3.86 AU (Absorbance Units) on Day-14 before stabilising. Notably, the flask group supplemented with OM (oligomannuronate) exhibited higher OD for yellow pigment than the control, OG, and MO groups on most fermentation days. The OM elicitor led to an overall increase in yellow pigment OD throughout the fermentation process, with the highest concentration observed on Day-14, reaching 8.13 AU. Similarly, the OG-supplemented flask group also showed enhanced yellow pigment production, especially during the later stages of fermentation, where the OD gradually increased, reaching 6.91 AU on Day-14. The flask group with MO showed higher yellow pigment production than the C, however, was generally lower than the OM and OG groups. The presence of the MO led to an increase in yellow pigmentation reaching a maximum OD of 5.63 AU at 400 nm on Day-14. These results highlight the impact of elicitors on enhancing yellow pigment production and suggest that OM and OG elicitors

have a particularly notable effect in increasing pigment levels compared to the control and MO groups. The observed increase in yellow pigment due to elicitor supplementation aligns with findings from other studies. Various research papers have reported the positive influence of elicitors on pigment production in *Monascus* cultures (Huang et al., 2017; Qian et al., 2021; Bai et al., 2022 and 2024), suggesting that the choice of elicitors can notably impact yellow pigment production.



Fig. 4.3. The effect of different elicitors (OG, OM, and MO) on yellow pigment production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10^6 spores/mL and incubated at 25°C with a shaking speed of 120 rpm. 150 mg/L of each elicitor (OG, OM and MO) was added to the respective set of flasks at 48 h of fermentation. Three sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with standard deviation error bars. Yellow pigment production was measured at 400 nm wavelength, with a *p*-value<0.01 indicating statistically significant variation among different flask groups in comparison to the control group.

Table 4.1 presents the yield of yellow pigment in shaken flask fermentation for different flask groups. The yield values (AU/g CDW) are as follows: the control flask group (C) yielded 0.61 AU/g CDW of yellow pigment, the OG flask group yielded 1.03 AU/g CDW, the OM flask

group yielded the highest amount with 1.24 AU/g CDW, and the MO flask group yielded 0.87 AU/g CDW of yellow pigment. This data demonstrates the variations in pigment production among different flask groups, with the OM group showing the highest yield. Compared to the Control flask group, the OM group displayed a 2.03-fold increase in yellow pigment production. Subsequently, the OG group demonstrated a 1.69-fold increase, while the MO group indicated a 1.42-fold increase, suggesting a significant difference in yellow pigment production between the flask groups (C, OG, OM, MO). Statistical analysis using one-way ANOVA indicated a significant difference in yellow pigment production among the flask groups (*p*-value<0.01). Tukey's post hoc test confirmed that the OM-supplemented group exhibited significantly higher pigment production than the control group. Furthermore, OM differed significantly from both OG and MO, while OG produced significantly more pigment than MO, indicating distinct levels of production across the treatments (*p*-value<0.01; Appendix–Table 8).

Table 4.1. Yellow 1	Pigment Yield in Diffe	erent Flask Groups	(C, OG, OM, MO)	during Shaken
Flask Fermentation	. The abbreviation "w	.r.t" denotes "With	Respect To."	

Flask Group (3 flasks/set)	Yellow Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.61	-
Oligoguluronate (OG)	1.03	1.69
Oligomannuronate (OM)	1.24	2.03
Mannan Oligosaccharides (MO)	0.87	1.42

While prior research specifically targeting the enhancement of yellow pigment using carbohydrate elicitors in *Monascus* sp. and similar organisms is limited, existing studies underscore the significance of elicitation in augmenting pigmentation in various organisms, including *Monascus* sp. For example, Raheem et al. (2022) utilised potato powder as a

carbohydrate elicitor and found an increase in both biomass concentration and pigment production in *M. purpureus* Went NRRL 1992. Likewise, in a study by Huang and colleagues (2017), the manipulation of oxidoreduction potential was investigated to influence yellow pigment production in *M. ruber* CGMCC 10910. Their findings indicated that oxidative and reductive substances can be strategically employed to enhance pigment yields by exerting control over the oxidative process. Furthermore, Chavan et al. (2010) investigated the influence of dried fungal cells, as biotic elicitors, including *Trametes versicolor, Mucor sp., Penicillium notatum, Rhizopus stolonifer, and Fusarium oxysporum*, on cell growth, α -tocopherol, and pigment production in *Carthamus tinctorius* cell cultures and highlighted their impact in enhancing pigment synthesis. Similarly, Savitha and colleagues (2006) investigated the effects of various biotic elicitors, including purified glycans, in enhancing betalain productivity, a class of yellow and red tyrosine-derived pigments found in hairy root cultures of *Beta vulgaris*. These diverse investigations provide valuable insights into the potential of elicitation strategies to enhance yellow pigment production in various organisms.

4.2.1.4 Effect of Elicitation on Orange Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

Orange pigment acts as the primary pigment, with red and yellow pigments subsequently derived through different reactions from the orange pigment. This sequence underscores the foundational role of orange pigment in the overall pigment formation process in *Monascus* cultures (Chen et al., 2017). The conditions in shaken flasks, such as fluctuating pH and variable aeration, can influence the concentration and quality of orange pigments, reflecting the dynamic nature of metabolic processes in these cultures. Figure 4.4 represents the average values of orange pigment production in different flask groups (C, OG, OM, MO) over the course of fermentation. In the control group, the orange pigment production gradually increased throughout the fermentation process, reaching its highest OD (470 nm) of 3.43 AU

on Day-14. After Day-14, the pigment production remains relatively stable until the end of the fermentation. For the flask group with OG, orange pigment production increased more rapidly and peaked at 8.2 AU on Day-14, before stabilising. Similarly, the OM group showed increased production, with OD values reaching a peak of 7.6 AU on Day-14. Like the other groups, the OM group maintained a stable OD after Day-14. The MO group also demonstrated an increase in orange pigment production, with a peak OD of 5.8 AU on Day 14, followed by a steady phase similar to the other groups. These findings indicate that all elicitor-treated groups enhanced orange pigment production compared to the control, with OG having the most pronounced effect, followed by OM and MO. These results align with previous studies demonstrating the ability of elicitors to stimulate secondary metabolism and activate pigment biosynthesis pathways in *Monascus* and other microbial systems (Embaby et al., 2018; Zhang et al., 2020; Lyu et al., 2022).



Fig. 4.4. Orange pigment production in different flask groups (C, OG, OM, MO) throughout the fermentation process of 18 Days in *M. purpureus* C322 cultures during shaken flask fermentation. Each fermentation experiment was carried out in a 500 mL Erlenmeyer flask with 100 mL medium, inoculated with 10^6 spores/mL, and maintained at 25°C at 120 rpm. 150 mg/L of each elicitor was added at 48 h to the respective flask group. Each flask group was composed of three flasks, with error bars illustrating the standard deviation. Orange pigment production was quantified at 470 nm, with a *p*-value<0.01 highlighting statistical significance.

Furthermore, Table 4.2 presents the yield (AU/g CDW) of orange pigment in shake flask fermentation for different flask groups as follows: 0.54 AU/g CDW for the Control, 1.22 AU/g CDW for OG, 1.16 AU/g CDW for OM, and 0.89 AU/g CDW for MO. In terms of fold increase, the OG group shows a 2.26-fold increase compared to the Control group, followed by the OM group with a 2.15-fold increase. The MO group also indicates a noticeable increase with a fold of 1.65. This data demonstrates the variations in orange pigment production among different flask groups, with the OG group showing the highest yield. One-way ANOVA confirmed that orange pigment production differed significantly between the treatment groups (p-value<0.01). According to Tukey's post hoc test, among the elicitors, OG induced the highest production levels, with OM and MO also resulting in significantly elevated pigment production relative to the control. While all elicitor groups outperformed the control, statistically significant differences were also observed between the elicitor treatments themselves, with OG exceeding OM, and OM surpassing MO (p-value<0.01; Appendix–Table 9).

Table 4.2.	Orange Pigment	Yield in Differen	t Flask Groups (C	C, OG, OM, MO)	during Shaken
Flask Ferm	nentation				

Flask Group (3 Flasks/Set)	Orange Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.54	-
Oligoguluronate (OG)	1.22	2.26
Oligomannuronate (OM)	1.16	2.15
Mannan Oligosaccharides (MO)	0.89	1.65

A few studies emphasise the role of elicitation in enhancing orange pigment production in various microbes, including *Monascus*. For instance, Liu et al. (2019) achieved enhanced pigment production in *M. purpureus* LQ-6 through elicitation via spore immobilisation using sodium alginate. In other organisms, Tang et al. (2019) observed increased orange-yellow carotenoid production in *Cordyceps militaris* with the application of dry cell matter of

Rhodotorula glutinis as a fungal elicitor. Furthermore, elicitation employing agro-industrial waste, such as residues from fruits like orange, palm date, pineapple, apple, loquat kernels, and pomegranate waste, has demonstrated its capacity to augment yellowish-orange pigments in various microorganisms, including *Chryseobacterium artocarpi, Rhodotorula rubra, Lactobacillus plantarum, Rhodotorula glutinis,* and *Sarcina* sp. (Aruldass et al., 2018).

4.2.1.5 Effect of Elicitation on Red Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The production of red pigments in *Monascus*, resulting from the amination of orange pigments, is indicative of specific metabolic pathways being active under the fermentation conditions present in shaken flasks, and the resulting red pigment levels can provide insights into the efficiency of these biosynthetic pathways (Shin et al., 1998; Embaby et al., 2018; Virk et al., 2020; Gong et al., 2023). Figure 4.5 illustrates the red pigment production in different flask groups (C, OG, OM, MO) throughout fermentation. In the control group, the red pigment production peaked, with a maximum OD of 3.3 AU observed on Day-14 at 510 nm. In the presence of the elicitor OG (oligoguluronate), the red pigment production follows a similar increasing trend as the control group, however, with consistently higher pigment production. The maximum OD observed in the OG group was 7.55 AU on Day-14. The OM group displayed the highest red pigment OD compared to the C, OG, and MO groups, reaching 8.87 AU on Day-14. The MO group showed a slightly lower pigment OD than the OG and OM groups; however, it was higher than the control group, reaching 6.35 AU on Day-14. These findings indicate that all three elicitors (OG, OM, and MO) enhanced red pigment production, with OM having the most pronounced effect. These results align with previous studies that have demonstrated the effectiveness of various elicitors; such as microbial pollysaccharidepullalan, purified glycans, calcium, raw agro-waste, red and blue light; in enhancing red pigment production in various microorganisms, including Monascus sp. (Savitha et al., 2006;

Embaby et al., 2018; Zhang et al., 2020). The data highlights the potential of utilising elicitors to modulate red pigment biosynthesis. The OM flask group exhibited the highest red pigment production in shaken flask fermentation, followed by the OG, MO, and finally, the control flask group.



Fig. 4.5. Red pigment production in different flask groups (C, OG, OM, MO) throughout the fermentation process of 18 days in *M. purpureus* C322 cultures during shaken flask fermentation. The fermentation setup included 500 mL Erlenmeyer flasks each containing 100 mL of growth medium, inoculated with 10^6 spores/mL, and incubated at 25° C with a shaking speed of 120 rpm. The concentration of each elicitor was 150 mg/L added at 48 h. Three sets of experiments were performed, and each experiment was conducted in triplicate, with error bars representing standard deviation of the mean values (*p*-value<0.01).

Table 4.3 presents the yield of red pigment in shaken flask fermentation for different flask groups. The yield values (AU/g CDW) are as follows: the control flask group (C) yielded 0.52 AU/g CDW of red pigment, the OG flask group yielded 1.13 AU/g CDW, the OM flask group yielded the highest amount with 1.35 AU/g CDW, and the MO flask group yielded 0.98 AU/g CDW of red pigment. The red pigment production in the OG, OM, and MO flask groups exhibited fold increases of 2.17, 2.60, and 1.88-fold, respectively, compared to the control flasks. This data demonstrates the variations in red pigment production among different

flask groups, with the OM group showing the highest yield. The variation in red pigment production among the flask groups was statistically significant, as determined by one-way ANOVA (*p*-value<0.01). Tukey's post hoc analysis showed that OM supplementation resulted in the highest red pigment production, surpassing all other treatments. OG and MO also led to significantly increased production compared to the control. Distinct differences were evident among the elicitor groups as well, with OM exceeding both OG and MO, and OG producing more than MO (*p*-value<0.01; Appendix–Table 10).

Table 4.3. Red Pigment Yield in Different Flask Groups (C, OG, OM, MO) during Shaken Flask Fermentation

Flask Group (3 Flasks/Set)	Red Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.52	-
Oligoguluronate (OG)	1.13	2.17
Oligomannuronate (OM)	1.35	2.60
Mannan Oligosaccharides (MO)	0.98	1.88

Research into enhancing red pigment production in *Monascus* sp. through elicitation using carbohydrate elicitors, especially OG, OM, and MO, remains limited. Nonetheless, some evidence suggests an increase in red pigment production in *Monascus* and other microorganisms using other types of elicitors. For instance, Sibir and Goksungur (2019) observed increased red pigment production by *M. purpureus* CMU001 when exposed to brewery waste hydrolysate (Brewer's spent grain) as an elicitor. Gong et al. (2023) outlined that agricultural byproducts such as potato pomace, corn starch, saba banana peel, residual beer, cheese whey, soybean meals, waste loquat kernels, date waste substrates, and whey can elicit pigment production in *M. purpureus*. Similarly, an increase in the red pigment, betalain, production in *Celosia cristata* was observed in the presence of dried *Fusarium oxyporum* cells (1 mg/mL) as elicitor (Warhade and Badere, 2018). These observations align with the outcomes

of the current study, further emphasising the importance of elicitors, specifically carbohydrate elicitors (OG, OM, and MO), in enhancing red pigment production.

4.2.1.6 Impact of Elicitation on Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The rate and pattern of carbohydrate consumption provide insights into the efficiency of the fermentation process and the influence of various cultivation conditions, such as the presence of specific elicitors, on the microorganism (Wong et al., 1981; Agboyibor et al., 2018; Mata et al., 2019). Figure 4.6 illustrates the difference in carbohydrate consumption within different flask groups (C, OG, OM, and MO) over the course of fermentation. Carbohydrate concentrations (g/L) were monitored across all flask groups (C, OG, OM, MO) from Day-1 to Day-18. The control group reached the lowest value of 6.21 g/L on Day-18. The elicitor-supplemented groups display similar patterns in carbohydrate consumption to control flask groups. The lowest values on Day-18 for flask groups OG, OM and MO were 5.99, 5.91, 6.15 g/L, respectively. Glucose concentrations were quantified by referencing a standard curve (Appendix–Fig. 4).

The carbohydrate consumption values in the flask groups are comparable to the standard glucose curve in terms of decreasing carbohydrate consumption with decreasing glucose concentration. Assuming the log phase of fermentation lies between Day-5 and Day-15 in all flask groups, the carbohydrate consumption rates for all flask groups (C, OG, OM, MO) are 0.73, 0.74, 0.75 and 0.73 g/L/day, respectively. No statistically significant differences in carbohydrate consumption were observed between flask groups (*p*-value>0.01; Appendix–Table 11).



Fig. 4.6. Carbohydrate consumption in different flask groups (C, OG, OM, MO) throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. Each 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10^6 spores/mL prior to incubation at 25 °C under 120 rpm. 150 mg/L of each elicitor was added to the respective flask group at 48 h of fermentation. Three sets of experiments were performed in triplicates, and error bars representing the standard deviation of the mean values (*p*-value>0.01).

As fermentation progresses, the availability of carbohydrates becomes a critical factor influencing both biomass accumulation and secondary metabolite synthesis. During early fermentation, when nutrients are abundant, microbial growth and metabolite production are typically enhanced (Agboyibor et al., 2018; Reddy et al., 2021). However, as carbohydrates are depleted, metabolic activity declines due to reduced energy availability. Simultaneously, the accumulation of toxic metabolic by-products can negatively affect microbial viability and productivity (Washburne et al., 1996; Llorens et al., 2010). Furthermore, under nutrient-limited conditions, oxygen transfer can become impaired, particularly in dense cultures, compounding metabolic stress (Cullen and Sprague, 2012; Shi et al., 2017; Lopez et al., 2022). This may lead to premature cessation of both biomass and pigment production. These factors underline the importance of optimising nutrient concentration, dissolved oxygen levels, and waste

management during microbial fermentations to maximise productivity and metabolic efficiency (Ruiz et al., 2010; Willemen et al., 2021; Cruz et al., 2021).

4.2.1.7 Comparison of pH, Pigment Production and Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The data presented in Figure 4.7 provides insights into the relationship between pH, pigment production (yellow, orange, and red), and carbohydrate consumption in each flask group (C, OG, OM, and MO) over the fermentation period of 18 days. In terms of pigment production, the concentration of all three pigments increased with time in all flask groups, reaching peak concentrations between Day 15-17 before stabilising. As evident from Figure 4.7, the elicitor-supplemented groups (OG, OM, and MO) consistently showed higher pigment production than the C group, indicating that elicitors positively influenced pigment biosynthesis. After reaching a stable level, the slight decline in pigment production may be attributed to pigment degradation, which can be affected by various environmental factors such as pH, light, and temperature. Several studies have reported the impact of higher pH on the stability of *Monascus* pigments (Jeon et al., 2013; Wei et al., 2023). Consistent with these findings, our study also observed a decrease in pigment production with increased pH.

In shaken flasks, the pigment yield displayed distinct patterns across different groups (C, OG, OM, MO). The control group showed a comparatively higher yellow pigment yield (0.61 AU/g CDW), followed by orange (0.54 AU/g CDW) and then red (0.52 AU/g CDW). This indicates that, without the elicitors, *M. purpureus* C322 cultures primarily engage in reduction reactions, resulting in higher yellow pigment, with some orange and red pigments as intermediates. Nonetheless, variations may occasionally occur. However, it was observed that the addition of elicitors (OG, OM, MO) triggered more consistent results in terms of pigment production; thus, indicating a notable effect of these carbohydrate elicitors on the pigment

production process. For details on the reduction and amination reactions, refer to Chapter I, Section 1.4.5.

The supplementation of oligosaccharide elicitors (OG, OM, and MO) brought about notable shifts. OG led to an increase in orange pigment yield (1.22 AU/g CDW), surpassing yellow (1.03 AU/g CDW) and red (1.13 AU/g CDW) by the end of fermentation i.e. Day-18. OM prompted a similar response, with red pigment yield (1.35 AU/g CDW) being the highest, followed by yellow (1.24 AU/g CDW), and then orange (1.16 AU/g CDW) by Day-18. This suggests that OM enhances amination reactions, leading to an increase in red pigments, while yellow and orange pigments are also present but less abundant. MO resulted in a shift where red pigment yield (0.98 AU/g CDW) was most abundant, followed by orange (0.89 AU/g CDW), and then yellow (0.87 AU/g CDW). Mannan oligosaccharides (MO) appear to strongly influence amination reactions, resulting in a notable increase in red pigments, with orange and yellow pigments also present but less prevalent.

On the other hand, carbohydrate consumption decreased over time in all flask groups during the log phase of fermentation. As the pH decreased over time, pigment production increased, and carbohydrate consumption accelerated. The enhanced pigment production in the elicitorsupplemented groups (OG, OM, and MO) is linked to the microbial response to elicitors, leading to more active metabolic processes and increased carbohydrate utilisation.









Fig. 4.7. Comparison between pH, pigment production, and carbohydrate consumption across different flask groups during shaken flask fermentation; where, a) Control (C), b) Oligoguluronate (OG), c) Oligomannuronate (OM), and d) Mannan Oligosaccharides (MO).

Chapter IV

4.2.1.8 Impact of Elicitation on Lovastatin Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The effectiveness of elicitation in enhancing lovastatin production is a key focus of this research. The biosynthesis of lovastatin is a complex process and can be influenced by growth factors. The results from shaken flasks provide preliminary insights into the conditions favouring lovastatin biosynthesis. Carbohydrate elicitors (OG, OM and MO) were used to enhance the production of lovastatin in *M. purpureus* C322. Figure 4.8 presents the average lovastatin concentrations (mg/L) across different flask groups. The C group recorded an average concentration of 31.82 mg/L. For the flask group supplemented with OG, the average lovastatin concentration of 79.54 mg/L. The flask group with MO elicitor shows an average lovastatin concentration of 58.33 mg/L. Comparing these values, it is evident that the groups with elicitor supplementation, particularly OM and OG, display higher average lovastatin concentrations than the control and MO groups.



Fig. 4.8. Lovastatin production in different flask groups (C, OG, OM, MO) on Day-18 of fermentation in *M. purpureus* C322 cultures during shaken flasks fermentation. Each fermentation experiment utilised a 500 mL Erlenmeyer flask with 100 mL growth medium,

inoculated with 10^6 spores/mL, and incubated at 25° C and 120 rpm. The experiments were conducted in triplicate, with error bars denoting standard deviation. Lovastatin concentration on Day 18 was quantified using High-Performance Liquid Chromatography (HPLC) (*p*-value<0.01).

Table 4.4 displays the lovastatin yield in various flask groups (C, OG, OM, MO) during the fermentation process in shaken flasks. The C group yielded 5.72 mg/g CDW of lovastatin. Comparatively, the OG flask group produced 10.46 mg/g CDW, the OM group yielded 12.11 mg/g CDW and the MO group yielded a lovastatin yield of 9.41 mg/g CDW. Compared to the control flasks, the lovastatin concentration in the flask groups OG, OM, and MO showed fold increases of 1.83, 2.12, and 1.64, respectively. These results illustrate the differences in lovastatin concentration across the different flask groups, with the OM group displaying the highest yield. The HPLC profiles for each flask group (C, OG, OM, MO) exhibit characteristic peaks corresponding to the retention time of lovastatin, allowing for quantitative analysis of the compound's concentration in the samples (Appendix-Fig. 7). Additionally, this analysis is further supported by correlation curves displaying lovastatin standards, provided in Appendix Figure. 5. The standard lovastatin curve displays a set of known lovastatin concentrations 0.01 mg/mL, 0.02 mg/mL, 0.05 mg/mL, and 1.0 mg/mL (Appendix-Fig. 6). These values helped to establish a correlation between peak area in HPLC chromatograms and actual lovastatin concentration. A significant variation in lovastatin production was observed among the different flask groups, as confirmed by one-way ANOVA (*p*-value<0.01). Tukey's post hoc test further demonstrated that the OM group produced the highest lovastatin levels, followed by OG and MO, with all elicitor-supplemented groups showing significantly higher production than the control. Notably, OM also differed significantly from both OG and MO, while OG produced significantly more lovastatin than MO, highlighting clear distinctions across all treatments (*p*-value<0.01Appendix–Table 12).

Flask Group (3 Flasks/set)	Lovastatin Yield (mg/g CDW)	Fold Increase w.r.t Control
Control (C)	5.72	-
Oligoguluronate (OG)	10.46	1.83
Oligomannuronate (OM)	12.11	2.12
Mannan Oligosaccharides (MO)	9.41	1.64

Table 4.4. Lovastatin Yield in Different Flask Groups (C, OG, OM, MO) during Shaken Flask Fermentation

Several studies have shown that lovastatin yields can vary ranging from 0.26 mg/g of Cell Dry Weight (CDW) to 20 mg/g CDW depending not only on the species and strains used but also on the growth conditions of the culture (Mohan-Kumari et al., 2012; Mansoori and Yazdian, 2014; Dikshit and Tallapragada, 2015; Seenivasan et al., 2016; Suraiya et al., 2018; Wen et al., 2020; Liu et al., 2022; Wu et al., 2023). In alignment with these findings, the lovastatin yield observed in the current study fell within this spectrum, especially for control flasks. For flasks supplemented with elicitors, the lovastatin concentration approached the end of this range. For instance, Dai et al. (2021) examined the potential of four strains of *M. pilosus* (MS-1, YDJ-1, YDJ-2, and K104061) to produce lovastatin in both solid-state and submerged fermentation, noting yields ranging from 0.30 mg/g CDW to 6.13 mg/g CDW depending on the fermentation from *M. sanguineus* through the optimisation of various factors using response surface methodology (Dikshit and Tallapragada, 2016). Additionally, Xu et al. (2005) managed to attain a total lovastatin yield of 4-6 mg/g CDW by fine-tuning the concentrations of carbon and nitrogen sources.

However, the application of elicitors, particularly OG, OM, and MO, as a strategy to enhance lovastatin concentration, represents an unexplored area. While there have been studies exploring various methods to increase lovastatin concentration in *Monascus* cultures, direct

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comparisons are challenging due to the novel approach of elicitation using OG, OM, and MO. Despite this, there are indications from research that adopting other elicitation strategies, encompassing both abiotic and biotic elicitors such as UV light, red and blue light, dried cell matter, etc, can enhance secondary metabolism, potentially resulting in enhanced lovastatin production (Sayyad et al., 2007; Ahmed et al., 2009). This suggests that while the approach of using these specific elicitors is relatively new, the concept of enhancing secondary metabolite production to boost lovastatin yield is a promising area of research.

Results and Discussion 2.5 L Stirred Tank Fermenters

4.2.2 Elicitation in 2.5 L Stirred Tank Fermenters

This section highlights the effect of carbohydrate elicitors (OM, OG, MO) on biomass concentration and secondary metabolite (pigments and lovastatin) production in 2.5 L Stirred Tank Fermenters. The bioreactors were incubated at 32 °C and 300 rpm, and the medium pH was 6.5. Elicitors were introduced at a concentration of 150 mg/L at 24 h of fermentation. The total working volume was 1,500 mL, including 150 mL seed culture. The fermentation process with elicitors was repeated thrice to ensure repeatability.

4.2.2.1 Effect of Elicitation on the Culture pH during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

Compared to shaken flask fermentation, fermenter-based cultivation offers more control over the growth conditions. Monitoring and understanding the pH variations during fermentation is crucial for deciphering the metabolic state of the culture. The data presented in Figure 4.9 provides a comprehensive insight into the pH variations throughout the fermentation period in 2.5 L bioreactors. In the control bioreactor (C), the pH decreased to 6.1 by Day-4. Subsequently, an increase in pH to 6.3 by Day-5 was observed. The bioreactor supplemented with the OG elicitor displayed a decrease to 5.8 on Day-4. Thereafter, an increase in pH to 6.2 on Day-5 was noticed. Similarly, the pH trend within the OM bioreactor declined to 5.7 by Day-4 and subsequently increased to 6.3 by Day-5. In a bioreactor supplemented with MO, the pH decreased to 5.8 by Day-4. By Day-5, the pH in a bioreactor groups (C, OG, OM, MO), the variations in pH levels do not demonstrate statistical significance, as indicated by a *p*-value greater than 0.05 (see Appendix- Table 13).


Fig. 4.9. pH variation in different bioreactor groups over time: C, OG, OM, and MO. The depicted average pH values are from three experimental runs in 2.5 L stirred tank bioreactors, each containing 1,500 mL growth medium and 150 mL seed culture, incubated at 32° C at 300 rpm. Standard deviation is shown as error bars (*p*-value>0.05).

Figure 4.10 presents a comparative analysis of pH variations during the fermentation process in both shake flasks (SF) and 2.5 L fermenters (2.5L_F). In shaken flask fermentation, the control group (C) exhibited a pH range from 6.0 (minimum) to 6.8 (maximum). In the elicitorsupplemented groups (OG, OM, MO), pH values ranged from 5.9–6.0 at the lowest, to 7.0–7.1 at the maximum by the end of fermentation. In fermenters, the C group maintained a narrower pH range, from 6.1 (minimum) to 6.3 (maximum). In the elicitor-supplemented fermenter groups, pH values ranged from 5.7–5.8 at the minimum to 6.1–6.3 at the maximum. These findings indicate that the transition from shaken flasks to fermenters in *M. purpureus* C322 cultures resulted in a consistent pattern of pH variation across all groups (C, OG, OM, MO), highlighting reproducibility and stability in pH control across different cultivation scales.



Fig. 4.10. Comparative analysis of the minimum and maximum pH values recorded during the fermentation process in both shake-flasks (SF) and 2.5 L fermenters (2.5L_F). Error bars represent the standard deviation of average pH measurements between three sets of experiments.

The pH variations typically reflect the natural progression of the fermentation process, influenced by the metabolic activities of the culture (Zaki et al., 2016; Zia et al., 2021; Voidaroa et al., 2021). Upon transitioning to fermenters, the observed consistency in pH trends between the shaken flasks and fermenters suggests that *M. purpureus* C322 cultures can maintain a similar metabolic profile even when scaled up. Thus, the observation of similar pH trends aligns with the notion that elicitation strategies employed in shaken flasks are effectively translated to larger bioreactor systems, preserving the pH dynamics.

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4.2.2.2 Effect of Elicitation on Biomass Concentration during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

Compared to shaken flasks, fermenters provide a more consistent environment for biomass concentration, allowing for optimisation and scalability of the fermentation process. Biomass concentration is a crucial parameter in fermentation processes, as it reflects the growth and productivity of the microbial culture. This section compares the biomass concentration across different bioreactor groups —C, OG, OM, and MO—measured at the end of fermentation (Day-5) to analyse if the presence of elicitors influences *M. purpureus* C322 biomass. Figure 4.11 showcases the biomass concentration, represented as cell dry weight (CDW) in grams per litre (g/L), across different bioreactor groups, including C, OG, OM, and MO. Among the bioreactor groups, OM exhibits the highest average CDW value of 8.0 g/L, indicating a relatively higher biomass concentration than the other groups. OG and MO groups follow with CDW values of 7.5 g/L and 7.0 g/L, respectively. The control group demonstrated the lowest mean biomass concentration with a CDW of 7.1 g/L.

To assess the efficacy of the selected bioreactor groups in responding to the upscaling process in terms of biomass, the total cell dry weight (CDW) (g/L) was compared between shaken flasks and bioreactors. The C group showed a 1.12-fold increase in CDW. Likewise, the groups supplemented with elicitors - OG, OM, and MO - displayed increased CDW by 1.12, 1.22, and 1.08 folds, respectively, upon transitioning from shaken flasks to bioreactors. The observed rise in biomass concentration during upscaling indicates that the cultivation strategies facilitated successful adaptation to the bioreactor environment. The calculated *p*-value for oneway ANOVA in biomass concentration among the bioreactor groups (C, OG, OM, MO) was greater than 0.05, indicating no significant differences in biomass concentration among the studied bioreactor groups (Appendix-Table 14).

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Fig. 4.11. Biomass concentration on Day-5 of fermentation in different bioreactor groups: C, OG, OM, and MO. In each 2.5 L stirred tank bioreactor, 1,500 mL of growth medium and 150 mL of seed culture were used, incubated at 32°C and stirred at 300 rpm. Each elicitor was added at a concentration of 150 mg/L concentration at 24 h to the respective bioreactor group. The error bars denote the standard deviation based on three experimental sets (*p*-value>0.05).

4.2.2.3 Effect of Elicitation on Yellow Pigment Production during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The provided data in Figure 4.12 illustrates the production of yellow pigment in 2.5 L stirred tank fermenters across different bioreactor groups (C, OG, OM, and MO) over a span of five days (D1 to D5). The production of yellow pigment for each group showed a continuous increase throughout the observed days. The optical density measured for yellow pigment production in the C group was 5.01 by the end of fermentation in bioreactors (Day-5). Likewise, the bioreactors with added elicitors (OG, OM and MO) also displayed an increase in yellow pigment, reaching an OD (400 nm) of 10.21, 12.67, and 7.27 AU at 400 nm, respectively, by Day-5 of fermentation.



Fig. 4.12. Yellow Pigment production in different bioreactor groups: C, OG, OM, and MO. Each 2.5 L stirred tank bioreactor contained 1,500 mL growth medium, including 150 mL seed culture prior to incubation at 32 °C under 300 rpm. Each elicitor (OG, OM, MO) was added at a concentration of 150 mg/L to the respective bioreactor group at 24 h of fermentation. Three sets of experiments were performed, and the error bars represent the standard deviation (*p*-value<0.01).

Table 4.5 comprehensively compares yellow pigment yield and the influence of upscaling among various bioreactor groups (C, OG, OM, MO) during 2.5 L Bioreactor Fermentation. "F/SF" indicates the transition from "Shaken flasks to Fermenters". The yellow pigment yields are measured in Absorbance Units per Cell Dry Weight (AU/g CDW). The data reveals both the pigment yield in 2.5 L Stirred Tank Bioreactors as well as the fold increase resulting from the transition from shaken flasks to fermenters. The C bioreactor group yielded 0.71 AU/g CDW yellow pigment. Comparatively, the bioreactor groups supplemented with elicitors yielded notably higher levels of yellow pigment yields of 1.36, 1.58, and 1.04 AU/g CDW respectively. Notably, the MO group exhibited the highest yellow pigment yield among all the groups. In comparing each bioreactor group to the C group, the OM group displayed the highest increment in yellow pigment yield with a fold increase of 2.22. The OG and MO groups

followed with fold increases of 1.91 and 1.46, respectively. These outcomes underscore the potency of the OG and OM elicitors in notably boosting yellow pigment production, while MO also exhibited a substantial increase (Table 4.5). The trend observed in yellow pigment production during shaken flask experiments was successfully replicated in 2.5 L stirred tank fermenters. One-way ANOVA confirmed that yellow pigment production differed significantly among the bioreactor groups (*p*-value<0.01). According to Tukey's post hoc test, the OM-supplemented group exhibited the highest production, followed by OG and MO, all of which resulted in greater pigment production than the control. Statistically supported differences were also observed among the elicitor groups themselves, with OM producing more than both OG and MO, and OG exceeding MO (*p*-value<0.01; Appendix–Table 15).

Table 4.5. Comparison of Yellow Pigment Yield and Impact of Upscaling across different Bioreactor Groups (C, OG, OM, MO) during 2.5 L Bioreactor Fermentation, where F/SF refers to "Shaken flasks to Fermenters".

Bioreactor Group	Yellow Pigment Yield- Bioreactor (AU/g CDW)	Fold Increase w.r.t Control Bioreactor	Yellow Pigment Yield- Shaken Flasks (AU/g CDW)	Fold Increase in Yield: F/SF
Control (C)	0.71	-	0.61	1.16
Oligoguluronate (OG)	1.36	1.91	1.03	1.32
Oligomannuronate (OM)	1.58	2.22	1.24	1.27
Mannan Oligosaccharides (MO)	1.04	1.46	0.87	1.19

The notable fold increases in pigment yield observed for each bioreactor group demonstrate the efficacy of the upscaling process in enhancing pigment production potential. This phenomenon aligns with findings in other studies. For example, Zhou et al. (2009) reported an increase in yellow pigment production in *M. anka*, from 87.24 to 92.45 OD, following upscaling from shaken flasks to 5 L fermenters. Similarly, Zhang et al. (2017) and Kim et al.

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(2002) recorded comparable enhancements in *Monascus* pigment yields when scaling up from smaller bioreactors to larger pilot vessels of 200 L and 300 L, respectively.

4.2.2.4 Influence of Elicitation on Orange Pigment Production in 2.5 L Stirred Tank Fermenters during Submerged Fermentation of *M. purpureus* C322

Monascus pigments are recognised for their scalability and ease of production, making them suitable for industrial applications, highlighting their versatility and practicality in various sectors (Chaudhary et al., 2022). The pattern observed in the 2.5 L stirred tank fermentation for orange pigment production was similar to the shaken flask cultures. The OD (470 nm) by the end of fermentation (Day-5) for the Bioreactor Groups (C, OG, OM, and MO) were 3.9, 12.5, 11.4, and 7.8 AU, respectively. These results highlight a consistent trend in orange pigment production over the fermentation period, with elicitors (OG, OM, MO) enhancing higher pigment than the control group. The highest orange pigment production was observed in bioreactors supplemented with OG, followed by OM and MO, respectively (Fig. 4.13). Table 4.6 comprehensively compares orange pigment yield and upscaling effects across various bioreactor groups (C, OG, OM, MO) during the 2.5 L bioreactor fermentation. The notation F/SF signifies the transition from shaken flasks to fermenters. The orange pigment yield in the C bioreactor group stood at 0.55 AU/g CDW. The bioreactor groups supplemented with elicitors displayed higher yields of orange pigment. The OG group exhibited a yield of 1.67 AU/g CDW, signifying a notable enhancement. Similarly, the OM group yielded 1.42 AU/g CDW, while the MO group yielded 1.11 AU/g CDW.



Fig. 4.13. Concentration of Orange Pigment in Various Bioreactor Groups: C, OG, OM, and MO. Each 2.5 L bioreactor, contained 1,500 mL growth medium and 150 mL seed culture, maintained at 32° C with agitation at 300 rpm. 150 mg/L of each elicitor (OG, OM, MO) was added to the respective bioreactor group at 24 h of fermentation. Error bars indicate standard deviation from three experimental runs (*p*-value<0.01).

The pattern observed in 2.5 L stirred tank fermenters reflected the trend established in shaken flask experiments, with elicitor-supplemented groups producing more orange pigment than the control. OG exhibited the highest mean orange pigment concentration, followed by OM, MO, and C in both systems. One-way ANOVA confirmed that orange pigment production differed significantly across bioreactor groups (*p*-value<0.01). Tukey's post hoc test showed that OG and OM both produced significantly more pigment than MO and the C group, with OG exhibiting comparatively higher production than OM. Both OG and OM outperformed MO and C, maintaining the expected trend across scale-up (*p*-value<0.01; Appendix–Table 16). The comparative analysis in this study also reveals clear improvements in orange pigment production in the OG, OM, and MO bioreactor groups compared to the control. Specifically, orange pigment yield increased by 3.04-fold in the OG group, 2.6-fold in the OM group.

Bioreactor Group	Orange Pigment Yield-Bioreactor (AU/g CDW)	Fold Increase w.r.t Control Brioreactor	Orange Pigment Yield-Shaken Flasks (AU/g CDW)	Fold Increase in Yield: F/SF
Control (C)	0.55	-	0.55	1.0
Oligoguluronate (OG)	1.67	3.04	1.22	1.37
Oligomannuronate (OM)	1.42	2.6	1.16	1.22
Mannan Oligosaccharides (MO)	1.11	2.02	0.89	1.25

Table 4.6. Comparison of Orange Pigment Yield and Impact of Upscaling across different Bioreactor Groups (C, OG, OM, MO) during 2.5 L Bioreactor Fermentation.

In this study, the addition of carbohydrate elicitors (OG, OM, MO) increased the orange pigment yield in *M. purpureus* C322 cultures as compared to control groups. In addition, the production of orange pigment was successfully scaled up, with an enhanced yield noted when transitioning from shaken flasks (500 mL) to 2.5 L stirred tank bioreactors. This observation is consistent with the findings in several reports suggesting that elicitation in Monascus sp. leads to the overproduction of pigments (Wang et al., 2013; Gmoser et al., 2017; Liu et al, 2020; Lyu et al., 2022). For example, Embaby et al., 2018 observed a 174% increase in orange pigment production by using corncob agro-industrial waste as elicitors. The transition to fermenters not only maintained the consistency of *Monascus* orange pigment production but, in many cases, enhanced it, which can be attributed to the more efficient mixing, aeration, and nutrient distribution in fermenters. The scalability of pigment production in fermenters paves the way for commercial applications, as demonstrated by our research. Several studies noted an enhanced production of pigments in fermenters, confirming the general trend observed in filamentous fungal cultures, including Monascus (Choe et al., 2020; Venkatachalam et al., 2023). Kim et al, 2002, for instance, observed a successful scale-up in *Monascus* pigment production when transitioning from a 5 L jar to a 300 L pilot vessel yielding 37.5 OD.

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4.2.2.5 Effect of Elicitors on Red Pigment Production in 2.5 L Stirred Tank Fermenters during Submerged Fermentation of *M. purpureus C322*

In the context of 2.5 L stirred tank bioreactors, the data reveal the dynamics of red pigment production over the course of fermentation. As observed in shaken flasks, the trend continued in the bioreactor setup, with all groups showing a progressive increase in pigment production. In the control group, the red pigment OD (510 nm) reached 4.18 AU by Day-5. In comparison, the OG group exhibited a significant increase to 12.0 AU, while the OM group showed the highest production, reaching 14.41 AU. The MO group also demonstrated enhanced pigment production, however, achieved lower values compared to OG and OM, reaching an OD of 8.1 AU by the end of fermentation (Day 5). These findings indicate that the addition of elicitors, particularly OM, resulted in a substantial enhancement in red pigment synthesis. The consistent trend with shaken flask results reinforces the reliability of the observed pattern in the more controlled environment of stirred tank bioreactors (Fig. 4.14).

Table 4.7 presents a comparative analysis of red pigment yield among the different bioreactor groups: C, OG, OM, and MO, during 2.5 L bioreactor fermentation. Yield values, expressed in absorbance units per cell dry weight (AU/g CDW), are accompanied by fold increases relative to the control group. The control group yielded 0.60 AU/g CDW, while the OM group achieved the highest yield of 1.80 AU/g CDW. OG and MO yielded 1.60 and 1.20 AU/g CDW, respectively, further supporting the impact of elicitation. The pattern of red pigment production observed in the 2.5 L stirred tank fermenters mirrored the trend established in the shaken flask experiments, with all elicitor-supplemented groups producing higher pigment levels than the control. One-way ANOVA confirmed a significant difference in red pigment production across bioreactor groups (p-value<0.01). According to Tukey's post hoc test, the OM group produced the highest pigment levels overall, exceeding both OG and MO. OG also showed greater

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production than MO, and all three elicitor groups outperformed the control, confirming distinct differences among all treatments (*p*-value<0.01; Appendix–Table 17).



Fig. 4.14. Concentration of Red Pigment in Various Bioreactor Groups: C, OG, OM, and MO. Each 2.5 L bioreactor comprised 1,500 mL growth medium and 150 mL seed culture, incubated at 32 °C and agitated at 300 rpm. Each elicitor (OG, OM, MO) was added at a concentration of 150 mg/L to the respective bioreactor group at 24 h of fermentation. The standard deviation between three sets of experiments is indicated by error bars (*p*-value<0.01).

Fold increases in pigment yield were notable. The red pigment yield for the OM group increased by 3.03 fold compared to the control. Similarly, the application of OG and MO led to a 2.7-fold and 1.97-fold increase in pigment yield, respectively. The MO group showed an increase in pigment yield compared to the control. The outcomes from 2.5 L fermenters confirmed that elicitor supplementation, particularly with OM and OG, substantially improved red pigment production in *M. purpureus* C322, consistent with trends observed during shake flask experiments.

Bioreactor Group	Red Pigment Yield- Bioreactors (AU/g CDW)	Fold Increase w.r.t Control Bioreactor	Red Pigment Yield-Shaken Flasks (AU/g CDW)	Fold Increase in Yield: F/SF
Control (C)	0.6	-	0.52	1.15
Oligoguluronate (OG)	1.6	2.7	1.13	1.41
Oligomannuronate (OM)	1.8	3.0	1.35	1.33
Mannan Oligosaccharides (MO)	1.2	2	0.98	1.22

Table 4.7. Comparison of Red Pigment Yield and Impact of Upscaling across different Bioreactor Groups (C, OG, OM, MO) during 2.5 L Bioreactor Fermentation.

Some studies have focused on optimising the production of *Monascus* red pigments, especially when transitioning from laboratory-scale processes, such as shaken flasks, to larger fermenters and demonstrated a direct correlation between the upscaling process and the increased yield of pigments (Kim et al., 2002; Agboyibor et al., 2018; Sibir and Goksungur, 2019; Srianta et al., 2014; Chaudhary et al., 2023; Venkatachalam et al., 2023). Moreover, the addition of various elicitors have been reported to notably enhance the yield of red pigments (Carvalho et al., 2003; Embaby et al., 2018; Mehri et al., 2021; Raheam et al., 2022). For instance, Oliveira et al. (2022) have noted that the addition of maltose as elicitor has led to notable increases in red pigment production from 6 AU/g CDW to 15 AU/g CDW in *M. ruber* CCT 3802 cultures. Similarly, elicitation with potato pomace enhanced the red pigment production in *M. purpureus* CHO1 and *M. purpureus* CICC 5,016 cultures by 35.2% and 67.1%, respectively during fed-batch fermentation (Chen et al., 2021).

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4.2.2.6 Effect of Elicitation on Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The data presented in Figure 4.15 displays the trend of carbohydrate consumption over the fermentation period in 2.5 L stirred tank bioreactors for different bioreactor groups, C, OG, OM, and MO. In the C group, carbohydrate consumption decreased from 20 g/L to 9.38 g/L by Day-5. Similar patterns are observed in bioreactors supplemented with elicitors, where the carbohydrate consumption decreased from 20 g/L to 9.19, 9.47, and 9.28 g/L for OG, OM and MO bioreactor groups, respectively. The carbohydrate consumption rate over the 5-day fermentation period for each respective bioreactor group was 2.0, 2.04, 2.0 and 2.02 g/L/day for C, OG, OM and MO, respectively as estimated from the standard glucose curve (Appendix-Fig. 4). The variance between the carbohydrate consumption levels among the different bioreactor groups C, OG, OM, and MO, corresponds to a *p*-value>0.05 indicating that there is no statistically significant difference in carbohydrate consumption among these bioreactor groups (Appendix-Table 18).

The results suggest that upon transitioning to the 2.5 L fermenters, the observed carbohydrate consumption trend was consistent with the earlier shaken flask data. This similarity in trends indicates that the findings from the shaken flask experiments could be reliably scaled up to the larger fermenter setup. This is a crucial aspect in the field of bioprocessing, as it provides validation that the results obtained in smaller volumes are applicable and consistent in larger industrial-scale setups. In essence, the parallel trends between shaken flask and fermenter experiments signify the successful upscaling of the fermentation process.



Fig. 4.15. Carbohydrate consumption in different bioreactor groups: C, OG, OM, and MO. In each 2.5 L bioreactor, 1,500 mL of growth medium and 150 mL of seed culture were used, incubated at 32°C and stirred at 300 rpm. The concentration of each elicitor (OG, OM, MO) was 150 mg/L at 24 h of fermentation. Error bars show the standard deviation from three experimental runs. The *p*-value>0.05 indicates no significant difference.

4.2.2.7 Comparison of pH, Pigment Production and Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in Stirred Tank Fermenters

The data presented in Figure 4.16, derived from two sets of experiments, provides a comprehensive overview of the correlation between pH levels, carbohydrate consumption, and pigment (yellow, orange, and red) production across different bioreactor groups (C, OG, OM, MO) over a span of five days. Notably, the observed patterns in 2.5 L stirred tank fermenters closely resemble those observed in shaken flasks, reinforcing the consistency of the correlation between pH, pigment production (Yellow, Orange, Red) and carbohydrate consumption across different bioreactor groups (C, OG, OM, and MO) (Fig. 4.6). The data reflects a gradual decline in pH, which corresponded with an increase in pigment production and carbohydrate consumption. Regarding pigment production, it is evident that all bioreactor groups exhibit an

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increase in yellow, orange, and red pigments as the fermentation progresses from D1 to D5; however, the bioreactors supplemented with elicitors (OG, OM and MO) resulted in a higher pigment production in comparison to the C group. Interestingly, the OM group demonstrates the highest production of yellow and red pigments, followed by the OG, MO, and C groups. In contrast, the OG group exhibits the highest production of orange pigment, followed by the OM, MO, and C groups.

The trend observed in the control group was not always consistent; however, the addition of elicitors consistently triggered more predictable outcomes. This was evident in shaken flasks as well as fermenters, where the occurrence of both reduction and amination reactions notably influenced the relative concentrations of yellow, orange, and red pigments. For details on the reduction and amination reactions, refer to Chapter I, Section 1.4.5. In the control group, the prevalence of yellow pigment (0.71 AU/g CDW) suggests a dominant reduction reaction, converting orange pigments into yellow pigments. This was followed by red (0.59 AU/g CDW) and orange pigments (0.55 AU/g CDW), indicating a lesser extent of amination. With the addition of OG, there was a notable shift, with orange pigment (1.67 AU/g CDW) becoming the most abundant. This implies a reduced level of reduction reactions, maintaining higher levels of orange pigment, while still supporting significant amounts of yellow (1.36 AU/g CDW) and red pigments (1.6 AU/g CDW) through both reduction and amination. In the OM group, red pigment yield (1.8 AU/g CDW) was the highest, suggesting an enhanced amination process where orange pigments were converted to red. This was followed by yellow (1.6 AU/g CDW) and then orange pigments (1.42 AU/g CDW), indicating a complex interplay of both reactions. Interestingly, in the MO group, there was a noticeable increase in both red (1.16 AU/g CDW) and orange pigments (1.12 AU/g CDW), followed by yellow pigment yield (1.04 AU/g CDW). This pattern suggests a balanced occurrence of amination and reduction,

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leading to similar levels of red and orange pigments, with a slight preference for the amination process as evidenced by the slightly higher red pigment production.





Fig. 4.16. Correlation between pH, Pigment Production, and Carbohydrate Consumption in 2.5 L Stirred Tank Fermentation between different Bioreactor Groups, namely a) Control (C), b) Oligoguluronate (OG), c) Oligomannuronate (OM) and d) Mannan Oligosaccharides (MO).

The data, therefore, reveals a consistent correlation between pH levels, carbohydrate consumption, and pigment production across all bioreactor groups (C, OG, OM, MO). It is noteworthy that the presence of elicitors (OG, OM, and MO) induced the highest pigment production, suggesting the efficacy of the mentioned elicitors, especially OM, in enhancing pigment biosynthesis in *M. purpureus* C322 in small-scale shake-flasks as well as larger-scale 2.5 L stirred tank fermentation.

4.2.2.8 Effect of Elicitation on Lovastatin Production during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

At the end of the 2.5 L stirred tank fermentation, lovastatin concentrations were measured for each bioreactor group (Fig. 4.17). In the C group, the concentration reached 36.93 mg/L. The addition of OG notably increased lovastatin production to 84.66 mg/L, while the OM group showed an even greater enhancement, reaching 98.29 mg/L. MO elicitation also improved lovastatin concentration, yielding 67.04 mg/L by Day-5. These results highlight the potential of all three elicitors, particularly OM and OG, in promoting lovastatin biosynthesis in *M. purpureus* C322 during 2.5 L stirred tank fermentation. Appendix Figure 8 presents the HPLC chromatograms, showing the characteristic peaks corresponding to lovastatin in each bioreactor group (C, OG, OM, MO). One-way ANOVA confirmed a statistically significant difference in lovastatin production across the bioreactor groups (*p*-value<0.01). According to Tukey's post hoc test, the OM group produced significantly more lovastatin than all other groups. OG also showed significantly higher production than MO and the control, while MO differed significantly from the control, confirming distinct differences between all treatments (*p*-value<0.01; Appendix–Table 19).



Fig. 4.17. Lovastatin concentration in different bioreactor groups on Day-5 of fermentation: C, OG, OM, and MO. Each 2.5 L bioreactor contained 1,500 mL of growth medium and 150 mL of seed culture, incubated at 32°C with 300 rpm agitation. Each elicitor (OG, OM, MO) was added at a concentration of 150 mg/L at 24 h of fermentation. Error bars denote standard deviation within the sets of experiments (*p*-value <0.01).

Table 4.8 compares lovastatin yield and the scaling-up impact across various bioreactor groups (C, OG, OM, and MO) during 2.5 L Bioreactor Fermentation. The estimated lovastatin yield in control group was 5.27 mg/g CDW. Compared to the control group, lovastatin yield in the oligoguluronate (OG) group increased to 11.29 mg/g CDW, corresponding to a fold increase of 2.14 after transitioning from shaken flasks to bioreactors (F/SF). Similarly, the oligomannuronate (OM) group exhibited an increased lovastatin yield of 12.6 mg/g CDW with a fold increase 2.39, and the mannan oligosaccharides (MO) group yielded 9.58 mg/g CDW with a fold increase of 1.82. These results highlight the potential of elicitors, particularly OG and OM, in enhancing lovastatin production, as evidenced by the increased lovastatin yield and the positive impact of upscaling from shaken flasks to bioreactors.

Bioreactor Group	Lovastatin Yield- Bioreactors (mg/g CDW)	Fold Increase w.r.t Control Bioreactor	Lovastatin Yield- Shaken Flasks (mg/g CDW)	Fold Increase in Yield: F/SF
Control (C)	5.27	-	5.72	0.92
Oligoguluronate (OG)	11.29	2.14	10.46	1.08
Oligomannuronate (OM)	12.6	2.39	12.11	1.04
Mannan Oligosaccharides (MO)	9.58	1.82	9.41	1.02

Table 4.8. Comparison of Lovastatin Yield and Impact of Upscaling across different Bioreactor Groups (C, OG, OM, MO) during 2.5 L Bioreactor Fermentation.

In the current study, the transition from shake-flask experiments to fermenter-based processes has demonstrated consistent outcomes in terms of lovastatin yield within *Monascus* cultures, particularly when elicited with carbohydrate elicitors, namely OG, OM, and MO. This consistency in lovastatin production is a crucial finding, as it validates the scalability of the process from a laboratory setting to a more industrially relevant environment. The successful upscaling of lovastatin production in *M. purpureus* C322 aligns with findings from previous studies (Seenivasan et al., 2008; Lia et al., 2004; Mohan-Kumari et al., 2012; Mukhtar et al., 2014). Several reports have indicated that the production of lovastatin in filamentous fungi such as *Monascus* and *Aspergillus* can be influenced by the scale of cultivation, with various parameters needing careful optimisation to maintain yield (Xu et al., 2005; Zhang et al., 2014; Dikshit and Tallapragada, 2015; Liu et al., 2022). In this context, the ability of the current study to maintain lovastatin yields following scale-up is particularly noteworthy. The application of carbohydrate elicitors in this context is particularly significant. While the literature on using specific elicitors like OG, OM, and MO in *Monascus* cultures is limited, the concept of using elicitors to enhance lovastatin production has been reported (Zhang et al., 2014; Suraiya et al., 2018; Wu et al., 2023). The ability to maintain increased lovastatin production during scale-up

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supports the applicability of elicitor-based strategies in larger-scale fermentations of *M. purpureus* C322.

4.3 Summary

The elicitation experiment conducted in both shaken flasks and 2.5 L stirred tank fermenters has provided valuable insights into the modulation of pigment production and lovastatin yield in *M. purpureus* C322. The results demonstrated that the addition of elicitors (OG, OM, and MO) notably enhanced the production of yellow, orange, and red pigments, as well as lovastatin. These enhancements were evident in both shaken flask and 2.5 L stirred tank fermentation where groups supplemented with specific elicitors - OG, OM, and MO consistently exhibited higher pigment yields compared to the control group, indicating the scalability and reproducibility of the elicitation. In both small-scale shaken flasks and largescale 2.5 L stirred tank fermenters, both pigment (Yellow, Orange, and Red) and lovastatin were upscaled successfully. The highest yellow and red pigments were produced in the OM group, followed closely by the OG group and then the MO group; whereas the highest orange pigment was produced by the OG group, followed by OM and MO groups. The C groups in both shaken flasks and 2.5 L stirred tank bioreactors produced the lowest pigment (Yellow, Orange, and Red) and lovastatin yield. The highest lovastatin was produced by OM group followed by OG, MO and lastly by C groups. Overall, this experiment underscores the potential of elicitation as a bioprocess optimisation for augmenting pigment production and lovastatin yields in M. purpureus C322.

Chapter V

Enhancing Production of *M. purpureus* C322 Lovastatin and Pigments via Quorum Sensing



Chapter V

Quorum Sensing

5.1 Introduction

This chapter presents the outcomes of the quorum sensing (QS) experiments carried out using specific quorum sensing molecules (QSM), namely butyrolactone I (B), tyrosol (T), farnesol (F), and linoleic acid (LA), on pigment and lovastatin production in liquid cultures of *M. purpureus* C322. Quorum sensing has shown promising results in enhancing secondary metabolite production in microbial cultures including fungi. Therefore, QS has gained popularity as a cost-effective method for enhancing gene expression and controlling various activities like secondary metabolite production, biofilm formation, sporulation, and more in microorganisms, including filamentous fungi (Choudhary and Dannert, 2010).

A series of experiments were conducted in shaken flasks to assess the effects of quorum sensing molecules (QSMs) on pigment (Yellow, Red, and Orange) and lovastatin production of *M. purpureus* C322. These experiments, referred to as Shaken Flasks Run I, II, and III, were sequentially tailored based on the outcomes of each prior run to refine the conditions and concentrations of QSMs for optimal results in terms of enhancing pigment and lovastatin production. To ensure the reliability and reproducibility of results, two sets of experiments were conducted for Shaken Flask Run I and II, and three sets for Shaken Flask Run III (considered the final experiment). Each run consisted of three flasks per group to validate consistency across replicates. Shaken Flasks Run I was supplemented with 1% DMSO and QSMs: 100 nM B, 10 mM T, and 10 mM F introduced at 0 H. Since F was diluted in DMSO, it was important to first analyse whether DMSO alone affected *M. purpureus* C322 cultures. Shaken Flasks Run II included supplementation with 1 mM F, 1 mM T, 150 nM B, and 3.57 mM LA at 48 h. Shaken Flasks Run III featured QSM concentrations adjusted to 0.3 mM T, 0.5 mM T, 0.2 mM Farnesol (F), 300 nM B, and 0.4 mM LA introduced at 48 h. These

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sequential adjustments aimed to optimise pigment and lovastatin production by refining the concentrations and timing of QSM additions.

For shaken flasks, 500 mL Erlenmeyer flasks containing 100 mL of inoculated growth medium were used and incubated at 25 °C and 120 rpm for 18 days. pH, pigment production, and carbohydrate consumption were monitored regularly, and biomass levels and lovastatin concentration were assessed at the end of fermentation. Following shaken flask experiments, the study progressed to upscaling in 2.5 L stirred tank fermenters. Similar to shaken flasks, three experiments were conducted for confirmation, with bioreactors containing 1,350 mL of growth medium supplemented with 150 mL of seed culture. The fermenters were maintained at 32 °C and 300 rpm. The fermenters were supplemented with 0.3 mM T, 0.2 mM F, and 0.4 mM LA. This transition from small-scale to large-scale cultivation provided a broader perspective for the investigation.

Results and Discussion

Shaken Flask Fermentation

5.2 Results and Discussion

5.2.1 Quorum Sensing in Shaken Flasks

This section highlights the outcome of the effects of quorum sensing using quorum sensing molecules (butyrolactone I, tyrosol, farnesol, linoleic acid) on biomass and production of secondary metabolites namely lovastatin and pigments (Yellow, Red, and Orange) in shaken flasks. The flasks were incubated at 25 °C and 120 rpm, and the pH of the medium was 6.5. Each experiment was performed with three flasks per group, and error bars were applied to all plots based on the standard deviation of each sample set.

5.2.1.1 Effect of Quorum Sensing on Growth and Morphology during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The impact of various quorum sensing molecules, including butyrolactone-I and farnesol, as well as the solvent dimethyl sulfoxide (DMSO), on the growth and morphology of *M. purpureus* C322 cultures was examined. The initial investigation aimed to determine whether the presence of 1% DMSO (CD) had any independent effect on *M. purpureus* C322, given that F was diluted in this solvent. Results indicated that neither B nor F induced any noticeable changes in the growth or morphology of the cultures when compared to the C samples. Similarly, the addition of CD did not yield any discernible alterations.

In contrast, introducing 10 mM T had a marked impact on the cultures. Notably, samples demonstrated accelerated growth kinetics, with the *M. purpureus* C322 culture reaching full maturity (4-5 mm) by Day-10 of the experiment (Fig. 5.1 a), whereas the control samples formed small pellets by Day-10 (1-2 mm). Additionally, a noticeable colour change was observed in flasks supplemented with 10 mM T, with the cultures beginning to develop a subtle brownish tint from Day-13 onwards (Fig. 5.1 c). This pigmentation intensified progressively, leading to a complete browning of the culture by Day-18 (Fig. 5.1 e, f). In contrast, C flasks

exhibited deep pigmentation by Day-14 of fermentation (Fig. 5.1 d), and by Day-18, the C samples had formed mature pellets (4-5 mm) (Fig. 5.1 g, h).





Fig. 5.1. Visual observations of QS experiment during shaken flask fermentation, where a) Flask with 10 mM tyrosol on Day-9 of fermentation; b) Control flasks on Day-9 of fermentation; c) Flask with 10 mM tyrosol on Day-14 of fermentation; d) Control flasks on Day-14 of fermentation; e) and f) Flask with 10 mM tyrosol on Day-18 of fermentation; g) and h) Control flasks on Day-18 of fermentation.

Regarding morphology, changes were observed in the hyphal structure. Specifically, the hyphae appeared elongated, with notably fewer branching patterns observed (Fig. 5.2). Upon reducing the T concentration to 5 mM and beyond, no discernible differences were noted in either the growth pattern or the morphology when compared to the control samples. Multiple studies have demonstrated the impact of tyrosol on the growth and morphology of filamentous fungi (Chen et al., 2004; Nickerson et al., 2006; Albuquerque and Casadevall; 2012; Jakab et al., 2019). For example, exogenous addition of 20 μ M tyrosol during early stages of biofilm formation in *C. albicans* GDH 2346 stimulating germ tube and hypha formation between 1-6 h of inoculation (Alem et al., 2006). Additionally, research has indicated that tyrosol can influence the augmentation of fungal cell density (Chen et al., 2004; Alem et al., 2006).





Fig. 5.2. Microscopic observations of QS experiment during shaken flask fermentation, where a) and b) Control samples at 4x and 10x objective magnifications; c), d) and e) Flask with 10 mM tyrosol at 4x, 10x, and 40x objective magnification on Day-14 of fermentation; f) and g) Flask with 10 mM tyrosol at 4x objective magnification on Day-18 of fermentation.

5.2.1.2 Effect of Quorum Sensing on pH of the Culture during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shaken Flasks Run I

In the control flasks, the pH began to decline from Day-3 onwards, reaching 5.6 on Day-13. Thereafter, the pH steadily increased, reaching 6.7 by Day-18. A similar trend was observed in the flasks supplemented with 1% DMSO (CD), where the pH dropped to 5.7 on Day-13, followed by a gradual increase to 6.7 by Day-18. In the flasks treated with B (100 nM), the pH

also decreased gradually, reaching 5.7 on Day-13, and subsequently increased to 6.8 by the end of fermentation. The flasks supplemented with T (10 mM) exhibited a more rapid decline in pH, reaching 6.1 on Day-9, before rising to 6.8 by Day-18. The flasks treated with F (10 mM) showed negligible pH variation due to a lack of microbial growth (Fig. 5.3). Statistical analysis revealed no significant difference in pH variation between the control, B, and CD flasks (*p*-value>0.05) (Appendix–Table 20). However, flasks supplemented with T (10 mM) demonstrated a statistically significant deviation in pH trend when compared to the C (*p*-value<0.05) suggesting a possible concentration-dependent effect on growth (Appendix– Table 21) rather than direct modulation of acid production, as similar effects have not been reported in previous studies (Chen & Fink, 2006; Raina, 2008; Sorrentino, 2009; Amache, 2014).



Fig. 5.3. pH variation during QS optimisation in shaken flasks. Five different treatments were evaluated: the control (C), 1% DMSO (CD), 100 nM butyrolactone I (B), 10 mM tyrosol (T), and 10 mM Farnesol (F). Each experiment utilised a 500 mL Erlenmeyer flask with 100 mL of growth medium, inoculated with a concentration of 10⁶ spores/mL, and incubated at 25°C at a shaking speed of 120 rpm. Data points in the graph are average values of two sets of experiments from Day-1 to Day-18 of fermentation, and error bars show the standard deviation. Each quorum sensing molecule (B, T, F) and CD were introduced at 0 h.

B) Shaken Flasks- Run II

The minimum pH value observed in the C flasks was 5.9 on Day-13. Subsequently, the pH began to increase, reaching 7.0 by Day-18. In flasks supplemented with B (150 nM), the pH decreased from Day-3 onwards, reaching a minimum of 5.9 on Day-13. From Day-14 to Day-18, the pH consistently increased, reaching 6.9 on Day-18. The flasks supplemented with T (1 mM) also exhibited a decline in pH, reaching a minimum of 5.8 on Day-13. The pH started to increase after Day-14 and reached 7.01 on Day-18. The flasks with F (1 mM) and LA (3.57 mM) showed negligible pH variation, as no growth was observed (Fig. 5.4). There was no significant difference in pH variation between the control and the flasks supplemented with B and T (*p*-value>0.05) (Appendix–Table 22). This supports the interpretation that tyrosol's effect in Run I was growth-related and not a direct consequence of quorum sensing modulation.



Fig. 5.4. pH variation during QS optimisation in shaken flasks. The treatments assessed include control (C), 100 nM butyrolactone I (B), 1 mM tyrosol (T), 1 mM farnesol (F), and 3.57 mM linoleic acid (LA). Each experiment was conducted in a 500 mL Erlenmeyer flask containing 100 mL of growth medium, inoculated with a concentration of 10⁶ spores/mL, and maintained at 25°C with a shaking speed of 120 rpm. The data points on the graph represent the average values of two sets of experiments, with three samples per flask group, spanning from Day-1 to Day-18 of fermentation. Error bars indicate the standard deviation. Quorum sensing molecules (B, T, F, LA) were introduced at 48 h into each experiment.

C) Shaken Flasks-Run III

In the C flasks, the pH exhibited a declining trend starting from Day-3, reaching 6.0 on Day-13. Following this, a gradual increase was observed, with the pH reaching 6.9 by Day-18. For the flasks supplemented with B (300 nM), the minimum pH of 5.9 was recorded on Day-13. Subsequently, from Day-14 to Day-18, a consistent upward trend was observed, with the pH reaching 7.0 by Day-18. In the case of flasks treated with 0.3 mM tyrosol (T1), a decline in pH was observed, with the lowest value of 5.8 on Day-13. Following this decline, the pH increased steadily, reaching 7.1 by Day-18. Similarly, for flasks supplemented with 0.5 mM tyrosol (T2), the pH dropped to 5.9 on Day-13 and then gradually increased to 7.0 by Day-18 (Fig. 5.5).



Fig. 5.5. pH variation during quorum sensing experiment (Shaken Flasks-Run III) conducted in shaken flasks. The flask groups include control (C), 300 nM of butyrolactone I (B), two concentrations of tyrosol (0.3 mM - T1 and 0.5 mM - T2), 1 mM of farnesol (F), and 0.4 mM linoleic acid (LA). Each experiment was carried out in a 500 mL Erlenmeyer flask containing 100 mL of growth medium, with an initial inoculation concentration of 10^6 spores/mL. The flasks were maintained at a temperature of 25 °C and a shaking speed of 120 rpm throughout the fermentation period. Data points on the graph represent the average values obtained from two sets of experiments covering the duration from Day-1 to Day-18 of fermentation. Error bars are included to indicate the standard deviation. Quorum sensing molecules (B, T, F, LA) were introduced into each experiment 48 h after initiation (*p*-value<0.05).

Flasks supplemented with 0.2 mM F exhibited a similar pattern, with the lowest pH value of 5.8 recorded on Day-13. By the end of the fermentation period (Day-18), the pH had increased to 7.1. Similarly, in the flasks supplemented with 0.4 mM LA, the lowest pH of 5.9 was observed on Day-13, followed by a rise to pH 7.0 by Day-18. Statistical analysis revealed no significant difference in pH variation among the flask groups (*p*-value>0.05), as indicated in Appendix–Table 23. These results further confirm that quorum sensing molecules do not alter extracellular pH under the tested conditions. Minor variations in timing or amplitude of pH change were attributable to differences in growth patterns rather than any specific pH-regulating role of the QSMs. Similar findings have been reported in previous studies, where QSMs such as butyrolactone and linoleic acid showed no measurable impact on pH during fungal fermentations (Raina, 2008; Sorrentino, 2009; Amache, 2014).

5.2.1.3 Effect of Quorum Sensing on Biomass Concentration during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shaken Flasks-Run I

The initial quorum sensing experiment was conducted by testing three QSMs: T, F, and B. Figure 5.6 illustrates the average biomass, represented as total cell dry weight measured in grams per litre (g/L), at Day 18 of fermentation for various treatments including 1% DMSO (CD), and three different quorum sensing molecules (QSM): 10 mM T, 10 mM F, and 100 nM B and the C with no added QSM. The average biomass concentration in the C group on Day-18 was 4.75 g/L. Similarly, the flasks supplemented with CD and B showed similar biomass concentrations, i.e. 4.75 and 4.71, respectively on Day-18. On the other hand, the group treated with T showed an average biomass concentration of 4.17 g/L. The flask groups treated with F did not exhibit any growth. No significant difference between C, CD, and B flask groups was found (p-value>0.05) (Appendix-Table 24); whereas the difference between T and control flasks was significant (p-value<0.05) (Appendix-Table 25).



Fig. 5.6. Average biomass concentration (Total Cell Dry Weight) on Day-18 of fermentation for *M. purpureus* C322 cultures supplemented with B (100 mM), T (10 mM), and F (10 mM), alongside C and CD (1% DMSO) groups. Fermentation was conducted in 500 mL Erlenmeyer flasks, each containing 100 mL growth medium and inoculated with 10^6 spores/mL, at 25 °C and 120 rpm. Results are based on two sets of experiments performed in triplicates., with standard deviation depicted as error bars on the mean values (*p*-value>0.05). Each quorum sensing molecule (B, T, F) and CD was introduced 0 h into the fermentation process.

B) Shaken Flasks- Run II

Figure 5.7 depicts the average biomass concentration, quantified as total cell dry weight measured in grams per litre (g/L), at Day 18 of fermentation for various treatments, with QSM: 1 mM T, 1 mM F, and 150 nM B, alongside the C with no added QSM. In the C group, the average biomass concentration on Day 18 was 6.2 g/L. Similarly, the flasks supplemented with B and T exhibited comparable biomass concentrations, at 6.65 g/L and 6.15 g/L, respectively, on Day 18. Conversely, the group treated with F and LA displayed minimal growth, with average biomass reaching 0.7 g/L and 0.25 g/L, respectively. The difference in biomass concentration was not significant between C, B, and T flask groups (p-value>0.05) (Appendix-Table 26).



Fig. 5.7. The average biomass (total cell dry weight) at Day-18 of fermentation for *M. purpureus* C322 cultures with and without supplementation of quorum sensing molecules (B, T, F and LA). The fermentation process was conducted in 500 mL Erlenmeyer flasks, each containing 100 mL growth medium, and inoculated with 10^6 spores/mL. The flasks were maintained at 25°C and agitated at 120 rpm. The results are derived from two sets of experiments, each performed in triplicate, with error bars depicting the standard deviation on the mean values (*p*-value>0.05). Each quorum sensing molecule (B, T, F, LA) was introduced 48 h into the fermentation process.

C) Shaken Flasks- Run III

Figure 5.8 depicts the average biomass concentration (g/L), at Day 18 of fermentation for various QSM treatments, including: 0.3 mM T1, 0.5 mM T2, 0.2 mM F, 0.4 mM LA, and 300 nM B, alongside the C with no added QSM. For the C group, the average biomass concentration on Day 18 of fermentation was 5.35 g/L. Similarly, the flasks supplemented with B, T1, T2, F and LA exhibited comparable biomass concentrations, at 5.35 g/L, 5.44 g/L, 5.38 g/L, 5.33 g/L and 5.41 g/L, respectively, on Day 18, indicating that the difference in biomass was not significant between the flask groups (*p*-value>0.05) (Appendix-Table 27).

Research has shown that certain QSMs, especially tyrosol and farnesol, exert notable effects on the morphology and growth of microorganisms, particularly fungi. However, the impacts of butyrolactone and linoleic acid on morphology and biomass remain less explored. Nevertheless, all these QSMs are recognised for their involvement in regulating various cellular
processes that influence secondary metabolism in both bacteria and fungi. For example, tyrosol has been demonstrated to induce hyphal growth and modulate biofilm formation in fungi such as *C. albicans* and *A. fumigatus* (Hogan and Kolter, 2002; Rodrigues and Cernakova, 2020, Sauer et al., 2022). Additionally, tyrosol can influence the transition of yeast cells to filamentous forms in *C. albicans* (Sebaa et al., 2019; Marton et al., 2023).



Fig. 5.8. The average biomass concentration (total cell dry weight) at Day-18 of fermentation for *M. purpureus C322* cultures with and without supplementation of quorum sensing molecules (B, T, F and LA) introduced 48 h into the fermentation process. The fermentation process was conducted in 500 mL Erlenmeyer flasks, each containing 100 mL growth medium, and inoculated with 10^6 spores/mL. The flasks were maintained at 25° C and agitated at 120 rpm. The results are derived from two sets of experiments, each performed in triplicate, with error bars depicting the standard deviation on the mean values (*p*-value>0.05).

Similarly, farnesol is known to regulate the morphological transition of certain fungi (Ramage et al., 2001 and 2002). In *C. albicans*, farnesol acts as a quorum sensing molecule that inhibits the transition of yeast cells to filamentous forms, favouring instead the formation of biofilms. This modulation of cellular morphology can influence the proportion of yeast cells versus filamentous forms and the architecture of the biofilm, thereby potentially affecting biomass concentration. However, the effects of the QSMs may vary depending on factors such as their concentration, growth medium composition, and the specific strain being studied (Wall et al.,

2019; Hogan and Kolter, 2002). In this study, although an effect on morphology and growth rate was evident, especially when 10 mM T was used, no notable effect on overall biomass concentration was observed at the specified concentrations of QSMs.

5.2.1.4 Effect of Quorum Sensing on Yellow Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shake Flasks-Run I

In this investigation, the highest production of yellow pigments across all flask groups occurred between Days 12 and 14, followed by a stabilisation of production levels thereafter. The optical density (400nm) observed in C, CD, and B (100 nM) flasks exhibited similar trends. Although, the B flask group showed a similar trend to control group, overall the yellow pigment production was comparatively lower. The flasks supplemented with T (10 mM) demonstrated lower yellow pigment production, while those supplemented with F showed nearly negligible levels of yellow pigment production (Fig. 5.9). The OD values at 400 nm recorded from C, CD, and B flask groups were 3.0, 3.2, and 2.8 AU (Absorbance Units), respectively, on Day-14 of fermentation before reaching a plateau. Notably, the flask group supplemented with T (10 mM) displayed lower OD values compared to the control, CD, and B groups on most fermentation days, with the highest OD observed on Day-12, reaching 1.55 AU before stabilising. The variation in yellow pigment production between C and CD was not significant (p-value>0.05) (Appendix-Table 28); however, the variation between C and flask groups supplemented with B (100 nM) and T (10 mM) was significant (p-value<0.01) (Appendix-Table 29). The flasks supplemented with F (10 mM) had negligible growth and pigmentation. In terms of yellow pigment yield, C, CD, and B were similar and measured 0.63, 0.67, and 0.61 AU/g CDW respectively. The flasks supplemented with T yielded 0.35 AU/g CDW.



Fig. 5.9. The effect of different quorum sensing molecules (B, T, and F) and CD on yellow pigment production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars representing standard deviation.

The initial results obtained for the quorum sensing molecules (QSMs) were not favourable. At the tested concentrations of 10 mM for T and F, and 100 nM for B, yellow pigment production in the treated cultures was lower than in the control group. Notably, cultures supplemented with farnesol exhibited no growth or pigment production, whereas tyrosol-supplemented flasks demonstrated altered growth morphology and reduced pigmentation. These observations suggested potential cytotoxic effects of T and F at the concentrations employed. As for B, although growth was similar to C, pigmentation was slightly lower (C: 3.0 AU, B: 2.88 AU, OD at 400 nm), prompting an increase in the concentration of B to evaluate its potential for improvement.

B) Shaken Flasks-Run II

Based on the results from Shaken Flasks Run I, the concentrations selected for Run II were 150 nM B, 1 mM T, 1 mM F, and 3.57 mM LA, administered at 48 h. This experiment also included testing the effect of linoleic acid. The highest production of yellow pigments across all flask groups was measured on Day-14, followed by a stabilisation thereafter (Fig. 5.10). In terms of yellow pigment production, the optical density (400 nm) observed in C, B (150 nM) and T (1 mM) flasks exhibited similar trends. The OD values recorded on Day-14 from C, B, and T flask groups were 4.4, 4.1, and 3.88 AU, respectively. The yield was 0.71, 0.61, and 0.63 AU/g CDW respectively (Fig. 5.10). Although the trend was similar, the variation between yellow pigment production was significant between control, B, and T flask groups (*p*-value<0.05) (Appendix-Table 30).



Fig. 5.10. The impact of various quorum sensing molecules (B, T, F, and LA) on yellow pigment production in *M. purpureus* C322 cultures during shaken flask fermentation over an 18-day period. Each 500 mL Erlenmeyer flasks contained 100 mL of growth medium, inoculated with 10⁶ spores/mL, and incubated at 25°C under 120 rpm. Each quorum sensing molecule was introduced into the corresponding set of flasks 48 h into the fermentation process. Two sets of experiments were conducted, with each experiment performed in triplicate. The mean values are presented along with error bars representing the standard deviation.

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The flasks supplemented with F (1 mM) and LA (3.57 mM) did not show any noticeable pigmentation. However, the flasks treated with T displayed a marked improvement in pigmentation compared to earlier experiments, suggesting that the reduction in concentration was an appropriate adjustment. Similar to the current study, Kiziler et al. (2022) discovered that introducing the quorum sensing molecules (QSMs) farnesol and tyrosol to cultures of *Rhodotorula glutinis* and *Serratia marcencens* on Day-3, rather than Day-1, led to more substantial increases in pigment synthesis; thus, confirming that the timing of QSM addition plays a crucial role.

C) Shaken Flasks-Run III

Following Run II, the concentrations of QSMs were further reduced to 0.3 mM and 0.5 mM for tyrosol, 0.2 mM for farnesol, and 0.4 mM for linoleic acid, while the concentration of butyrolactone-I was increased to 300 nM. On Day-14 of fermentation, the C group reached a maximum yellow pigment optical density (OD at 400 nm) of 3.81 AU. The OD values recorded for the QSM-treated groups were 3.55 (B), 5.80 (T1), 3.90 (T2), 7.10 (F), and 4.39 AU (LA) (Fig. 5.11), with farnesol yielding the highest absorbance. One-way ANOVA confirmed that yellow pigment production differed significantly between flask groups (*p*-value<0.01). Tukey's post hoc test indicated that F (0.2 mM) significantly enhanced yellow pigment production compared to all other treatments, followed by T1 (0.3 mM), LA (0.4 mM), and T2 (0.5 mM), all of which yielded significantly more pigment than the control. In contrast, butyrolactone-I exhibited the lowest pigment level and did not significantly differ from the control group (*p*-value<0.01, Appendix–Table 31).

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Fig. 5.11. The influence of various quorum sensing molecules, namely B (300 nM), T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (4 mM), on yellow pigment production in *M. purpureus* C322 cultures during shaken flask fermentation over an 18-day period. The experimental setup comprised 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium, inoculated with 10^6 spores/mL, and maintained at 25° C with a shaking speed of 120 rpm. Each QSM was introduced into the corresponding set of flasks 48 h into the fermentation process. Three sets of experiments were conducted, with each experiment performed in triplicate. The mean values are presented along with error bars representing the standard deviation (*p*-value<0.01).

The yellow pigment yield (AU/g CDW) reflected these trends. As shown in Table 5.1, the control group produced 0.71 AU/g CDW. Among QSM-treated groups, farnesol supplementation resulted in the highest yield at 1.33 AU/g CDW, representing a 1.87-fold increase over the control. Following closely, the T1 (0.3 mM) group demonstrated a 1.51-fold increase in pigment yield, with a concentration of 1.07 AU/g CDW. In contrast, the T2 (0.5 mM) group exhibited a more modest increase, showing a 1.01-fold rise in pigment production. Linoleic acid (0.4 mM) produced 0.82 AU/g CDW (1.15-fold), and butyrolactone-I resulted in a marginal increase of 0.66 AU/g CDW (0.93-fold).

Flask Group (3 flasks/set)	Yellow Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.71	-
Butyrolactone-I – 300 nM (B)	0.66	0.92
Tyrosol - 0.3 mM (T1)	1.07	1.51
Tyrosol - 0.5 mM (T2)	0.72	1.01
Farnesol -0.2 mM (F)	1.33	1.87
Linoleic Acid – 0.4 mM (LA)	0.82	1.15

Table 5.1. Yellow pigment yield in different flask groups (C, B, T1, T2, F, LA) during shaken flask fermentation

These outcomes support the decision to adjust QSM concentrations in this phase, as pigment production was positively influenced by the optimised levels of tyrosol, farnesol, and linoleic acid. Similar trends were reported by Erkaya et al. (2020), where tyrosol and farnesol increased yellow pigment production by 3.16-fold in *M. purpureus* ATCC16365. While existing studies have demonstrated that γ -butyrolactone enhances *Monascus* pigment production, the current use of butyrolactone-I showed limited efficacy, likely reflecting molecular specificity or strain sensitivity (Liu and Chen, 2022; Shi et al., 2022). At present, the role of linoleic acid in *Monascus* pigment biosynthesis remains underexplored and warrants further investigation.

5.2.1.5 Effect of Quorum Sensing on Orange Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shaken Flasks-Run I

During this investigation, the highest production of orange pigment across all flask groups was observed between Days 12 and 14, followed by a stabilisation of production levels thereafter. Notably, the trends in orange pigment production were similar in the C, CD, and B (100 nM) flask groups, indicating similar patterns in pigment production. However, the B flask group exhibited higher orange pigment production compared to the control group. Conversely, the flasks supplemented with T (10 mM) displayed reduced levels of orange pigment, while those

supplemented with F showed minimal to negligible levels of pigment production (Fig. 5.12). The maximum OD values at 470 nm recorded from the C, CD, and B flask groups were 3.1, 3.0, and 3.4 AU, respectively, on Day 14 of fermentation before reaching a plateau. The flask group supplemented with T exhibited lower optical densities compared to the C, CD, and B groups on most fermentation days, with the highest concentration observed on Day 12, reaching 1.67 AU before stabilising. In terms of yellow pigment yield, the C and CD, demonstrated similar yields, measuring 0.65, and 0.63 AU/g CDW respectively, while B yielded 0.72 AU/g CDW. The flasks supplemented with T yielded a lower yield of 0.4 AU/g CDW. Statistical analysis revealed that the variation in orange pigment production between the C and CD groups was not significant (*p*-value > 0.05) (Appendix-Table 32). However, significant variations were observed between the control group and the flask groups supplemented with B (100 nM) and T (10 mM) (*p*-value < 0.01) (Appendix-Table 33). The flasks supplemented with F (10 mM) exhibited negligible growth and pigmentation.



Fig. 5.12. The effect of different quorum sensing molecules (B, T, and F) and CD on orange pigment production in *M. purpureus* C322 cultures in shaken flask fermentation conducted for 18 days. The setup involved 500 mL Erlenmeyer flasks each with 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of

experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars depicting standard deviation.

B) Shaken Flasks-Run II

The highest production of orange pigments across all flask groups was measured on Day-14, followed by a stabilisation thereafter (Fig. 5.13). In terms of orange pigment production, the optical density observed in C, B (150 nM) and T (1 mM) flasks exhibited similar trends. The OD values at 470 nm recorded from control, B, and T flask groups were 4.36, 4.27, and 4.15 AU, respectively, on Day-14 of fermentation before reaching a plateau. The yield was 0.70, 0.64, and 0.67 AU/g CDW respectively. Although the trend was similar, the variation between orange pigment production was significant between control, B, and T flask groups (*p*-value<0.05) (Appendix-Table 34). The flasks supplemented with F (1 mM) and LA (3.57 mM) did not show any noticeable pigmentation.



Fig. 5.13. The effect of different quorum sensing molecules (B, T F, and LA) on orange pigment production in *M. purpureus* C322 cultures was investigated during shaken flask fermentation over an 18-day period. The experimental setup comprised 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium and inoculated with 10⁶ spores/mL. The flasks were maintained at 25 °C with a shaking speed of 120 rpm throughout the fermentation process. Each QSM was introduced into the corresponding set of flasks 48 h into the fermentation process. Two sets of experiments were conducted, with each experiment

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performed in triplicate. The mean values are presented, along with error bars representing the standard deviation

C) Shaken Flasks- Run III

In the C group, the maximum orange pigment optical density (OD at 470 nm) reached 3.77 AU on Day-14. For the QSM-treated groups, the OD values were 3.88 (B 300 nM), 6.10 (T1 0.3 mM), 4.50 (T2 0.5 mM), 8.00 (F 0.2 mM), and 4.92 AU (LA 0.4mM) (Fig. 5.14). Among all treatments, farnesol (0.2 mM) produced the most pronounced increase in orange pigment accumulation, followed by T1, LA, T2, and B. Statistical analysis using one-way ANOVA revealed a significant variation in orange pigment production among the flask groups (*p*-value<0.01). Post hoc analysis (Tukey's test) demonstrated that all QSM-supplemented groups differed significantly from the control, with farnesol outperforming all other treatments. T1 also resulted in a greater pigment level than T2, LA, and B. Meanwhile, B did not substantially differ from the control (Appendix–Table 35).



Fig. 5.14. The impact of different quorum sensing molecules, including B (300 nM), T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (0.4 mM), on orange pigment production in *M. purpureus* C322 cultures was examined during shaken flask fermentation over an 18-day duration. The experimental setup consisted of 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium, inoculated with 10^6 spores/mL, and incubated at 25°C with a shaking

speed of 120 rpm. Introduction of each quorum sensing molecule occurred 48 h into the fermentation process in the respective flask sets. The experiments were replicated three times, with each experiment performed in triplicate. Mean values are depicted with error bars indicating the standard deviation (*p*-value < 0.01).

The yield data (AU/g CDW) were consistent with the OD results. As shown in Table 5.2, the orange pigment yield (AU/g CDW) across the flask groups. The control group yielded 0.70 AU/g CDW, while the F group exhibited the highest yield at 1.50 AU/g CDW, corresponding to a 2.14-fold increase. The T1 group showed a 1.60-fold increase, yielding 1.12 AU/g CDW. In comparison, the T2 group produced 0.84 AU/g CDW, indicating a 1.20-fold increase. LA supplementation resulted in a moderate enhancement (0.91 AU/g CDW; 1.30-fold), whereas B yielded 0.73 AU/g CDW (1.04-fold).

Table 5.2. Orange pigment yield	in different flask groups (C, B, T1, T2, F	F, LA) during shaken
flask fermentation			

Flask Group (3 flasks/set)	Orange Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.7	-
Butyrolactone-I – 300 nM (B)	0.73	1.04
Tyrosol – 0.3 mM (T1)	1.12	1.6
Tyrosol – 0.5 mM (T2)	0.84	1.2
Farnesol – 0.2 mM (F)	1.5	2.14
Linoleic Acid – 0.4 mM (LA)	0.91	1.3

These findings confirm the strong stimulatory effect of farnesol and, to a lesser extent, tyrosol on orange pigment production in *M. purpureus* C322. Comparable results were reported by Erkaya et al. (2020), where the addition of tyrosol and farnesol led to a 2.68-fold increase in orange pigment in *M. purpureus* ATCC16365. Additionally, Shi et al. (2022) noted increases of 56.60% (red), 46.26% (orange), and 31.34% (yellow) in *M. purpureus* M1 with γ -butyrolactone supplementation at 25 µmol/L. However, the present study employed

butyrolactone-I (300 nM), which yielded only a marginal improvement in orange pigment production, suggesting potential specificity differences. The modest effect of LA highlights its possible secondary influence on *Monascus* pigment biosynthesis.

5.2.1.6 Effect of Quorum Sensing on Red Pigment Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shaken Flasks-Run I

In this study, the highest production of red pigments across all flask groups was observed between Days 12 and 14 depending on the flask group, followed by stabilisation. Regarding red pigment production, the OD observed in the C, CD, and B (100 nM) flask groups exhibited similar trends. However, the B flask group showed slightly lower overall red pigment production compared to the C group (Fig. 5.15). The OD values at 510 nm recorded from the C, CD, and B flask groups were 3.0, 3.0, and 2.99 AU, respectively, on Day 14 of fermentation before reaching a plateau. Notably, the flask group supplemented with T (10 mM) displayed lower optical densities compared to the C, CD, and B groups on most fermentation days, with the highest concentration observed on Day 12, reaching 1.57 before stabilising. The variation in red pigment production between the C, CD, and B groups was not significant (*p*-value>0.05) (Appendix-Table 36); however, the variation between the control and flask groups supplemented with T (10 mM) was significant (*p*-value<0.01) (Appendix-Table 37). The flasks supplemented not pigment with F (10 mM) exhibited negligible growth and pigmentation. In terms of red pigment yield, the C, CD, and B groups were similar and measured 0.64, 0.63, and 0.63 AU/g CDW, respectively. The flasks supplemented with T yielded 0.37 AU/g CDW.



Fig. 5.15. The effect of different quorum sensing molecules (B, T, and F) and CD on red pigment production in *M. purpureus*_C322 cultures in shaken flask fermentation conducted for 18 days. Each 500 mL Erlenmeyer flasks contained 100 mL growth medium, inoculated with 10⁶ spores/mL and incubated at 25°C with a shaking speed of 120 rpm. Each QSM and CD were added to the respective set of flasks at 0 h of fermentation. Two sets of experiments were performed, and each experiment was performed in triplicates. The mean values were presented with error bars representing standard deviation.

B) Shaken Flasks- Run II

The highest production of red pigments across all flask groups was measured on Day-14, followed by a stabilisation thereafter (Fig. 5.16). The optical density at 510 nm observed in C, B (150 nM) and T (1 mM) flasks exhibited similar trends. The OD values for C, B, and T flask groups were 4.33, 4.1, and 3.96 AU, respectively. The yield was 0.70, 0.62, and 0.64 AU/g CDW respectively. Although the trend was similar, the variation between red pigment production was significant between C, B, and T flask groups (*p*-value<0.05) (Appendix-Table 38). The flasks supplemented with F (1 mM) and LA (3.57 mM) did not show any noticeable pigmentation.



Fig. 5.16. The effect of different quorum sensing molecules (B, T, F, and LA) on red pigment production in *M. purpureus* C322 cultures was investigated during shaken flask fermentation over an 18-day period. The experimental setup comprised 500 mL Erlenmeyer flasks, each containing 100 mL of growth medium and inoculated with 10⁶ spores/mL. The flasks were maintained at 25 °C with a shaking speed of 120 rpm throughout the fermentation process. Each QSM was introduced into the corresponding set of flasks 48 h into the fermentation process. Two sets of experiments were conducted, with each experiment performed in triplicate. The mean values are presented, along with error bars representing the standard deviation.

C) Shaken Flasks-Run III

The C group reached a maximum optical density (OD at 510 nm) of 3.75 AU on Day-14 before stabilising. In terms of flasks supplemented with quorum sensing molecules, B (300 nM), T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (0.4 mM), the red pigment production on Day-14 of fermentation displayed an OD of 3.71, 5.93, 4.1, 7.8, and 5.0 AU, respectively (Fig. 5.17). The highest red pigment was produced by F supplemented flask group followed by T1, LA, T2, and lastly B. One-way ANOVA indicated that red pigment production differed significantly between the groups (*p*-value<0.01). Post hoc comparisons confirmed that F significantly outperformed all other treatments, while T1 also resulted in notably higher

pigment levels than its T2 counterpart, LA, B, and C. Butyrolactone-I did not show a statistically significant difference compared to the control group (Appendix–Table 37).



Fig. 5.17. The influence of various quorum sensing molecules, including B (300 nM), T1 (0.3 mM), T2 (0.5 mM), F (0.2 mM), and LA (0.4 mM), on red pigment production in *M. purpureus* C322 cultures during shaken flask fermentation spanning an 18-day period. Each 500 mL Erlenmeyer flasks contained 100 mL of growth medium inoculated with 10^6 spores/mL, and maintained at 25°C with a shaking speed of 120 rpm. The QSMs were introduced into the corresponding flask sets 48 hours into the fermentation process. The experiments were replicated three times, with each experiment performed in triplicate. Mean values were presented with error bars indicating the standard deviation (*p*-value < 0.01).

The quantitative yield data (AU/g CDW) reflected these trends. As shown in Table 5.3, the C group yielded 0.71 AU/g CDW of red pigment. The highest yield was observed in the F group, at 1.46 AU/g CDW, representing a 2.09-fold increase over the control. Tyrosol at 0.3 mM produced a yield of 1.10 AU/g CDW (1.57-fold increase), while the 0.5 mM concentration yielded 0.76 AU/g CDW (1.09-fold). LA resulted in a moderate yield of 0.92 AU/g CDW (1.31-fold), and B produced 0.69 AU/g CDW (0.99-fold), essentially comparable to the control. These results underscore the superior enhancing effect of farnesol on red pigment biosynthesis in *M. purpureus* C322 under the tested conditions. Supporting findings were reported by

Erkaya et al. (2020), who observed a 2.87-fold increase in red pigment in the presence of farnesol and tyrosol in *M. purpureus* ATCC16365. Similarly, Liu and Chen (2022) and Shi et al. (2022) reported increases in *Monascus* pigments following γ -butyrolactone supplementation at 25 µmol/L. However, in this study, butyrolactone-I at 300 nM showed no meaningful enhancement, potentially pointing to differences in the structural specificity or bioactivity of γ -butyrolactone analogues in *Monascus* cultures.

Table 5.3. Red pigment yield in different flask groups (C, B, T1, T2, F, LA) during shaken flask fermentation

Flask Group (3 flasks/set)	Red Pigment Yield (AU/g CDW)	Fold Increase w.r.t Control
Control (C)	0.70	-
Butyrolactone-I – 300 nM (B)	0.69	0.99
Tyrosol – 0.3 mM (T1)	1.10	1.57
Tyrosol – 0.5 mM (T2)	0.76	1.09
Farnesol – 0.2 mM (F)	1.46	2.09
Linoleic Acid – 0.4 mM (LA)	0.92	1.31

5.2.1.7 Impact of Quorum Sensing on Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

A) Shaken Flasks-Run I

Figure 5.18 illustrates the difference in carbohydrate consumption across the flask groups (C, CD, B, T, and F) throughout the fermentation period. Carbohydrate concentrations are expressed in grams per litre (g/L) from Day 1 to Day 18. By Day 18, the final carbohydrate concentration in the C group was 6.21 g/L. The QSM-supplemented groups exhibited similar consumption patterns to the control. On Day 18, the final concentrations for groups CD, B, and T were 6.08, 6.14, and 5.46 g/L, respectively. In contrast, the F-supplemented group showed negligible carbohydrate utilisation due to lack of growth, with a concentration of 19.32 g/L at

Day 18. The standard glucose curve (Appendix–Fig. 4) was used to estimate carbohydrate concentrations in all flask groups.



Fig. 5.18. Carbohydrate consumption in different flask groups (C, CD, B, T, F) throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. Each 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10⁶ spores/mL prior to incubation at 25 °C under 120 rpm. Each QSM was added to the respective flask group at 0 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values.

The carbohydrate consumption rates for all flask groups (C, CD, B, T) are 0.73, 0.73, 0.73, and 0.77 g/L/day respectively. Statistical analysis reveals that the difference in carbohydrate consumption between the control and QSM-supplemented flask groups (C, CD, B) is not statistically significant (*p*-value > 0.05) (Appendix- Table 40). However, a significant variation in carbohydrate consumption is observed between the C and T flask groups (*p*-value < 0.01), indicating a distinct effect of T supplementation on carbohydrate utilisation (Appendix-Table 41). This may be attributed to earlier growth initiation or increased metabolic activity rather than a quorum sensing-mediated enhancement of sugar uptake. Tyrosol has previously been associated with altered growth dynamics and morphological transitions in fungi such as

C. albicans (Sebaa et al., 2019; Rodrigues & Cernakova, 2020), which may explain the shift in consumption pattern even though the endpoint was comparable to the other groups.

B) Shaken Flasks-Run II

Figure 5.19 illustrates the patterns of carbohydrate consumption across the flask groups (C, B, T, F, and LA) throughout the fermentation period. By Day-18, the final carbohydrate concentration in the C group was 6.11 g/L. The groups supplemented with B and T exhibited comparable consumption patterns to the control, with final concentrations of 6.14 g/L and 6.08 g/L, respectively. In contrast, the flasks treated with F and LA displayed negligible carbohydrate utilisation due to an absence of growth, maintaining concentrations of 19.3 g/L at Day-18.



Fig. 5.19. Carbohydrate consumption in different flask groups (C, B, T, F, LA) throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. Each 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10⁶ spores/mL prior to incubation at 25 °C under 120 rpm. The QSMs were added to the respective flask group at 48 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values.

The carbohydrate consumption values in the flask groups are comparable to the standard glucose curve in terms of decreasing carbohydrate consumption with decreasing glucose concentration. In all flask groups, the carbohydrate consumption rates for all flask groups (C,

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B, and T) are 0.74, 0.73, and 0.74 g/L/day respectively. The difference in carbohydrate consumption between the flask groups (C, B, T) is not statistically significant (*p*-value>0.05) (Appendix- Table 42). This further supports the notion that these QSMs do not influence carbohydrate metabolism in *M. purpureus* C322, as previously reported in other *Monascus* and *Aspergillus* systems (Raina, 2008; Amache, 2014).

C) Shaken Flasks-Run III

Figure 5.20 presents the dynamics of carbohydrate consumption across different flask groups (C, B, T1, T2, F, and LA) throughout the fermentation process. On Day-18, the carbohydrate concentration in the C group was 6.1 g/L. Similarly, the final concentrations for the B, T1, T2, F, and LA groups were 5.94, 6.1, 5.98, 6.11, and 6.02 g/L, respectively, reflecting comparable consumption trends to the C. These findings indicate that the presence of quorum sensing molecules (QSMs) did not significantly influence carbohydrate utilisation during fermentation (*p*-values>0.05) (Appendix–Table 43).

The observed carbohydrate consumption values in all flask groups align with the expected trend of decreasing carbohydrate consumption as glucose concentration decreases over time. This is consistent with the standard glucose curve (Appendix-Table 4). In all flask groups, the carbohydrate consumption rates for all flask groups (C, B, T1, T2, F, and LA) are 0.74, 0.75, 0.74, 0.74, 0.74, and 0.74 g/L/day respectively. Despite the presence of different QSMs, carbohydrate consumption followed similar trends across all active cultures, suggesting that these molecules did not affect primary substrate utilisation under the tested conditions. The similarity in consumption rates implies that carbohydrate uptake was more closely linked to biomass development rather than directly influenced by quorum sensing signals. This observation is consistent with earlier studies in submerged fungal fermentations, where carbohydrate utilisation remained unaffected by QSM supplementation (Raina, 2008; Sorrentino, 2009; Amache, 2014).

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Fig. 5.20. Carbohydrate consumption in different flask groups (C, B, T, F, LA) throughout the fermentation process in *M. purpureus* C322 cultures in shaken flask fermentation. The experimental setup comprised of 500 mL Erlenmeyer flask containing 100 mL growth medium was incubated with 10^6 spores/mL prior to incubation at 25 °C under 120 rpm. The QSMs were added to the respective flask group at 48 h of fermentation. Two sets of experiments were performed in triplicates, and error bars representing the standard deviation were applied to the mean values (*p*-values>0.05).

5.2.2.8 Comparison of pH, Pigment Production, and Carbohydrate Consumption during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The data presented in Figure 5.21, is derived from three sets of Shaken Flasks-Run III, and provides a comprehensive overview of the correlation between pH levels, carbohydrate consumption, and pigment (yellow, orange, and red) production across different flask groups (C, B, T1, T2, F, LA) over a span of eighteen days. Similar to elicitation experiments, the data in the quorum sensing experiments reflected a gradual decline in pH, which corresponded with an increase in pigment production and carbohydrate consumption.

It is evident that all flask groups exhibit an increase in yellow, orange, and red pigments as the fermentation progressed from D1 to D18; however, the flasks supplemented with QSMs (T1, F, and LA) resulted in a higher pigment production in comparison to the C group. The

B (300 nM) and T2 (0.5 mM) flask groups showed similar results in terms of pigment production to that of the control group. Interestingly, the F (0.2 mM) group demonstrates the highest pigment (yellow, orange, red) production, followed by the T1 (0.03 mM), and LA (0.4 mM). The T2, B, and C groups displayed similar results in terms of pigment production.







Fig. 5.21. Correlation between pH, Pigment Production, and Carbohydrate Consumption in Shaken Flask Fermentation between different Flask Groups, namely a) control (C), b) butyrolactone-I 300 nM (B), c) tyrosol 0.3 mM (T1), d) tyrosol 0.5 mM (T2), e) farnesol 0.2 mM (F), and f) 0.4 mM linoleic acid (LA).

Similar to elicitation experiments, the trend observed in the C group was not always consistent; however, the addition of QSMs consistently triggered more predictable outcomes. In general, the C group, displayed higher yellow pigment than orange pigments and yellow pigments, suggesting predominant reduction reactions. For details on the reduction and amination reactions, refer to Chapter I, Section 1.4.5. In this case, the control group yielding 0.71 AU/g CDW which was followed by orange and red pigments, yielded 0.7 AU/g CDW. The flasks with QSMs, B, T, and F all showed predominant orange pigment yield (B-0.73 AU/g CDW, T1-1.12 AU/g CDW, T2-0.84 AU/g CDW, F-1.5 AU/g CDW) followed by red (B-0.69 AU/g CDW, T1-1.1 AU/g CDW, T2-0.76 AU/g CDW, F-1.46 AU/g CDW) and yellow (B-0.66 AU/g CDW, T1-1.07 AU/g CDW, T2-0.72 AU/g CDW, F-1.33 AU/g CDW) pigment. However, flasks with LA showed higher red (1.31 AU/g CDW) pigment yield followed by orange (1.3 AU/g CDW) pigment and lastly by yellow pigment (0.81 AU/g CDW), suggesting more amination reactions than reduction reactions. The data, therefore, reveals a consistent correlation between pH levels, carbohydrate consumption, and pigment production across all flask groups (C, B, T1, T2, F, LA). It is noteworthy that the presence of OSMs (T, F, LA) induced increased pigment production, in *M. purpureus* C322 with F inducing the highest pigmentation.

5.2.1.9 Impact of Quorum Sensing on Lovastatin Production during Submerged Fermentation of *M. purpureus* C322 in Shaken Flasks

The results from shaken flasks provide preliminary insights into the conditions favouring lovastatin biosynthesis. QSMs (T, F, and LA) were used to enhance the production of lovastatin in *M. purpureus* C322. The results depicted in Figure 5.22 represent the average readings of lovastatin concentration (mg/L) across different flask groups. The average lovastatin concentration in the C flask group was 36.36 mg/L. For the flask group supplemented with 0.03 mM T1, the average lovastatin concentration notably increased to 68.18 mg/L. Similarly,

the F (0.02 mM) group exhibited a higher average lovastatin concentration of 74.62 mg/L. The flask group with QSMs, B (300 nM), T2 (0.5 mM), and LA (0.4 mM) showed an average lovastatin concentration of 29.54, 54.24, and 51.14 mg/L (Fig. 5.22). Comparing these values, it is evident that the groups with QSM supplementation, particularly F and T1, display higher average lovastatin concentrations than the control and other groups.



Fig. 5.22. Lovastatin production in different flask groups (C, B, T1, T2, F, LA) on Day-18 of fermentation in *M. purpureus* C322 cultures during shaken flasks fermentation. Each fermentation experiment utilised a 500 mL Erlenmeyer flask with 100 mL growth medium, inoculated with 10^6 spores/mL, and incubated at 25°C and 120 rpm. The experiments were conducted in triplicate, with error bars denoting standard deviation. Lovastatin concentration on Day 18 was quantified using High-Performance Liquid Chromatography (HPLC). (*p*-value<0.01).

Table 27 displays the lovastatin yield in various flask groups (C, B, T1, T2, F, LA) during the fermentation process in shaken flasks. The C yielded 6.8 mg/g CDW of lovastatin. Comparatively, the flask groups with quorum sensing molecules, B, T1, T2, F, LA, produced 5.53, 12.54, 10.1, 14.0, and 9.46 mg/g CDW respectively. Compared to the C flasks, the lovastatin concentration in the flask groups B, T1, T2, F, and LA showed a fold increases of 0.81, 1.87, 1.49, 2.05, and 1.41, respectively. These results illustrate the differences in

lovastatin concentration across the different flask groups, with the F group displaying the

highest yield.

Table 5.4. Lovastatin	yield in different	t flask groups	(C, B, T1,	T2, F, LA)	during shake	n flask
fermentation						

	Lovastatin Yield	Fold Increase w.r.t
Flask Group (3 Flasks/set)	(mg/g CDW)	Control
Control (C)	6.8	-
Butyrolactone-I - 300 nM (B)	5.53	0.81
Tyrosol - 0.3 mM (T1)	12.54	1.87
Tyrosol - 0.5 mM (T2)	10.1	1.49
Farnesol – 0.2 mM (F)	14.0	2.05
Linoleic acid – 0.4 mM (LA)	9.46	1.41

The HPLC profiles obtained from each flask group (C, B, T1, T2, F, and LA) exhibit distinct peaks corresponding to the retention time of lovastatin, facilitating quantitative analysis of the compound's concentration in the samples (Appendix-Fig. 9). To further support this analysis, correlation curves displaying lovastatin standards are provided in Appendix Figure 5. The lovastatin standard curve includes known concentrations of lovastatin (0.01 mg/mL, 0.02 mg/mL, 0.05 mg/mL, and 1.0 mg/mL) (Appendix-Fig. 6), which enabled the establishment of a correlation between peak area in HPLC chromatograms and actual lovastatin concentration. Statistical analysis using one-way ANOVA revealed a significant difference in lovastatin production across the flask groups (*p*-value<0.01). Tukey's post hoc test indicated that flasks supplemented with F and T1 exhibited the highest lovastatin concentrations, with no significant difference between them. These were followed by T2 and LA, both of which yielded significantly more lovastatin than the control group. In contrast, B showed no significant difference from the control (*p*-value<0.01; Appendix–Table 44).

The application of quorum sensing molecules (QSMs), particularly butyrolactone-I, tyrosol, farnesol, and linoleic acid, to enhance lovastatin concentration in *Monascus* cultures represents

an unexplored area. While there have been studies exploring various methods to increase lovastatin concentration by different strains of *Monascus*, direct comparisons are challenging due to the novelty of using QSMs like B, T, F, and LA. Despite this, existing research indicates that adopting other QS strategies can enhance secondary metabolism, potentially resulting in increased lovastatin production. For instance, Palonen et al. (2014) reported that adding 100 nM of butyrolactone I to A. terreus MUCL38669 cultures resulted in a significant increase (up to two-fold) in lovastatin production, regardless of the growth stage. This increase was consistent when butyrolactone I was introduced at high cell density during the stationary phase. Similarly, Schimmel et al. (1998) observed an increase in secondary metabolism when butyrolactone I (500 µM) was added to cultures of A. terreus, resulting in enhanced production of lovastatin. Moreover, Sorrentino et al. (2010) conducted high-performance liquid chromatography analysis, revealing that the addition of linoleic acid at concentrations ranging from 0 to 320 µM (i.e., 0%, 1.79%, 8.97%, and 17.93% dissolved in 500 µM ethanol) to low cell density cultures of A. terreus enhanced lovastatin production by up to 1.8-fold. This suggests that while the approach of using these specific OSMs is new, the concept of enhancing secondary metabolite production to boost lovastatin yield is a promising area of research.

Results and Discussion

2.5 L Stirred Tank Fermenters

5.2.2 Quorum Sensing in 2.5 L Stirred Tank Fermenters

This section highlights the effect of QSMs (T, F, LA) on biomass and secondary metabolite (pigments and lovastatin) production in 2.5 L Stirred Tank Fermenters. The bioreactors were incubated at 32 °C and 300 rpm, and the medium pH was 6.5. QSMs were introduced at 24 h of fermentation. The total working volume was 1,500 mL, including 150 mL seed culture. The fermentation process with QSMs was repeated thrice to ensure repeatability.

5.2.2.1 Effect of Quorum Sensing on the Culture pH during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The data depicted in Figure 5.23 provides a comprehensive overview of pH dynamics during the fermentation process in 2.5 L bioreactors. In the C bioreactor (C), the pH initially decreased to 6.0 by Day-4, followed by a subsequent rise to 6.25 by Day-5. Conversely, the bioreactor supplemented with 0.3 mM T exhibited a decline to 5.8 on Day-4, with a subsequent increase to 6.4 on Day-5. Similarly, the pH trajectory within the 0.2 mM F bioreactor decreased to 5.8 on Day-4 and then ascended to 6.5 by Day-5. In the case of 0.4 mM LA-supplemented bioreactor, the pH decreased to 5.9 by Day-4, followed by a rise to 6.3 by Day-5. The observed variations in pH levels across the bioreactors was not statistically significance, as evidenced by a *p*-value greater than 0.05 (Appendix- Table 45). Minor variations in minimum and maximum pH values detected among bioreactors are likely attributable to normal metabolic progression rather than quorum sensing-mediated regulation. Similar outcomes were reported in previous studies involving fungal submerged cultures, where butyrolactone and linoleic acid had no observable effect on medium pH (Raina, 2008; Sorrentino, 2009; Amache, 2014).

Figure 5.24 presents a comparative assessment of pH fluctuations during the fermentation process in both shake-flasks (SF) and 2.5 L fermenters (2.5L_F). In shaken flask fermentation, the C group exhibited pH values ranging from 6.0 to 6.9. In the presence of QSMs (T, F, LA), the minimum pH values ranged from 5.8 to 6.0, while the maximum values reached between

7.0 and 7.1 by the end of fermentation. In fermenters, the control group exhibited pH values ranging from 6.0 to 6.25. With the addition of QSMs (0.3 mM T, 0.02 mM F, 0.4 mM LA), pH ranged from 5.8 to 5.9 at the minimum and 6.3 to 6.5 at the maximum. The transition from shaken flasks to fermenters in *M. purpureus* C322 cultures revealed a remarkable alignment in pH patterns across all groups (C, T, F, LA), indicating consistency across different cultivation scales. It can be noted that the fermentation process was carried out in flasks for 18 days and in fermenters for just 5 days, which may explain the observed variations in pH increase by the end of the fermentation period. This consistency suggests that *M. purpureus* C322 cultures maintain a similar metabolic profile even when scaled up to fermenters. Consequently, the observation of pH trends employed in shaken flasks, were successfully translated to larger bioreactor systems, thereby preserving pH dynamics.



Fig. 5.23. pH variation in different bioreactor groups over time: the control (C), 0.3 mM tyrosol (T), 0.2 mM farnesol (F), and 0.4 mM linoleic acid (LA). The depicted average pH values are from three experimental runs in 2.5 L stirred tank bioreactors, each containing 1,500 mL growth medium and 150 mL seed culture, incubated at 32°C at 300 rpm. Standard deviation is shown as error bars (*p*-value>0.05).



Fig. 5.24. Comparative analysis of the lowest and highest pH values recorded within each group during the fermentation process in both shake-flasks (SF) and 2.5 L fermenters (2.5L_F). Error bars represent the standard deviation of average pH measurements between three sets of experiments.

5.2.2.2 Effect of Quorum Sensing on Biomass Concentration during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

Compared to shaken flasks, fermenters provide a more consistent environment for biomass production, allowing for optimisation and scalability of the fermentation process. Biomass concentration is a crucial parameter in fermentation processes, as it reflects the growth and productivity of the microbial culture. This section compares the biomass concentration across different bioreactor groups, namely C, 0.3 mM T, 0.2 mM F, and 0.4 mM LA measured at the end of fermentation (Day-5) to analyse if the presence of QSMs influences *M. purpureus* C322 biomass. Figure 5.25 showcases the biomass concentration, represented as cell dry weight (CDW) in grams per litre (g/L), across different bioreactor groups, including C, T, F, and LA. Among the bioreactor groups, the C group demonstrated an average CDW value of 7.8 g/L,

whereas the bioreactor groups supplemented with QSMs, namely T, F, and LA, were followed by the CDW values of 7.8 g/L, 7.5 g/L, and 7.7 g/L, respectively.



Fig. 5.25. Biomass concentration on Day-5 of fermentation in different bioreactor groups: C, 0.3 mM T, 0.2 mM F, and 0.4 mM LA. In each 2.5 L stirred tank bioreactor, 1,500 mL of growth medium and 150 mL of seed culture were used, incubated at 32°C and stirred at 300 rpm. Each quorum sensing molecule was added at 24 h to the respective bioreactor group. The error bars denote the standard deviation based on three experimental sets (*p*-value>0.05).

To assess the efficacy of the selected bioreactor groups in responding to the upscaling process w.r.t biomass concentration, the total cell dry weight (CDW) (g/L) was compared between shaken flasks and bioreactors. The C group showed a 1.46-fold increase in CDW in bioreactors compared to shaken flasks. Likewise, the groups supplemented with QSMs - T, F, and LA - displayed increased CDW by 1.43, 1.41, and 1.42 folds, respectively, upon transitioning from shaken flasks to bioreactors. The observed rise in biomass concentration during upscaling indicates that the cultivation strategies facilitated successful adaptation to the bioreactor environment. The calculated *p*-value for the ANOVA in biomass concentration among the bioreactor groups (C, T, F, LA) was greater than 0.05, indicating no significant differences in biomass concentration among the studied bioreactor groups (Appendix-Table 46). This was consistent with trends observed in shaken flasks, where QSMs likewise did not affect biomass.

Similar observations have been made in earlier fungal studies, where QSMs did not significantly impact biomass production during submerged fermentation (Raina, 2008; Sorrentino, 2009; Amache, 2014).

5.2.2.3 Effect of Quorum Sensing on Yellow Pigment Production during Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The provided data in Figure 5.26 illustrates the production of yellow pigment in 2.5 L stirred tank fermenters across different bioreactor groups (C, T, F, and LA) over a span of five days (D1 to D5). The optical density (400 nm) measured for yellow pigment production in the C group reached 5.8 AU by the end of fermentation. Likewise, the bioreactors with added QSMs (T, F and LA) also displayed an increase in yellow pigment, with OD reaching 9.52, 11.95, and 7.16 AU, respectively, by Day-5 of fermentation. One-way ANOVA confirmed significant differences in pigment production across groups (*p*-value<0.01; Appendix–Table 47). Tukey's post hoc test revealed that F yielded the highest yellow pigment levels, followed by T and LA, all significantly higher than C. Differences among QSMs were also significant, with F outperforming both T and LA, and T exceeding LA. (*p*-value<0.01; Appendix–Table 47).

Table 5.5 summarises yellow pigment yield (AU/g CDW) and fold increase upon scale-up. "F/SF" indicates the transition from "Shaken flasks to Fermenters". The Control (C) bioreactor group yielded 0.74 AU/g CDW yellow pigment. Among QSMs, the F group produced the highest yield at 1.59 AU/g CDW (2.15-fold increase), followed by T (1.22 AU/g CDW; 1.65-fold) and LA (0.93 AU/g CDW; 1.27-fold). These findings reinforce the enhancing effect of QSMs on pigment biosynthesis in *M. purpureus* C322 under fermenter conditions. These outcomes underscore the potency of the QSMs in notably boosting yellow pigment production (Table 28). Although limited literature exists on QSM-induced pigmentation in bioreactors, some parallels can be drawn. For instance, Vila et al. (2019) reported that farnesol modulated yellow pigment production in *S. aureus* via oxidative stress responses. The current study builds

upon this premise, demonstrating that exogenous QSMs can enhance yellow pigment production in fungal cultures at scale.



Fig. 5.26. Yellow pigment production in different bioreactor groups: C, 0.3 mM T, 0.2 mM F, and 0.4 mM LA. Each 2.5 L stirred tank bioreactor contained 1,500 mL growth medium, including 150 mL seed culture prior to incubation at 32 °C under 300 rpm. Each QSM (T, F, LA) was added to the respective bioreactor group at 24 h of fermentation. Three sets of experiments were performed, and the error bars represent the standard deviation (*p*-value<0.01).

Table 5.5. Comparison of yellow pigment yield and impact of upscaling across different bioreactor groups (C, T, F, LA) during 2.5 L bioreactor fermentation.

Bioreactor Group	Yellow Pigment Yield- Bioreactor (AU/g CDW)	Fold Increase w.r.t Control Bioreactor	Yellow Pigment Yield- Shaken Flasks (AU/g CDW)	Fold Increase in Yield: F/SF
Control (C)	0.74	-	0.71	1.04
Tyrosol – 0.3 mM (T)	1.22	1.65	1.06	1.15
Farnesol – 0.2 mM (F)	1.56	2.11	1.33	1.17
Linoleic Acid 0.4 mM% (LA)	0.93	1.26	0.81	1.15

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5.2.2.4 Influence of Quorum Sensing on Orange Pigment Production in 2.5 L Stirred Tank Fermenters during Fermentation of *M. purpureus* C322

Monascus pigments are recognised for their scalability and ease of production, making them suitable for industrial applications, highlighting their versatility and practicality in various sectors (Chaudhary et al., 2022). The pattern observed in the 2.5 L stirred tank fermentation for orange pigment production was similar to the shaken flask cultures (Fig. 5.27). By Day-5, OD at 470 nm reached 5.58 AU in the C group. Bioreactors supplemented with QSMs showed increased pigment levels, with final OD values of 10.61 (T), 15.10 (F), and 8.11 AU (LA), respectively. One-way ANOVA demonstrated a significant difference in orange pigment production between the groups (*p*-value<0.01). Tukey's post hoc analysis indicated that F resulted in the highest pigment levels, significantly surpassing T, LA, and C. The T supplementation also enhanced orange pigment production significantly over LA and the control, and all pairwise comparisons between groups were statistically significant (*p*-value<0.01; Appendix–Table 48).



Fig. 5.27. Orange pigment production in various bioreactor groups: C, 0.3 mM T, 0.2 mM F, and 0.4 mM LA. The experimental set up was comprised of four 2.5 L bioreactor, containing 1,500 mL growth medium and 150 mL seed culture, maintained at 32° C with agitation at 300 rpm. Each QSM was added to the respective bioreactor group at 24 h of fermentation. Error bars indicate standard deviation from three experimental runs (*p*-value<0.01).

As shown in Table 5.6, the orange pigment yield in the C group was 0.72 AU/g CDW. Among the QSM-supplemented bioreactor groups, farnesol (0.2 mM) produced the highest yield at 2.01 AU/g CDW, corresponding to a 2.79-fold increase. The T group (0.3 mM) followed with 1.36 AU/g CDW (1.89-fold), while LA (0.4 mM) yielded 1.06 AU/g CDW, representing a 1.47-fold increase compared to the C. These findings highlight the superior performance of F in enhancing orange pigment biosynthesis under bioreactor conditions compared to T and LA.

Table 5.6. Comparison of orange pigment yield and impact of upscaling across different bioreactor groups (C, F, T, LA) during 2.5 L bioreactor fermentation.

Bioreactor Group	Orange Pigment Yield-Bioreactor (AU/g CDW)	Fold Increase w.r.t Control Brioreactor	Orange Pigment Yield-Shaken Flasks (AU/g CDW)	Fold Increase in Yield: F/SF
Control (C)	0.72	-	0.71	1.01
Tyrosol – 0.3 mM (T)	1.36	1.89	1.12	1.21
Farnesol – 0.2 mM (F)	2.01	2.79	1.5	1.34
Linoleic Acid -0.4 mM	1.06	2.05	0.91	1.16
(LA)				

These findings confirm that the introduction of QSMs, particularly F, significantly improved orange pigment biosynthesis in *M. purpureus* C322 under bioreactor conditions. Supporting studies demonstrate similar enhancement in related organisms. For example, Raina et al. (2010) reported a 6.4-fold increase in sclerotiorin production in *P. sclerotiorum* upon addition of γ -butyrolactone derivatives. Likewise, Amache et al. (2019) showed that crude QSM extracts from *P. sclerotiorum* led to over 2-fold enhancement of orange pigments in 2 L bioreactors. The present findings further substantiate the potential of QSMs as fermentation enhancers in scaled-up pigment production.
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5.2.2.5 Effect of Quorum Sensing on Red Pigment Production in 2.5 L Stirred Tank Fermenters during Fermentation of *M. purpureus* C322

The red pigment concentrations recorded in 2.5 L stirred tank bioreactors for groups C, T, F, and LA are shown in Figure 5.28. By Day-5, the OD at 510 nm for the control (C) group reached 5.55 AU. The addition of QSMs led to higher pigment production in all supplemented groups, with F (0.2 mM) reaching 13.70 AU, followed by T (0.3 mM) at 9.87 AU and LA (0.4 mM) at 8.63 AU. These values reflected a notable enhancement in pigment production compared to the C group, consistent with trends observed in shaken flask experiments. Statistical analysis using one-way ANOVA confirmed that red pigment production differed significantly across the bioreactor groups (*p*-value<0.01). Tukey's post hoc test revealed that F supplementation resulted in the highest red pigment concentration, significantly greater than all other treatments, followed by T, LA, and the C group. All pairwise comparisons between treatment groups were statistically significant, reinforcing the influence of QSMs on red pigment biosynthesis in fermenter conditions (*p*-value<0.01; Appendix–Table 49).



Fig. 5.28. Red pigment production in Various Bioreactor Groups: C, T, F, LA. Each 2.5 L bioreactor comprised 1,500 mL growth medium and 150 mL seed culture, incubated at 32 °C and agitated at 300 rpm. The QSMs were added at to the respective bioreactor group at 24 h of

fermentation. The standard deviation between three sets of experiments is indicated by error bars (*p*-value<0.01).

Table 5.7 outlines the red pigment yields observed across the bioreactor groups. The C group recorded a yield of 0.71 AU/g CDW. In comparison, the F group exhibited the highest yield at 1.82 AU/g CDW, equating to a 2.56-fold increase. The T group yielded 1.27 AU/g CDW, reflecting a 1.79-fold increase, while the LA group achieved 1.12 AU/g CDW, corresponding to a 1.58-fold increase. These results confirm the enhancing effect of QSMs on red pigment biosynthesis in fermenter systems, with F showing the greatest impact, followed by T and LA. Additionally, the results also underscore the successful transition from shaken flasks to bioreactors, as evidenced by the consistent increases in pigment yield observed across the various bioreactor groups.

Table 5.7. Comparison of red pigment yield and impact of upscaling across different bioreactor groups (C, T, F, LA) during 2.5 L bioreactor fermentation.

Bioreactor Group	Red Pigment	Fold	Red Pigment	Fold Increase in
	Yield-	Increase	Yield-	Yield: F/SF
	Bioreactors	w.r.t	Shaken	
	(AU/g CDW)	Control	Flasks	
		Bioreactor	(AU/g CDW)	
Control (C)	0.71	-	0.71	1.0
Tyrosol – 0.03 mM (T)	1.27	1.79	1.1	1.15
Farnesol – 0.02 mM (F)	1.82	2.56	1.46	1.25
Linoleic acid – 0.4 mM	1.12	1.58	0.92	1.22
(LA)				

The enhancing effect of QSMs observed in this study is consistent with previous findings. Kiziler et al. (2022) reported that farnesol supplementation led to a fivefold increase in prodigiosin (a red pigment) in *Serratia marcescens*, while tyrosol enhanced carotenoid synthesis in *Rhodotorula glutinis* by 2.13-fold. Similarly, Cheng et al. (2022) found that formic acid, an organic acid structurally similar to linoleic acid, increased violacein (a reddish-purple pigment) production by 20% in *Chromobacterium violaceum*. Collectively, these studies

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demonstrate the potential of QSMs—particularly farnesol and tyrosol—hold considerable potential to stimulate red pigment biosynthesis in microbial cultures during submerged fermentation.

5.2.2.6 Effect of Quorum Sensing on Carbohydrate Consumption during Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The data presented in Figure 5.29 illustrate the trend of carbohydrate consumption over the fermentation period in 2.5 L stirred tank bioreactors across different groups: C, T, F, LA. In the C group, carbohydrate consumption decreased from 20 g/L to 9.33 g/L by Day-5. A similar pattern was observed in bioreactors supplemented with quorum sensing molecules, where carbohydrate consumption dropped from 20 g/L to 9.42, 9.48, and 9.35 g/L for the T, F, and LA groups, respectively. The carbohydrate consumption rate over the 5-day fermentation period was estimated using the standard glucose curve, resulting in rates of 2.02, 2.0, 1.98, and 2.01 g/L/day for the C, T, F, and LA groups, respectively. The variance in carbohydrate consumption levels among these bioreactor groups was not statistically significant, as indicated by a p-value>0.05 (Appendix-Table 50).

The results suggest that upon transitioning to the 2.5 L fermenters, the observed carbohydrate consumption trend was consistent with the earlier shaken flask data. This similarity in trends indicates that the findings from the shaken flask experiments could be reliably scaled up to the larger fermenter setup. This is a crucial aspect in the field of bioprocessing, as it provides validation that the results obtained in smaller volumes are applicable and consistent in larger industrial-scale setups. In essence, the parallel trends between shaken flask and fermenter experiments signify the successful upscaling of the fermentation process. This is in line with previous work in fungal submerged cultures, where QSMs such as butyrolactone I and linoleic acid were not found to modulate carbohydrate utilisation (Raina, 2008; Sorrentino, 2009; Amache, 2014, Shi et al., 2022).



Fig. 5.29. Carbohydrate consumption in different bioreactor groups: C, T, F, LA. In each 2.5 L bioreactor, 1,500 mL of growth medium and 150 mL of seed culture were used, incubated at 32° C and stirred at 300 rpm. The concentration of each QSM was added at 24 h of fermentation. Error bars show the standard deviation from three experimental runs. The *p*-value>0.05 indicates no significant difference.

5.2.2.7 Comparison of pH, Pigment Production, and Carbohydrate Consumption during Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The data presented in Figure 5.30, derived from three sets of quorum sensing experiments in 2.5 L stirred tank fermenters, and provides a comprehensive overview of the correlation between pH levels, carbohydrate consumption, and pigment (yellow, orange, and red) production across different bioreactor groups (C, T, F, LA) over a span of five days. Notably, the observed patterns in 2.5 L stirred tank fermenters closely resemble those observed in shaken flasks, reinforcing the consistency of the correlation between pH, pigment production (Yellow, Orange, Red), and carbohydrate consumption across different bioreactor groups (C, T, F, and LA). The data reflects a gradual decline in pH, which corresponded with an increase in pigment production and carbohydrate consumption.

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Regarding pigment production, it is evident that all bioreactor groups exhibit an increase in vellow, orange, and red pigments as the fermentation progresses from D1 to D5; however, the bioreactors supplemented with QSMS (T, F and LA) resulted in a higher pigment production in comparison to the control group. Interestingly, the F group demonstrates the highest production of all Monascus pigments (Yellow, Orange, Red), followed by the T, LA, and C groups. The trend observed in the C group was not always consistent; however, the addition of QSMs consistently triggered more predictable outcomes. For details on the reduction and amination reactions, refer to Chapter I, Section 1.4.5. This was evident in shaken flasks as well as fermenters, where the occurrence of both reduction and amination reactions notably influenced the relative concentrations of yellow, orange, and red pigments. In the C group, the prevalence of yellow pigment (0.74 AU/g CDW) suggesting a pronounced reduction reaction, converting orange pigments into yellow pigments. This was followed by red (0.71 AU/g CDW) and orange pigments (0.72 AU/g CDW), indicating a lesser extent of amination. As observed in shaken flasks, the bioreactors with T and F both showed an increase in orange pigment followed by red and lastly yellow. The bioreactor group with F yielded 1.59 AU/g CDW 2.01 AU/g CDW orange, and 1.82 AU/g CDW red pigment. Similarly, the yellow, bioreactor group with T yielded 1.22 AU/g CDW yellow, 1.36 AU/g CDW orange, and 1.27 AU/g CDW red pigment. Although orange pigment yield was higher in bioreactors supplemented with T and F, the comparatively higher red pigment over yellow suggests a preference for the amination reaction over reduction. The bioreactor group with LA showed a higher red pigment (1.12 AU/g CDW) compared to orange (1.06 AU/g CDW)and yellow pigment (0.93 AU/g CDW), suggesting increased amination reactions. Overall, the bioreactors supplemented with F showed higher pigments (Yellow, Orange, Red) followed by T, LA, and lastly C group.

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Fig. 5.30. Correlation between pH, Pigment Production, and Carbohydrate Consumption in Shaken Flask Fermentation between different Bioreactor Groups, namely a) control (C), b) tyrosol 0.3 mM (T1), c) farnesol 0.2 mM (F), and d) linoleic acid 0.4 mM (LA).

The data, therefore, reveals a consistent correlation between pH levels, carbohydrate consumption, and pigment production across all bioreactor groups (C, T, F, LA). It is noteworthy that the presence of QSM (T, F, LA) induced the highest pigment production, suggesting the efficacy of the mentioned QSMs, especially F, in enhancing pigment biosynthesis in *M. purpureus* C322 in small-scale shake-flasks as well as larger-scale 2.5 L stirred tank fermentation.

5.2.2.8 Effect of Quorum Sensing on Lovastatin Production during Submerged Fermentation of *M. purpureus* C322 in 2.5 L Stirred Tank Fermenters

The lovastatin concentrations were measured at the end of fermentation for each bioreactor group (Fig. 5.31). In the C group, the lovastatin concentration reached 39.2 mg/L. The addition of the QSM, T led to a significantly higher lovastatin concentration of 78.4 mg/L, reflecting a substantial increase in production compared to the control. Similarly, the F group exhibited an even greater enhancement, recording a lovastatin concentration of 97.16 mg/L. LA also influenced lovastatin production, resulting in a concentration of 55.68 mg/L. These outcomes underscore the potency of these QSMs in boosting lovastatin biosynthesis in *M. purpureus* C322 cultures within the 2.5 L stirred tank fermentation setup. Appendix-Figure 10 illustrates chromatograms exhibiting peaks corresponding to lovastatin concentrations for each bioreactor group (C, T, F, LA). One-way ANOVA confirmed that lovastatin concentrations differed significantly among the bioreactor groups (*p*-value<0.01). Tukey's post hoc analysis revealed that farnesol (0.2 mM) supplementation resulted in the highest lovastatin production, significantly surpassing all other treatments. Tyrosol (0.3 mM) also led to a substantial increase in lovastatin levels compared to the control and linoleic acid (0.4 mM), with all comparisons showing statistical significance. Linoleic acid produced moderately elevated levels of lovastatin, while the control group exhibited the lowest yield. These findings confirm that

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quorum sensing molecules, particularly farnesol and tyrosol, significantly enhance lovastatin biosynthesis during fermentation (*p*-value<0.01; Appendix–Table 51).

Table 5.8 compares lovastatin yield and the scaling-up impact across various bioreactor groups (C, T, F, LA) during 2.5 L Bioreactor Fermentation. The estimated lovastatin yield in control group was 5.27 mg/g CDW. Compared to the C group, lovastatin yield in the T group increased to 11.29 mg/g CDW, corresponding to a fold increase of 2.14 after transitioning from shaken flasks to bioreactors (F/SF). Similarly, the F group exhibited an increased lovastatin yield of 12.6 mg/g CDW with a fold increase 2.39, and the LA group yielded 9.58 mg/g CDW with a fold increase of 1.81. Thus, reiterating the potential of QSMs, particularly F and T, in enhancing lovastatin production, as evidenced by the increased lovastatin yield and the positive impact of upscaling from shaken flasks to bioreactors.



Fig. 5.31. Lovastatin concentration in different bioreactor groups on Day-5 of fermentation: C, T, F, LA. Each 2.5 L bioreactor contained 1,500 mL of growth medium and 150 mL of seed culture, incubated at 32°C with 300 rpm agitation. Each QSM (T, F, LA) was added at a concentration at 24 h of fermentation. Error bars denote standard deviation within the set of experiments (*p*-value <0.01).

Bioreactor Group	Lovastatin Yield-	Fold	Lovastatin	Fold Increase in
	Bioreactors	Increase	Yield- Shaken	Yield: F/SF
	(mg/g CDW)	w.r.t Control	Flasks	
		Bioreactor	(mg/g CDW)	
Control (C)	5.27	-	5.72	0.92
Tyrosol – 0.3 mM (T)	11.29	2.14	10.46	1.08
Farnesol – 0.2 mM (F)	12.6	2.39	12.11	1.04
Linoleic acid – 0.4 mM	9.58	1.81	9.41	1.02
(LA)				

Table 5.8. Comparison of lovastatin yield and impact of upscaling across different bioreactor groups (C, T, F, LA) during 2.5 L bioreactor fermentation

Similar to this study, in submerged cultures of *A. terreus* in 5 L bioreactors, the exogenous addition of 100 nM butyrolactone I at 96 hours post-inoculation led to a 2.5-fold increase in lovastatin production compared to control cultures at 168 hours. Raina et al. (2012). Raina also observed a concurrent increase in endogenous butyrolactone I levels (2.5-fold) in cultures supplemented with butyrolactone I, indicating a potential auto-stimulatory function. Additionally, real-time PCR analysis conducted by Sorrentino et al. (2010) demonstrated that supplementation of linoleic acid resulted in elevated transcriptional levels of the lovastatin biosynthetic genes lovB and lovF in cultures of *A. terreus*. This finding suggests that QSMs, such as B, T, F, LA, exert transcriptional control over genes that can potentially influence lovastatin production.

5.3 Summary

The QS experiment conducted in both shaken flasks and 2.5 L stirred tank fermenters has provided valuable insights into the modulation of pigment production and lovastatin yield in *M. purpureus* C322. The results demonstrated that the addition of QSMs (T, F, and LA) notably enhanced the production of yellow, orange, and red pigments, as well as lovastatin, indicating the scalability and reproducibility of the QS. The QSM, butyrolactone-I between 100-300 nM concentration, did not shown any impact on pigment or lovastatin production. In both small-scale shaken flasks and large-scale 2.5 L stirred tank fermenters, both pigment (Yellow,

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Orange, and Red) and lovastatin were upscaled successfully. The highest pigments (Yellow, Orange, Red) were produced in the F (0.2 mM) group, followed by the T (0.3 mM) group and then the LA (3.57 mM) group. The control groups in both shaken flasks and 2.5 L stirred tank bioreactors produced the lowest pigment (Yellow, Orange, and Red) and lovastatin yield. The B group displayed similar pigment and lovastatin yield to the control groups. The highest lovastatin concentration was produced by F (0.2 mM) group followed by T (0.3 mM), LA (0.4 mM), and lastly by C groups. Overall, this experiment underscores the potential of QS as a bioprocess optimisation for enhancing pigment and lovastatin production and lovastatin yields in *M. purpureus* C322.

Chapter VI

Insights from Whole Genome Sequencing, Assembly, Annotation, and Transcriptomic Analysis of *M. purpureus* C322



6.1 Introduction

This chapter presents the results of a comprehensive investigation aimed at unravelling the genomic and transcriptomic landscape of *M. purpureus* C322, particularly in the production of bioactive compounds. The journey begins with the extraction of high-quality DNA from *M. purpureus* C322, a critical step in any genomic study. Two distinct methods were employed for DNA extraction: first, utilising Qiagen's ALLPrep Fungal DNA/RNA/Protein Kit, a robust and widely utilised kit renowned for its efficiency in extracting DNA from fungal species. Additionally, a traditional phenol-chloroform extraction method was employed to ensure the integrity and purity of the genomic DNA extracted.

Following DNA extraction, ultra-purification of the extracted DNA was performed prior to whole genome sequencing of the high-quality *M. purpureus* C322 genome, since no genome information was available for the inhouse strain. The sequencing raw data was analysed, and a comprehensive annotation of the genes present in the genome was conducted, shedding light on the genetic architecture and function of this potentially important strain. Subsequently, extraction and purification of total cellular RNA from *M. purpureus* C322 was performed using Qiagen's ALLPrep Fungal DNA/RNA/Protein Kit to find out quorum sensing capabilities. Furthermore, primer design for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was undertaken to validate the transcriptomic data obtained from the sequencing analysis. RT-qPCR, a powerful molecular biology technique, was employed to quantify QS gene expression levels accurately, providing valuable insights into the biological processes at play within *M. purpureus* C322. The assembly, annotation, and primer design were performed using Geneious Prime software.

The subsequent sections, delve into the results obtained from each stage of this multifaceted investigation, unravelling the genetic details and transcriptional dynamics of *M. purpureus*

C322. These findings, are aimed to deepen our understanding of this microorganism and pave the way for harnessing its biotechnological potential to its fullest extent.

6.2 Results and Discussion: Whole Genome Sequencing, Assembly, and Annotation of *M. purpureus* C322 Genome

This section presents the outcomes of experimental procedures and analysis performed on both the genome and transcriptome of *M. purpureus* C322. This includes DNA and RNA extraction and ultra-purification, followed by whole-genome sequencing, assembly, and annotation of the *M. purpureus* C322 genome. Additionally, results from RT-qPCR analyses examining the expression levels of quorum sensing genes are provided.

6.2.1 DNA Extraction

The Nanodrop readings indicated that all samples tested positive for both the DNA extraction procedures, as detailed in Table 6.1. However, significant differences were observed in the quality of the DNA. The 260/230 ratio for DNA extracted using Qiagen's ALLPrep Fungal DNA/RNA/Protein Kit was lower than that obtained via the phenol-chloroform extraction (PCE) method. This discrepancy suggests contamination with substances that absorb at a 230 nm wavelength, such as proteins. Conversely, the 260/280 ratio was lower in DNA extracted through the PCE method compared to that using Qiagen's kit, indicating the presence of contaminants such as phenol, that absorb wavelength at 280 nm. This type of contamination can typically be mitigated by thorough washing of the DNA pellet with alcohol.

Table 6.1. Quantification of DNA Using NanoDrop Spectrophotometry

Sample	Nucleic Acid	Unit	A260	A280	A260/A280	A260/A230
PCE	74.8	ng/ul	1.497	0.966	1.55	2.95
Qiagen	45.0	ng/ul	0.899	0.436	2.06	0.79

6.2.2 DNA Purification

The purification of DNA using the Cambridge Biosciences - Genomic DNA Clean & ConcentratorTM Kit yielded positive outcomes, achieving the necessary purity levels. Both the A260/230 and A260/280 ratios exceeded the threshold of 1.9 and 1.8, respectively. Specifically, the post-purification A260/280 ratio reached 2.54, while the A260/230 ratio was 3.24. The minimum DNA concentration necessary for whole genome sequencing as per Eurofins Ltd was 200.0 ng per 100 μ l buffer, equivalent to 2 ng/ μ l. The DNA concentration in the purified sample was 65.5 ng/ μ l in a 500 μ l stock solution, thereby exceeding the required threshold. Absorbance readings at 260 nm and 280 nm were 1.310 and 0.516, respectively. The ratios of A260/280 and A260/230, calculated as 2.54 and 3.24 respectively, signify a high level of purity, making the DNA suitable for sensitive procedures like sequencing and cloning. Details of these results are depicted in Figure 6.1.



Fig. 6.1. NanoDrop Spectrophotometry Results for DNA Quantification illustrating the absorbance ratios A260/280 and A260/230, indicating the purity levels of the DNA sample. The x-axis represents the wavelength in nanometers, spanning critical ranges from 220 nm to 340 nm, while the y-axis measures the absorbance, crucial for determining nucleic acid purity. This graph exemplifies the typical absorbance peak at 260 nm characteristic of pure DNA.

6.2.3 Total Cellular RNA Extraction & Purification from M. purpureus C322

The RNA extraction process was conducted utilising Qiagen's ALLPrep Fungal DNA/RNA/Protein Kit. The concentration of RNA obtained from the extraction was quantified at 23.5 ng/ μ l in a 300 μ l stock solution, demonstrating a successful extraction yield. The minimum suggested RNA concentration necessary for RT-PCR is 50 ng per 20 μ l reaction, which equals 2.5 ng/ μ l. The absorbance at 260 nm was recorded at 0.6, and at 280 nm it was 0.4. These measurements yielded an A260/280 ratio of 1.48 and an A260/230 ratio of 0.75 (Fig. 6.2). Following quantification and initial assessment, the extracted RNA was promptly utilised in reverse transcription-polymerase chain reaction (RT-PCR) experiments. This step is crucial for converting RNA into complementary DNA (cDNA), which is then amplified to facilitate the detailed study of gene expression.

User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
nanodrop	17/02/2022 19:51	6.7	ng/µl	0.167	0.118	1.42	0.25	RNA	40.00
nanodrop	17/02/2022 19:52	23.5	ng/µl	0.587	0.397	1.48	0.75	RNA	40.00

Fig. 6.2. RNA Quantification Using NanoDrop Spectrophotometry. The following parameters are displayed: the nucleic acid concentration (ng/μ), absorbance at 260 nm (A260), absorbance at 280 nm (A280), the ratio of absorbance at 260 nm to 280 nm (A260/280), and the ratio of absorbance at 260 nm to 230 nm (A260/230), providing comparative insights into the quality and concentration of the extracted RNA.

6.2.4 Whole Genome Sequencing and Assembly of *M. purpureus* C322 Genome

This section provides a detailed summary of the results from the assembly and annotation of raw sequencing data for *M. purpureus* C322. Utilising Geneious Prime software, the assembly phase employed sophisticated algorithms to construct a coherent and continuous genomic sequence, including the assembly of individual chromosomes from the sequenced reads. This constructed sequence served as a scaffold for subsequent analyses and annotations, setting the foundation for comprehensive genomic insights.

6.2.4.1 Quality Assessment and Report of Sequenced Raw Data

In the comprehensive sequencing project of *Monascus purpureus* C322, Eurofins Genomics Europe Sequencing GmbH utilised the Illumina HiSeq technology, specifically employing the NovaSeq 6000 S2 PE150 XP platform (Project number NG-21878. This advanced sequencing method provides a dual approach with paired-end reads, allowing for a more detailed and accurate assembly of the genome. The ultra-purified DNA sample proceeded through rigorous quality control and successfully passed. The sequencing process began immediately following sample approval.

Fast-QC, accessed via Usegalaxy.org, was employed to perform a thorough quality assessment of the raw sequencing data. The analysis revealed a total of 5,926,160 sequenced reads, which collectively amounted to 1.778 billion sequenced bases. This extensive data collection is critical for ensuring comprehensive coverage and depth, facilitating detailed genomic analysis and insights. The raw sequencing data was outputted in two primary FASTQ files, labelled as NG-21878_*Monascus*_purpureus_lib353627_6573_1_1.fastq.gz and

NG-21878_Monascus_purpureus_lib353627_6573_1_2.fastq.gz.

The integrity and authenticity of each file were verified via MD5 checksums to ensure data accuracy and reliability post-transfer. The results documented in this sequencing report are pivotal for advancing the understanding of the *M. purpureus* C322 genome. The data not only enhances our genomic databases but also lays the groundwork for further biological and genetic studies. Such detailed genomic data is essential for exploring the genetic underpinnings of traits and potential applications in biotechnology and other fields. The completion of this sequencing project marks a significant achievement in fungal genomics, contributing valuable genetic information to the scientific community.

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6.2.4.2 Genome Assembly of *M. purpureus* C322: Constructing a Coherent Chromosomal Structure

In the process of assembling the whole genome and chromosomes of *M. purpureus* C322, two primary computational techniques were employed: de novo assembly and mapping to a reference genome, executed using Geneious Prime software. The de novo assembly began with preprocessing steps to prepare the raw sequencing data for assembly. Initially, reads were paired, setting the framework for accurate reconstruction. The ends of these sequences were then trimmed to eliminate possible sequencing errors or adapters, involving a total of 11,852,314 sequences. This step is vital for improving the quality of the reads that contribute to the assembly, as cleaner reads significantly enhance the accuracy of the assembled genome. Subsequently, 1,550,843 of these trimmed reads were successfully merged, while 8,750,628 could not be merged. Merging paired reads helps in extending read length, thereby improving the contiguity of the assembly.

Duplicate reads were removed from the dataset, resulting in 7,177,723 merged and 7,861,152 unmerged sequences. This step is critical as it eliminates redundant data that could otherwise distort the assembly results. Following this, error correction and normalisation were performed, yielding 5,778,232 sequences from the merged group and 6,815,454 from the unmerged sequences. This process is essential for reducing the computational resources needed for the assembly while enhancing both the efficiency and the accuracy of the resulting genomic assembly (Appendix-Fig. 11). The cleaned and optimised data set was then subjected to the de novo assembly, resulting in the formation of 1000 base pair contigs. The contigs provide a preliminary glimpse into the genomic architecture of *M. purpureus* C322. However, additional processing was required to integrate these contigs into a fully resolved and complete genome.

Once the contigs were generated, they, along with the initial raw reads, were aligned against the reference genome, *M. purpureus* YY1 (Wu et al., 2023). This step is critical as it allows for the placement of contigs in their correct genomic locations, while the raw data helped in filling gaps if any from contigs and correct misassemblies from the de novo process. The mapping to the reference genome not only validates the de novo assembly but also enhances its accuracy by anchoring the contigs to a known genomic sequence. This procedure is essential for assembling the contigs into a coherent model of the genomic DNA and accurately reconstructing the chromosomal architecture. The combined use of de novo assembly and reference mapping methodologies allowed for a comprehensive analysis of the genomic structure and variations, such as insertions, deletions, and rearrangements compared to the reference.

The genome assembly of *M. purpureus* C322 using Geneious Prime software, culminated in a complete genome measuring 23,818,993 base pairs (23.82 Mb) and features a GC contentment of 49.1%. This genome was systematically organised into eight distinct chromosomes, the sizes of which are detailed in a specific Table 6.2 (Appendix-Fig. 12).

MP C322 Chromosome	Length (bp)
Chr 1	4,110,925
Chr 2	3,845,047
Chr 3	3,436,822
Chr 4	2,822,337
Chr 5	2,740,421
Chr 6	2,685,663
Chr 7	2,250,132
Chr 8	1,927,653

Table 6.2. Chromosomal Length Distribution after Assembly of *M. purpureus* C322 Genome.

Comparative genomic analyses, as detailed by Wu et al. (2023), demonstrated that *M. purpureus* YY-1 possesses a genome size of 24.1 Mb, which is 13.6–40% smaller compared to other species within the Eurotiales order. These species include *Aspergillus clavatus*, *A. flavus, A. fumigatus, A. nidulans, A. niger, A. oryzae, A. terreus, Neosartorya fischeri, Penicillium chrysogenum, P. marneffei, and P. stipitatus*, with genome sizes of 27.9, 36.8, 28.8, 30.1, 34.9, 36.5, 29.2, 31.8, 31.3, 28.5, and 35.6 Mb respectively. A similar trend is observed in the newly assembled genome of *M. purpureus* C322, which measures 23.82 Mb, further underscoring the trend of smaller genomes within this genus compared to other Eurotiales species.

Wu et al. (2023) also noted that *M. purpureus* YY-1 harbours the fewest predicted open reading frames among the studied Eurotiales, though other genomic characteristics such as gene density, average gene length, average number of exons per gene, and average exon size align closely with those of other Eurotiales species. This suggests that the reduced genome size of *M. purpureus* YY-1 may result from the evolutionary loss of dispensable genes, a pattern likely shared by *M. purpureus* C322.

Further investigation into paralogous gene families and protein-coding genes across these species highlighted gene losses in *M. purpureus* YY-1, indicating both absent gene families and reduced numbers of genes within many existing families. Giovannoni et al. (2005) suggested that such genomic reductions could offer a selective advantage by increasing metabolic efficiency and reducing nutrient and energy demands. This evolutionary strategy may also contribute to the distinctive economic traits of *M. purpureus* C322, such as its efficient production of valuable secondary metabolites like lovastatin and pigments.

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The divergence of *M. purpureus* sp. is thought to have occurred around 90 million years ago, as suggested by Beimforde et al. in 2014. Historical records trace the use of *Monascus* sp., particularly in the production of Hongqu (red yeast rice), a staple in traditional fermentation and medicine, back to roughly a millennium ago. It is hypothesised that the association of *Monascus* sp. with human activities like food production could date back even further. A recurring pattern in the evolutionary history of *M. purpureus* involves gene loss, specifically genes involved in various metabolic pathways. This trend is indicative of its adaptation to rice-based environments. This phenomenon of gene loss mirrors patterns observed in other culinary-associated microorganisms such as *Streptococcus thermophilus* and various lactic acid bacteria, which have similarly lost numerous genes for carbon utilisation and amino acid biosynthesis due to their specialised roles in dairy environments, as noted by Bolotin et al. (2004), Pfeiler et al. (2007), and Makarova et al. (2007). In contrast, the genome of *Aspergillus oryzae*, pivotal in the production of traditional fermented foods, shows significant expansion. According to Machida et al. (2005), this expansion is likely due to selective breeding for strains capable of enhancing the flavor profiles in products like soy sauce.

6.2.4.3 Comparative Genomic Analysis of *M. purpureus* C322: Insights into Genetic Similarity within the Fungi Kingdom

To further explore the genetic landscape of *M. purpureus* C322, a comparative genomic analysis was conducted using Geneious Prime software-LASTZ plugin. This analysis revealed a 93.8% pairwise similarity between *M. purpureus* C322 and *M. purpureus* YY1, indicating minimal genetic variation between these closely related strains. Similarly, *M. ruber* M7 and *M. pilosus* IFO 4488, another species within the *Monascus* genus, showed a very high similarity of 94.4% and 94.0% respectively with *M. purpureus* C322, emphasising close evolutionary relationships within the genus. These insights are crucial for understanding the genetic relationships and evolutionary history of *M. purpureus* C322. They not only aid in

comprehending the genetic diversity within the *Monascus* genus but also help identify the genetic basis of phenotypic traits shared with other fungi (Appendix-Fig. 13).

Additionally, the *M. purpureus* C322 genome was pairwise aligned with other model fungal organisms to evaluate similarities, the results of which are compiled in Table 6.3. The comparative genomic analysis included several fungal species, revealing varying levels of genetic similarity. *C. albicans* CBS 562 and *S. cerevisiae* NRRL Y-12632, despite their distinct phylogenetic distances, showed moderate similarities of 72.5% and 72.3%, respectively. Other fungi like *Neurospora crassa* OR74A, *F. oxysporum* f.sp. lycopersici 4287, and *P. chrysogenum* IBT 35668 also displayed significant genetic resemblances with 73.6%, 70.6%, and 74.5% respectively, illustrating shared evolutionary traits and possible functional conservation. Notably, *A. terreus* NIH2624 and *A. niger* CBS 513.88, both of which are industrially significant fungi with proven quorum sensing activities, showed 75.1% and 75.2% similarity, respectively, highlighting a broad spectrum of genetic sharing across different fungal genera.

Table 6.3 Comparative genomic analysis via Geneious Prime software, showing the percentage of pairwise identity between the genome of *M. purpureus* C322 and various other fungal genomes.

Fungal Species	Pairwise Similarity (%)
Candida albicans CBS 562	72.5
Saccharomyces cerevisiae NRRL Y-12632	72.3
Neurospora crassa OR74A	73.6
Fusarium oxysporum f.sp. lycopersici 4287	70.6
Penicillium chrysogenum IBT 35668	74.5
Aspergillus niger CBS 513.88	75.2
Aspergillus terreus NIH2624	75.1
Monascus purpureus YY1	93.8
Monascus pilosus IFO 4488	94.0
Monascus ruber M7	94.4

This dual approach of detailed de novo assembly and rigorous comparative genomic analysis ensuring a thorough and reliable assembly of the *M. purpureus* C322 genome. Such comprehensive genomic research is critical for subsequent biological and biotechnological research applications, paving the way for future studies aimed at exploiting the genetic characteristics of *M. purpureus* C322 for industrial and medical purposes.

6.2.4.4 Comprehensive Genome Annotation of *M. purpureus* C322 Using Geneious Prime: Insights into Genetic Elements and Functional Genomics

The genome annotation of *M. purpureus* C322 performed using Geneious Prime software has yielded a detailed mapping of various genomic features (Table 6.4, Fig. 6.3). This annotation, leveraging the reference features embedded within the software, has been instrumental in identifying a wide array of genetic elements, which are crucial for understanding the organism's genome structure and its functional capacities.

The annotation process revealed 21,502 coding sequences (CDS), which are vital for predicting the protein-coding potential of the genome. Additionally, untranslated regions (UTRs) which play a role in post-transcriptional regulation of gene expression, were identified, with 1,243 regions in the 5' UTR and 84 in the 3' UTR. Introns, non-coding sections of DNA that are important for gene regulation and alternative splicing, were found in 300 locations across the genome.

Significant regulatory elements such as promoters and terminators, which are essential for the initiation and termination of transcription, were abundantly annotated with 6,888 promoters and 6996 terminators identified. This highlights the complex regulatory landscape of the *M. purpureus* C322 genome. Enhancers, which increase the transcription of associated genes, were found in 2,404 regions, suggesting additional layers of transcriptional control.

Moreover, a large number of miscellaneous features totalling 78,852 regions, along with 2,690 misc RNA annotations, underscore the complexity and diversity of non-coding RNA functions within the genome. Ribosome binding sites, critical for the translation initiation, were annotated in 710 regions, indicating potential starting points for protein synthesis. Regulators were prominently represented, with 8,080 regions annotated in the *M. purpureus* C322 genome, highlighting the complex network of gene regulation. Additionally, 817 repeat regions were identified.

Additionally, the genome's structural aspects were addressed by identifying 19, 907 open reading frames that indicate potential coding sequences starting with common start codons (TTG, CTG, ATG), providing insights into the coding strategy of the organism. The origin of replication, identified in 332 regions, is crucial for understanding the replication mechanisms and cell division processes. This comprehensive annotation not only provides a blueprint of the genetic and regulatory architecture of *M. purpureus* C322 but also sets the stage for further functional genomics studies and biotechnological applications.

Table 6.4. Detailed Annotation of <i>M. purpureus</i> C322 Genome Using Geneious Prime:	
Distribution of Genetic and Regulatory Elements	

Annotation	Regions found
3' UTR (Untranslated Regions)	84
5'UTR (Untranslated Regions)	1,244
Coding Sequences (CDS)	6,949
Enhancers	2,404
Introns	299
Misc. Features	78,852
Misc RNA	2,690
Open Reading Frames (Start Codons/ TTG, CTG, ATG)	19,907
Origin of Replication	332
Promoter	6,888
Ribosome Binding Sites	710
Regulator	8,080
Terminator	6,996
Repeat Regions	817



Fig. 6.3. Graphical representation of *M. purpureus* C322 genome assembly and annotation (Geneious Reference Features) via Geneious Prime Software.

6.2.4.5 Identification of rRNA Genes in M. purpureus C322

This project was also focused on identifying the highly conserved 18S and 28S rRNA gene sequences within the *M. purpureus* C322 genome, regions that are known for their preservation across eukaryotes. Using the NCBI database, these gene sequences were retrieved from other *Monascus* sp. as reference. This step was crucial for ensuring the accuracy of the subsequent genomic alignments. The reference rRNA sequences were aligned against the newly assembled genome of *M. purpureus* C322 using Geneious Prime software. The alignments confirmed the presence and correct assembly of the 18S and 28S rRNA genes within the genome. To further validate these results, NCBI's nucleotide BLAST was employed, which corroborated the findings from Geneious Prime. The successful alignments and verifications are detailed in the Appendix section IV, further underscoring the reliability of the genome assembly.

6.2.4.6 Comparative Phylogenetic Analysis of *M. purpureus* C322 Based on 18S rRNA Gene Sequences

Following the precise identification and validation of the 18S rRNA gene sequences in *M. purpureus* C322, the research focused on comprehensive phylogenetic analysis to elucidate the evolutionary relationships of *M. purpureus* C322 with a selection of fungal species, which included *Monascus pilosus* IFO 4488, *Aspergillus terreus* ATCC 1012, *Aspergillus nidulans* ATCC 10074, *Saccharomyces cerevisiae* NRRL Y-12632, *Candida albicans* CBS 562, *Neurospora crassa* OR74A, and *Fusarium oxysporum f.sp. lycopersici* 4287.

Using Clustal W, a tool renowned for its effectiveness in producing accurate multiple sequence alignments essential for phylogenetic studies, the 18S rRNA gene sequence of *M. purpureus* C322 was aligned with those of the listed fungal species. The resulting alignment displayed the sequence similarities and differences among these species, providing a foundation for further evolutionary analysis (Fig. 6.4). The phylogenetic alignment revealed that *M. purpureus* C322 shares a particularly high genetic similarity with *M. pilosus* IFO 4488, at 96.06%. This

significant similarity underscores the close genetic relationship within the *Monascus* genus, suggesting a recent common ancestry or strong evolutionary conservation of essential genetic elements. Similarly, the *Aspergillus* sp., *A. nidulans* ATCC 10074, and *A. terreus* ATCC 1012, demonstrated similarities of 95.84% and 96.06% respectively with *M. purpureus C322*, indicating a notable genetic closeness that might reflect shared evolutionary histories or adaptation to similar ecological niches.

Conversely, the similarities with yeast species such as *S. cerevisiae* NRRL Y-12632 and *C. albicans* CBS 562 were relatively lower, at 82.93% and 85.82% respectively. This is expected, given the broader taxonomic distances between these yeasts and the mold, *Monascus*. Despite being more evolutionarily distant, *N. crassa* OR74A and *F. oxysporum f.sp. lycopersici* 4287 still exhibited respectable genetic similarities of 70.75% and 88.84% respectively with *M. purpureus* C322. These figures suggest the presence of conserved genomic regions that are likely crucial for fundamental cellular functions and overall survival, transcending the taxonomic boundaries.

This comprehensive phylogenetic analysis not only deepens our understanding of the genetic structures and evolutionary paths of *M. purpureus* C322 but also emphasizes the genetic interconnectedness among diverse fungal species through shared genetic legacies. This knowledge is crucial for further studies in fungal biology, helping to frame future research into their evolutionary biology and potential applications.

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	Neurospor	Candida al	Saccharom	Fusarium o	Aspergillus	Aspergillus	Monascus	Monascus
Neurospora crassa OR7	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	68.51%	67.02%	72.58%	70.56%	70.54%	70.75%	70.03%
Candida albicans CBS 5	68.51%	\geq	91.46%	82.74%	84.29%	84.23%	85.82%	84.29%
Saccharomyces cerevis	67.02%	91.46%	$>\!\!\!<\!\!\!<$	81.69%	83.27%	83.40%	82.93%	82.81%
Fusarium oxysporum f	72.58%	82.74%	81.69%	$>\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!\!$	87.67%	87.69%	88.84%	87.72%
Aspergillus nidulans AT	70.56%	84.29%	83.27%	87.67%	$>\!\!<$	97.67%	95.84%	96.92%
Aspergillus terreus ATC	70.54%	84.23%	83.40%	87.69%	97.67%	$>\!\!\!<$	96.06%	97.12%
Monascus purpureus C	70.75%	85.82%	82.93%	88.84%	95.84%	96.06%	\geq	98.14%
Monascus pilosus IFO 4	70.03%	84.29%	82.81%	87.72%	96.92%	97.12%	98.14%	$>\!\!\!<$

Fig. 6.4. Pairwise comparison of the 18S rRNA gene sequences across various fungal species, showing the percentage of genetic similarity between each pair. The species compared include several molds and yeasts, such as *Monascus purpureus C322, Monascus pilosus IFO 4488, Aspergillus terreus ATCC 1012, Aspergillus nidulans ATCC 10074, Saccharomyces cerevisiae NRRL Y-12632, Candida albicans CBS 562, Neurospora crassa OR74A, and Fusarium oxysporum f.sp. lycopersici 4287. Each cell in the table shows the percentage similarity between the 18S rRNA sequences of the species listed on the left column and the top row.*

6.2.3.7 Phylogenetic Tree Analysis and Evolutionary Relationships of *M. purpureus* C322

Following the phylogenic analysis using ClustalW, a phylogenetic tree was constructed to elucidate the evolutionary relationships between *M. purpureus* C322 and other fungi, namely *M. pilosus* IFO 4488, *A. terreus* ATCC 1012, *A. nidulans* ATCC 10074, *S. cerevisiae* NRRL Y-12632, *C. albicans* CBS 562, *N. crassa* OR74A, *F. oxysporum f.sp. lycopersici* 4287. This analysis was conducted using the FastTree plugin within Geneious Prime software, which is designed to efficiently generate phylogenetic trees based on sequence alignment data (Fig. 6.5). The branch lengths on the tree represent genetic distances, which indicate the amount of genetic change. These distances are noted by numerical values next to the branching points, reflecting the evolutionary divergence between the lineages.

The phylogenetic tree shows *M. purpureus* C322 very closely aligned with *M. pilosus* IFO 4488, reflecting a tight genetic relationship within the same genus. This close association signifies a high level of genetic similarity, implying that these two *Monascus* sp. likely share a

recent common ancestor and have preserved key genetic elements throughout their evolutionary history. In terms of other fungal species, *M. purpureus* C322 is more distantly related to *A. nidulans* ATCC 10074 and *A. terreus* ATCC 1012, as evidenced by longer branch lengths. Although they belong to different genera, the genetic distances are relatively modest, reflecting some level of shared genetic heritage and possibly similar biochemical pathways. Thus, species within the same genus (*Monascus* and *Aspergillus*) show high conservation of the 18S rRNA gene, underscoring the close genetic relationships.



Fig. 6.5. Phylogenetic tree illustrating the evolutionary relationships among different fungal species based on 18S rRNA gene sequences. Each branch represents a species, and the branch lengths are proportional to the genetic distances, indicating the amount of genetic change. The scale bar at the bottom (0.05) represents genetic distance as substitutions per site.

The genetic distance between *M. purpureus* C322 and yeast species such as *S. cerevisiae* NRRL Y-12632 and *C. albicans* CBS 562 is considerably larger. This is indicative of the significant evolutionary divergence between the mold species of the genus *Monascus* and these yeasts,

highlighting the vast differences in their genetic makeup and evolutionary histories. Among the species analysed, *N. crassa* OR74A shows the greatest genetic distance from *M. purpureus* C322, underscored by its position as an outlier on the tree. This distance is a testament to its distinct evolutionary path and ecological adaptations. Similarly, *F. oxysporum f.sp. lycopersici* 4287, while closer than *N. crassa* OR74A, still exhibits a significant divergence from *M. purpureus* C322, reflecting different evolutionary pressures and ecological roles.

The phylogenetic analysis detailed by Wu et al. (2023) provides a profound insight into the evolutionary context of *M. purpureus* YY1 within the Eurotiomycetes class. This study conducted a comprehensive phylogenetic examination using 2,053 single-copy orthologs and included 16 species from Eurotiomycetes—including *Aspergillus flavus*, *A. oryzae*, *A. terreus*, *A. niger*, *A. fumigatus*, *A. nidulans*, *A. clavatus*, *M. purpureus*, *Penicillium chrysogenum*, *P. marneffi*, *P. stipitatus*, *Neosartorya fischeri*, *Coccidioides immitis*, *Uncinocarpus reesii*, *Trichophyton verrucosum*, and *Arthroderma gypseum*, among others—and two *Sordariomycetes* sp. as out-groups, *Neurospora crassa* and *Gibberella zeae*. The resulting phylogenetic tree reaffirms the close evolutionary connections between *M. purpureus* YY1 and nine species within the Trichocomaceae family, thereby supporting prior studies that have noted similar relationships with *Aspergillus* sp. (Wu et al., 2023).

Further, the taxonomic revision proposed by Houbraken et al. (2011), which suggests the division of the Trichocomaceae family into Aspergillaceae, Thermoascaceae, and Trichocomaceae, finds substantial support from this study. Specifically, this phylogenetic data places *M. purpureus* YY1 within the Aspergillaceae family, a categorisation that not only helps in accurately defining its taxonomic position but also aligns with its genetic and functional characteristics. Similar results were noticed in the phylogenic analysis of this study where *Aspergillus* sp. was closely related to *Monascus* sp.

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This phylogenetic analysis not only clarifies the position of *M. purpureus* C322 within the fungal phylogenetic tree but also provides insights into its evolutionary dynamics. The tree helps illustrate how *M. purpureus* C322, despite its distinctiveness, shares a fundamental genetic connection with both closely and distantly related fungal species. Understanding these relationships can help in leveraging its unique properties and in exploring potential genetic and metabolic capabilities that are rooted in its evolutionary past.

6.2.5 Investigating Quorum Sensing Genes in Monascus purpureus C322

The research focused on investigating the presence of QSM genes within the genome of *M. purpureus* C322. Following initial annotations using Geneious Prime software for features like ORFs, promoters, RBS, origin of replications, CDSs, etc., attention was directed towards identifying genes associated with the biosynthesis of specific QSMs, including farnesol, tyrosol, butyrolactone, and linoleic acid.

6.2.5.1 Biosynthetic Pathway Analysis: Investigating QSM Synthesis via NCBI Database Searches

To establish a foundation for the genetic investigation, the study began by focusing on identifying the biosynthetic pathways for key quorum sensing molecules (QSMs), namely farnesol, tyrosol, butyrolactone, and linoleic acid. Amongst these, complete biosynthetic pathways for farnesol and tyrosol were successfully retrieved from the NCBI PubChem database, providing an overview of the enzymatic steps involved in the synthesis of these compounds (Fig. 6.6, 6.7). This comprehensive information forms the basis for tracing the genetic origins of the enzymes responsible for these biosynthetic reactions.



Fig. 6.6. Farnesol Biosynthesis Pathway: Illustrating the enzymatic pathway for the biosynthesis of Farnesol, detailing each enzymatic reaction involved from the initial substrate (3-methylbut-3-en-1-yl diphosphate) to the final product, Farnesol (Source: NCBI PubChem_MetaCyc, MetaCyc all-trans-farnesol biosynthesis (biocyc.org)).



Fig. 6.7. Enzymatic reactions leading to the production of Tyrosol, starting from L-tyrosine. Each step shows the conversion process, involving various enzymes such as aromatic amino acid aminotransferase and pyruvate decarboxylase, culminating in the formation of Tyrosol. (Source: NCBI PubChem_MetaCyc, Candida albicans SC5314 tyrosol biosynthesis (biocyc.org)).

In contrast, the pathways for butyrolactone and linoleic acid remained less clear. Despite extensive searches in the current literature and the NCBI database, full biosynthetic pathways for these compounds could not be established due to gaps in the available data. Nonetheless, the genes responsible for the production of these QSMs were identified from literature and NCBI database searches, laying the groundwork for further genomic investigations. All the quorum sensing genes investigated in this project are documented in the Appendix- Table 53.

6.2.5.2 Gene Identification and Alignment: Unveiling the Biosynthetic Capabilities of *M. purpureus* C322 for QSM Synthesis

With the biosynthetic pathway knowledge in hand, the next step involved investigating the gene sequences responsible for encoding the enzymes that produce pathway intermediates for both farnesol and tyrosol. This search extended to genes necessary for the production of butyrolactone (such as A-factor), linoleic acid, and other available QSM genes identified in NCBI.

These identified gene sequences were subsequently aligned pairwise against the newly assembled genome of *M. purpureus* C322 using the Geneious Prime software. This step was crucial to determine if there were any similarities between the known gene sequences and the *M. purpureus* C322 genome, which would suggest the organism's capability to synthesise the QSMs. The results of this alignment were significant. The genome of *M. purpureus* C322 contained all the genes involved in the biosynthetic pathways of both farnesol and tyrosol, as confirmed by the alignments (Table 6.5 and Fig. 6.8).

The Appendix-section IV provides a comprehensive display of all successful alignments for the quorum sensing genes explored in this study. This discovery not only validates the assembly and annotation processes but also underscores the metabolic versatility of this strain. Additionally, the analysis led to the discovery of several other genes implicated in the synthesis

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of quorum sensing molecules, including N-acyl homoserine lactone, sphingosine-1-phosphate, and related lipids, marking an important advancement in understanding the quorum sensing capabilities of *M. purpureus* C322.

However, direct gene sequences for butyrolactone and linoleic acid biosynthesis were not definitively identified. Given the limited information is currently available on these pathways, it is premature to draw definitive conclusions about their presence or absence in the *M. purpureus* C322 genome, suggesting that further studies are required, potentially involving genomic exploration to elucidate the biosynthetic pathways producing the quorum sensing molecules such as butyrolactone, linoleic acid, etc in different microorganisms.

Table 6.5. Results of QS Gene Annotations in *M. purpureus* C322 via Geneious Prime Software

		Length	Similarity				
Gene [NCBI Reference]	Organism	(bp)	(%)	Location	Coordinates		
	FADN	FSOT BIOS	VNTHESIS				
	FARM	ESOL DIOS	11111111111111111111111111111111111111				
Farnesyl pyrophosphate							
synthase [CJI97_003197	Candida			M. purpureus	289,675-		
– 40028343] [IspA]	auris	1053 bp	52.90%	C322-Chr 2	290,723		
Farnesyl-pyrophosphate							
synthetase	Aspergillus						
[AFUA_5G02450 -	fumigatus			M. purpureus	289,614-		
3505656] [IspA]	Af293	119 bp	75.69%	C322-Chr2	290,727		
tRNA							
isopentenyltransferase							
[ANOM_005322 -	Aspergillus				503,633-		
26807126	nomiae				503,583;		
[DIMETHYLALLYLT	NRRL			M. purpureus	503,467-		
RANSFERASE]	13137	1458 bp	65.05%	C322-Chr3	502,129		
PHO8: alkaline							
phosphatase PHO8							
[PHO8 - 852092]							
[FARNASYL	Saccharomyc						
DIPHOSPHATE	es cerevisiae			M. purpureus	1,948,332-		
PHOSPHATASE]	S288C	1701 bp	47.17%	C322 -Chr4	1,946,653		
TYROSYL BIOSYNTHESIS							
	Fusarium						
Alcohol dehydrogenase	oxysporum						
[FOYG_00855 -	NRRL			M. purpureus	625,656-		
42008387]	32931	2722 bp	55.53%	C322 -Chr6	624,526		

Deleted to ADUS electrol					
Related to ADH5-alconol	.				
dehydrogenase V	Fusarium				
[FPRO_06554 -	proliferatum			M. purpureus	50,545-
42051433]	ET1	1183 bp	62.41%	C322-Chr5	49,328
					2,911,254-
					2,910,604;
					2,910,529-
ADH1_EMENI Alcohol	Aspergillus				2,910,175;
dehydrogenase I (ADH I)	nidulans			M. purpureus	2,842,527-
[AN8979.2 - 2868277]	FGSC A4	1168 bp	69.42%	C322 -Chr1	2,842,481
ADH1: alcohol	Saccharomyc				
dehydrogenase ADH1 [M nurnureus	2 910 208-
ADH1 = 8540681	S288C	1047 bp	18 13%	C322-Chr1	2,910,200
ADH2: alashal	Szeekanomye	1047.00	40.1370		2,711,234
ADH2. alcollol	Saccharomyc			14	2 011 254
denydrogenase ADH2	es cereviside	10471	17 700/	M. purpureus	2,911,254-
ADH2 - 855349]	S288C	1047 bp	47.79%	C322 -Chr1	2,910,208
ADH2: alcohol	Candida				
dehydrogenase [ADH2 -	albicans			M. purpureus	2,910,208-
3640751]	SC5314	1047 bp	46.21%	C322 -Chr1	2,911,254
					3,488,793-
					3,488,668;
					3,191,562-
					3,191,519;
ADH2 EMENI Alcohol					3.140.321-
dehydrogenase II (ADH	Aspergillus				3.140.165:
II) [AN3741 2 - 2873163	nidulans			M nurnureus	3 140 080-
	FGSC A4	1320 hn	68 69%	C322 -Chr1	3 139 380
	TODE III	1320 00	00.0770		3,137,500
					247,314-
A remetic emine soid					347,388,
	A				347,289-
aminotransierase	Aspergilius			14	346,715;
[BO96DRAF1_41/1/9 -	niger CBS			M. purpureus	346,647-
3/101885];	101883	2623 bp	62.06%	C322 -Chr6	345,591
Putative tyrosine					1,446,363-
decarboxylase					1,446,282;
[BO66DRAFT_379788 -	Aspergillus			M. purpureus	650,080-
37148948]	aculeatinus	1900 bp	56.18%	C322 -Chr7	648,310
AMO1_ASPNG Copper	Aspergillus				
amine oxidase 1 [ty oxi]	nidulans			M. purpureus	3,726,667-
[AN2532.2 - 2875160	FGSC A4	2185 bp	65.18%	C322 -Chr1	3,728,874
					3 726 667-
					3 726 997
					3,727,061-
					3 727 627
Connon amina avidada					3,727,037,
putativa [4			67 600/ -		3,121,101-
	A		07.09%;	14	5,728,077,
[AFUA_3G14390 -	Aspergillus	01771	/8.33%	M. purpureus	3,128,141-
3512134]	fumigatus	21// bp	[rest 3]	C322 -Chr1	3,728,876

Lipid-Derived QS Molecule Biosynthesis							
PAP2 domain protein [Aspergillus						
AFUA_6G04240 -	fumigatus			M. purpureus	2,140,044-		
3505155 [PgpB]	Af293	830 bp	60.36%	C322 -Chr5	2,140,850		
					983,326-		
					983,380;		
					1,471,444-		
					1,471,660;		
Sphingosine-1-phosphate					1,471,720-		
phosphohydrolase					1,472,490;		
[NFIA_072940 - 4586038	Aspergillus			M. purpureus	1,472,553-		
] [PgpB]	fischeri	1808 bp	58.61%	C322 -Chr6	1,473,076		
					582,839-		
					583,395;		
Hypothetical protein					583,457-		
[AN7316.2 - 2869849]	Aspergillus				583,616;		
[N-acyl homoserine	nidulans			M. purpureus	583,673-		
lactone]	FGSC A4	1016 bp	73.33%	C322 -Chr5	583,840		
					582,839-		
					583,395;		
Putative C-8 sterol	Aspergillus				583,457-		
isomerase [Erg-1] [novofumigat				583,616;		
P174DRAFT_442147 -	us IBT			M. purpureus	583,673-		
36534834]	16806	1099 bp	73.79%	C322 -Chr5	583,840		
Probable C-8 sterol					223,525-		
isomerase [erg-1]	Fusarium				223,892;		
[FFUJ_02631 - 35396113	fujikuroi IMI			M. purpureus	223,930-		
]	58289	760 bp	63.29%	C322 -Chr6	224,267		
					1,445,524-		
Hypothetical protein	Aspergillus				1,445,927;		
[AN4991.2 - 2872788]	nidulans			M. purpureus	1,445,928-		
[PgpB }	FGSC A4	1376 bp	69.52%	C322 -Chr3	1,446,906		


Fig. 6.8. This Circos plot, created using Circa software, visualises the complex genomic architecture of *M. purpureus* C322. Track 1 shows scale ticks for genomic positioning. Track 2 delineates the chromosomes, each represented by distinct colours for clarity. Track 3 identifies open reading frames (ORFs), suggesting potential protein-coding regions, while Track 4 highlights regulatory regions involved in gene expression control. Track 5 displays terminator sequences critical for ending transcription, and Track 6 marks promoter sites essential for initiating transcription. Track 7 features ribosome binding sites, origins of replication, coding sequences, and enhancers, key for protein synthesis and gene regulation. Track 8 encompasses untranslated regions (UTRs) and repeat regions, introns and miscellaneous RNA, providing insight into post-transcriptional regulation, indicating non-coding segments and diverse RNA molecules. Lastly, Track 9 focuses on quorum sensing (QS) genes, vital for understanding microbial communication and gene regulation in response to population density changes. This plot serves as a detailed representation of genomic elements,

facilitating a deeper understanding of genetic and regulatory frameworks in *M. purpureus* C322.

6.2.5.3 Validation of Gene Expression and Functional Analysis of QS Pathways in *M. purpureus* C322

To confirm the genomic findings related to *M. purpureus* C322, a series of primers were designed to target genes that showed sequence similarities in the genome (Chapter II, Table 10). These primers were utilised in reverse transcription polymerase chain reaction (RT-PCR) experiments aimed at verifying whether these gene sequences were indeed being transcribed into mRNA. The successful transcription of these genes into mRNA would validate their functional presence in the genome and underline their potential roles in *M. purpureus* C322 metabolic processes, particularly in the biosynthesis of quorum sensing molecules (QSMs).

The RT-PCR analysis yielded significant insights into the expression levels of several genes of interest (Table 6.6). Specifically, enzymes such as dimethylallyltransferase, farnesol diphosphate phosphatase, ADH5, ADH1, copper amine oxidase I, Erg-1, and aromatic amino acid aminotransferase demonstrated moderate expression levels, with Cq values ranging between 35 and 37.16. In contrast, genes like IspA, PgpB, N-acylhomoserine lactone, putative tyrosine decarboxylase, and ADH exhibited higher activity, showing moderate to high expression levels with Cq values between 30 and 35. Notably, ADH2 with very low Cq values (22.85) suggest very high expression levels, consistent across replicates. However, several instances were recorded where no amplification occurred, as indicated by a Cq value of 0.00. These instances, observed in wells A04, A08, B01, C04, and C05, might suggest potential issues such as primer failure, the absence of the target gene in the sample, or other technical problems during the assay setup (Schmerker, 2024).

Well	Fluor	Gene	Content	Sample	Cq	Cq Mean	Cq Std. Dev	Set Point
A01	SYBR	IspA	Unkn	E11-12	31.04	31.04	0.000	60.0
A02	SYBR	IspA	Unkn	E11-12	30.38	30.38	0.000	60.0
A03	SYBR	Dimethylallyltransferase	Unkn	F01- E01	37.16	37.16	0.000	60.0
A04	SYBR	Dimethylallyltransferase	Unkn	F01- E01		0.00	0.000	60.0
A05	SYBR	Farnasyl Diphosphate Phasphatase	Unkn	F03-04	36.86	36.86	0.000	60.0
A06	SYBR	Farnasyl Diphosphate Phasphatase	Unkn	F03-04	38.36	38.36	0.000	60.0
A07	SYBR	ADH5	Unkn	F05-06	36.31	36.31	0.000	60.0
A08	SYBR	ADH5	Unkn	F05-06		0.00	0.000	60.0
B01	SYBR	ADH1	Unkn	F07-08		0.00	0.000	59.9
B02	SYBR	ADH1	Unkn	F07-08	38.25	38.25	0.000	59.9
B03	SYBR	ADH2	Unkn	F09-10	22.85	22.85	0.000	59.9
B04	SYBR	ADH2	Unkn	F09-10	22.83	22.83	0.000	59.9
B05	SYBR	ADH	Unkn	F11-12	33.82	33.82	0.000	59.9
B06	SYBR	ADH	Unkn	F11-12	36.56	36.56	0.000	59.9
B07	SYBR	Aromatic amino acid aminotransferase	Unkn	G01-02	37.99	37.99	0.000	59.9
B08	SYBR	Aromatic amino acid aminotransferase	Unkn	G01-02	35.89	35.89	0.000	59.9
C01	SYBR	Putative tyrosine decarboxylase	Unkn	G03-04	33.21	33.21	0.000	59.7
C02	SYBR	Putative tyrosine decarboxylase	Unkn	G03-04	33.64	33.64	0.000	59.7
C03	SYBR	Copper amine oxidase 1	Unkn	G05-06	37.48	37.48	0.000	59.7
C04	SYBR	Copper amine oxidase 1	Unkn	G05-06		0.00	0.000	59.7
C05	SYBR	PgpB	Unkn	G07-08		0.00	0.000	59.7
C06	SYBR	PgpB	Unkn	G07-08	35.33	35.33	0.000	59.7
C07	SYBR	PgpB	Unkn	G09-10	35.91	35.91	0.000	59.7
C08	SYBR	PgpB	Unkn	G09-10	34.52	34.52	0.000	59.7
D01	SYBR	N-acylhomoserine lactone	Unkn	G11-12	32.38	32.38	0.000	59.3
D02	SYBR	N-acylhomoserine lactone	Unkn	G11-12	34.96	34.96	0.000	59.3
D03	SYBR	Erg-1	Unkn	H01-02	37.17	37.17	0.000	59.3
D04	SYBR	Erg-1	Unkn	H01-02	36.98	36.98	0.000	59.3

Table 6.6. RT-PCR	Expression .	Analysis of	QS Genes	in <i>M</i> . <i>p</i>	urpureus	C322
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The RT-PCR results confirm not only the successful amplification and expression of several key genes involved in metabolic pathways and potentially in quorum sensing but also provide valuable insights into the gene expression profile of *M. purpureus* C322 (Appendix-Fig. 15). This comprehensive approach—from genomic annotation through pathway analysis to experimental validation—highlights the intricate connection between genetic potential and metabolic output in *M. purpureus* C322.

Quorum sensing (QS) is a well-studied mechanism in many microorganisms, yet it remains a novel concept within the *Monascus* sp., where QS genes have not previously been identified. This study represents a pioneering exploration into the quorum sensing capabilities of *M. purpureus* C322, marking the first time QS genes have been uncovered in this species. The discovery of these genes in *M. purpureus* C322 not only expands our understanding of the genetic repertoire and regulatory mechanisms in *Monascus*, but also suggests potential avenues for studying how QS influences the metabolic and physiological processes in this fungus. Such insights could lead to innovative applications in industrial and biotechnological contexts, where *Monascus* sp. are valued for their biochemical products.

6.3 Summary

The whole genome sequencing and assembly of *M. purpureus* C322 resulted in a fully assembled 23.82 Mb genome organised into 8 chromosomes. Comparative analysis was conducted using pairwise similarity assessments with several fungal species, including *C. albicans* CBS 562, *S. cerevisiae* NRRL Y-12632, *N. crassa* OR74A, *F. oxysporum* f.sp. lycopersici 4287, *P. chrysogenum* IBT 35668, *A. niger* CBS 513.88, *A. terreus* NIH2624, and *M. purpureus* YY1. Phylogenetic analysis, based on 18S RNA, provided insights into the evolutionary distances between these species. Extensive genomic annotations performed using Geneious Reference Features revealed detailed information on untranslated regions (UTRs), coding sequences (CDS), promoters, regulators, terminators, enhancers, origins of replication, ribosome binding sites (RBS), open reading frames (ORFs), miscellaneous RNA, introns, and repeat regions. Additionally, several quorum sensing genes were identified and their expressions confirmed through RT-PCR, enhancing our understanding of the microbial communication within *M. purpureus* C322.

Chapter VII

Examination and Characterisation of *M. purpureus* C322 Morphology Chapter VII

Microscopy

7.1 Introduction

The chapter offers insights from microscopic analysis conducted on *M. purpureus* C322 cultures, aimed at unravelling the complex morphological transformations inherent in the growth and development of *M. purpureus* C322, focussing on sporulation, mycelium, and pellet formation.

Throughout this chapter, detailed observations spanning from Day 1 to Day 9 of the fermentation period are presented. These observations were conducted using 500 mL Erlenmeyer flasks containing 100 mL of a growth medium, inoculated with 10⁶ spores/mL, and subjected to shaken flask fermentation under controlled conditions of 25°C, 120 rpm, and pH 6.5. Microscopic analysis was conducted using a Nikon light microscope at various magnifications (4k, 10k, 100k), providing insights into fungal growth dynamics. This approach unveiled subtle shifts in morphology, secondary metabolite production, and the progressive evolution of pellet structures.

Given that pellet formation significantly influences *Monascus* pigment production and optimising this process could result in higher pigment yields, microscopic analysis assumes a critical role. It aids in comprehending the growth, morphology, and pigment production of *Monascus* sp. during submerged fermentation, while also facilitating the optimisation of fermentation conditions to enhance productivity.

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7.2 Results and Discussion

7.2.1 Overview of Dual-Reproduction Modes in *M. purpureus* C322

Within the *M. purpureus* C322 cultures, a dynamic interplay of both sexual and asexual reproductive mechanisms was evident, reflecting the fungal culture's adaptability and reproductive versatility. Figure 7.1 (d) captures this dual reproduction process within a single visual frame, highlighting the initiation of reproductive cycle of this fungus. The presence of aleurioconidia, depicted in Figure 7.1 (a), showcases single conidia measuring approximately 7 μ m at the tip, while chains of conidia, or catenula conidia, as observed in Figures 7.1 (c), underline the asexual proliferation aspect of *Monascus* sp. This phenomenon is consistent with the findings of Shu and Shin (2000) and Pordel et al. (2015), who noted the rapid emergence of conidia, ranging in size from 7-12 μ m, within 6 h of fermentation.

Moreover, the initial stages of sexual reproduction are hinted at in Figure 7.1 (b), possibly illustrating the development of trichogyne or the swelling of ascogonium, marking the commencement of the sexual phase (Wong and Chien, 1986; Patakova, 2013). This multifaceted reproductive strategy, incorporating both asexual and sexual modalities, has been extensively documented across various studies, underscoring the genetic and morphological diversity within *Monascus* cultures (Carels and Shepherd., 1975; Rasheva et al., 1997, 1998; Shu and Shin, 2000; Vasilyeva et al., 2012; Pan and Hsu., 2014; Portel et al., 2015; Mannan and Arif., 2017; Guerrero et al., 2021; Husakova et al., 2021).

The depiction of early cleistothecia development (~13 μ m) as shown in Figure 7.1 (e), echoes the rapid onset of sexual structures within the first 24 h of fermentation, a phenomenon previously highlighted by Carels and Shepherd (1975). This early formation of cleistothecia is indicative of *Monascus*' efficient reproductive strategy, enabling the swift transition from vegetative growth to reproductive maturity. Such early development is critical for the lifecycle progression of *Monascus*, facilitating spore production and dispersal, essential for the perpetuation of the species.

These observations not only validate the dual reproductive capabilities inherent in *M. purpureus* C322 cultures but also provide valuable insights into the temporal dynamics of fungal growth and development during submerged fermentation. The simultaneous occurrence of asexual and sexual reproduction within these cultures underscores the complex biological mechanisms that *Monascus* employs for survival and propagation. This multifaceted approach to reproduction enhances the fungal culture's adaptability and resilience, offering a rich avenue for further exploration in the context of industrial fermentation processes aimed at the production of pigments, secondary metabolites, and other biotechnologically significant compounds.



Fig. 7.1. Sexual and Asexual Reproduction in *M. purpureus* C322 cultures; capturing, a) Individual asexual aleurioconidia, b) Preliminary phase of sexual reproduction, c) Chain forming catenula conidia, d) Simultaneous asexual and sexual proliferation, e) Young Cleistothecia.

7.2.2 Morphology of M. purpureus C322 during Submerged Fermentation

This study documents the dynamic morphological development of *M. purpureus* C322 during a 9-day submerged fermentation process. Observations detail the transition from the spore state to the formation of mature mycelium, highlighting key stages such as hyphal elongation, conidia and aleurioconidia formation, cleistothecia development, and pellet formation. The investigation provides insights into the *M. purpureus* C322 growth cycle, emphasising changes in pigmentation and structural evolution, thus offering valuable data for optimising fermentation strategies in industrial applications.

7.2.2.1 Inoculation with M. purpureus C322 Spores

On Day-1 of fermentation, 100 mL growth medium in 500 mL Erlenmeyer flasks were inoculated with 10^6 spores/mL (Fig. 7.2). Spore concentration can significantly influence the morphological development of fungal cultures, particularly affecting pellet formation (Jimenez-Tobon et al., 1997; Znidarsic et al., 1998; Du et al., 2003). For instance, research by Du et al. (2003) on *R. chinesis* cultures showed that a high inoculum density of 10^9 spores/mL led to a predominantly hyphal growth pattern that entangled and subsequently reduced pellet formation. In contrast, a lower spore concentration of 10^5 spores/mL favoured the formation of larger pellets, albeit resulting in a reduced biomass yield. Conversely, Jimenez-Tobon et al. (1997) reported that increasing the spore concentration from 10^3 to 10^6 spores/mL in *Phanerochaete chrysosporium* cultures resulted in the formation of larger pellets, indicating that the impact of spore concentration on fungal morphology varies across different species and is not uniformly predictable. Thus, after conducting a series of in-house experiments, an inoculum density of 10^6 spores/mL was determined to be optimal for cultivating *M. purpureus* C322, aiming for a balanced outcome in terms of pellet morphology and biomass productivity.



Fig. 7.2 (a,b). *M. purpureus* C322 Spores at 40x objective magnification using different microscopic filters.

7.2.2.2 Morphology of *M. purpureus* C322 Cultures on Day-1 of Submerged Fermentation

On Day-1 of submerged fermentation, the *M. purpureus* C322 cultures demonstrated an onset of spore germination, observed around 7 h post-inoculation, with clear evidence of germ tube emergence as depicted in Figure 7.3. The prompt emergence of germ tubes is indicative of the spore's viability and the conducive conditions of the fermentation medium, setting a positive trajectory for the culture's development. An early and rapid germination of spores subsequently leading to mycelial development, plays a pivotal role in establishing the foundation for pellet formation, a desirable outcome for *Monascus* fermentation processes due to its implications for pigment production and secondary metabolite synthesis as detailed by Veiter et al. (2018). The successful germination and active growth observed on Day-1 are vital indicators of the health and vitality of the culture, essential for achieving the desired outcomes in secondary metabolite production and overall fermentation efficiency.



Fig. 7.3. Microscopic observation of *M. purpureus* C322 on the first day of submerged fermentation at 100x objective magnification, showcasing initial spore germination. The emergence of germinating spores is indicated by black arrows.

This swift transition from spore to germ tube formation aligns with findings from Ajdari et al. (2011), who highlighted the rapid germination capabilities of *M. purpureus* (strains FTC5391, FTC5400, FTC5354, FTC5357) spores as a testament to the effectiveness of the nutrient composition of the fermentation medium. Similarly, early mycelial initiation, within 6 hours of cultivation as observed by Suh and Shin (2000), further corroborates the propensity of *Monascus* cultures for quick growth initiation under optimal conditions. Furthermore, Carels and Shepherd (1975) observed the appearance of sexual structures within 8 h of inoculation, thus, emphasising the rapid and multifaceted growth phases *Monascus* cultures undergo from the very outset of fermentation.

7.2.2.3 Morphology of *M. purpureus* C322 Cultures on Day-2 of Submerged Fermentation

On the second day of submerged fermentation, *M. purpureus* C322 cultures exhibited notable developmental milestones, with spores transitioning into hyphae and the initiation of sporulation, as captured in Fig. 7.4. This period of growth was characterised by hyphae elongation, branching, and the commencement of aleurioconidia formation, indicating the onset of asexual reproduction.



Fig. 7.4. Microscopic images of *M. purpureus* C322 morphology on Day-2, depicting hyphae development and sporulation. The images were captured using 10x and 40x objective magnifications, with a) and b) Hyphal development at 10x magnification, and (c) Initiation of Hyphal branching and (d) Early aleuriconidia formation indicated by black arrows.

These results align with findings from Said (2010), who documented similar patterns of hyphal development and sporulation in the initial days of *M. ruber* ICMP 15220 submerged fermentation. Moreover, Suh and Shin (2000) highlighted the concurrent nature of hyphal

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branching and conidia formation during early fermentation in *Monascus* sp. J101, emphasising the dynamic interplay between growth and reproduction in optimising culture development. The simultaneous occurrence of hyphal development and sporulation within the initial days of fermentation, underscores the rapid adaptive mechanisms of *M. purpureus* C322 cultures to the submerged environment. Such early-stage morphological changes are pivotal, setting the stage for the formation of mycelium.

7.2.2.4 Morphology of *M. purpureus* C322 Cultures on Day-3 of Submerged Fermentation

By the third day of submerged fermentation, the *M. purpureus* C322 cultures exhibited mycelial development and continued sporulation, as evidenced in Fig. 7.5 (a-c). The formation of mycelium is characterised by the entwining of hyphae into a dense network, which not only facilitates the structural integrity of the fungal colony but also plays a critical role in the formation of pellets. Conidia was observed alongside mycelial formation, indicating the continuity of asexual reproduction, amid the expansion of biomass. The production of spores ensures propagation and long-term survival within the fermentation environment (Pirt, 1975). This stage of fermentation was also marked by the initial development of cleistothecia, signifying the onset of sexual reproduction—a more complex form of fungal propagation. The appearance of young cleistothecia by Day-3 aligns with observations by Carels and Shepherd (1975), indicating a well-timed progression towards sexual maturity within *Monascus* ATCC 16436 life cycle. Additionally, formation of septa within the hyphae was visible, indicating cellular differentiation (Fig. 7.5 d). Similar septum formation during submerged fermentation in *Monascus* development.



Fig. 7.5. Microscopic images of *M. purpureus* C322 growth on Day-3, showcasing a-c) mycelium formation and sporulation, d) Noticeable septum formation marked by red arrows. The images were captured at 4x, 10x, 40x and 100x objective magnification.

7.2.2.5 Morphology of *M. purpureus* C322 Cultures on Day-4 of Submerged Fermentation

On Day-4 of the submerged fermentation, *M. purpureus* C322 cultures displayed initial signs of pigmentation within the mycelium (Fig. 7.6 a). The pigmentation phase in *Monascus* can be influenced by a variety of environmental factors, such as pH levels, temperature settings, agitation speeds, levels of dissolved oxygen, and the availability of nutrients within the growth medium. For instance, a study by Lee et al. (2001) and Patrovsky et al. (2019) indicated that the production of pigments in *M. purpureus* sp. ATCC 16365 and DBM 4360 respectively, is not only a feature of the stationary growth phase but also heavily reliant on the type of nitrogen source supplied in the medium, affecting both pigment intensity and type of pigment.



Fig. 7.6. Microscopic observation of *M. purpureus* C322 growth on Day-4 at different magnification, a) mycelium with visible pigment development, b) lipid and/or vesicles formation highlighted by black arrows; c) evident sporulation, d) developing clestothecia and aleuriconidia. The images were captured at 10x, 40x and 100x objective magnification.

Alongside pigmentation, lipids molecules and/or vesicles also became visible within the hyphae (Fig. 7.6 b), highlighting the onset of lipid biosynthesis. Rasheva et al. (1997, 1998) similarly reported an accumulation of lipid molecules and pigmentation within the mycelium of *M. purpureus* 94-25 strain, noticeable through microscopic examination. Lipids can play various roles, including as energy reserves, formation of vesicles, transportation of metabolites, and in the structural formation of cell membranes, marking the importance in the synthesis of pigments and lipids (Muro et al., 2014).

The development of conidia and cleistothecia were continuing at this stage (Fig. 7.6 c, d), signifying the progression of *Monascus* life cycle displaying both asexual and sexual reproduction simultaneously. Similarly, the sustained mycelial expansion by Day-4 indicates that the cultures were likely still within the exponential growth phase, supported by sufficient nutrients conducive to both biomass increase and secondary metabolite synthesis. Similar observations in terms of simultaneous asexual and sexual sporulation within *Monascus* cultures were drawn by several researchers (Carels and Shepherd., 1975; Vasilyeva et al., 2012; Pordel et al., 2015; Manan and Arif, 2015).

7.2.2.6 Morphology of *M. purpureus* C322 Cultures on Day-5 of Submerged Fermentation

By Day-5 of submerged fermentation, *M. purpureus* C322 cultures notably increased pigmentation within and around hyphae, as observed in Figure 7.7 (a). This observation aligns with the findings of Sibirny and Goksungur (2019) and Pan and Hsu (2014), who also reported enhanced pigmentation in *Monascus* cultures by Day-5 of fermentation. The type of pigment predominantly present in the culture medium is an indication of the metabolic activity of *Monascus* sp., which can be altered based on the culture conditions, such as the presence of elicitors or quorum sensing molecules that can trigger specific biosynthetic pathways (Resheva et al., 1998; Embaby et al., 2018; Erkaya et al., 2020; Chen et al., 2023). For instance, conditions that favour reduction reactions lead to increased production of yellow pigments, whereas conditions that enhance amination reactions result in greater red pigment production, illustrating how environmental factors can dictate metabolic routes in *Monascus* cultures and affect pigment synthesis (Agboyibor et al., 2018).

At this phase, although conidia formation persisted, hyphal branching and mycelial expansion became more pronounced, significantly influencing pellet development as depicted in Figure 7.7 (b). The emphasis on pellet development is crucial, as it directly influences the efficiency of fermentation processes, affecting both the yield and quality of secondary metabolites produced. The observed mycelial architecture and pellet morphology could significantly impact the diffusion of nutrients and metabolites within the culture, as suggested by Veiter et al. (2018).



Fig. 7.7. Microscopic observations of *M. purpureus* C322 on Day-5, indicated by black arrows; where a) mycelium displaying pigmentation (4x objective), b) hyphal branching with active sporulation (10x objective); c-d) developing ascogenous hyphae/cleistothecia (100x and 40x objective respectively). The images depict the development of mycelium using different magnifications and filters.

The hyphal tips, devoid of organelles, serve as pivotal sites for the secretion of extracellular proteins and metabolic products, facilitated by lipid vesicles delivering contents to the plasma membrane (Wösten et al., 1991; Steinberg, 2007; Riquelme et al., 2016). This focus on hyphal elongation and branching underpins the fungal strategy for efficient nutrient utilisation and

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metabolic output, essential for pellet maturation. Multiple ascogenous hyphae and cleistothecia with developing spores were observed underscoring the culture's progression into a asexual and sexually reproductive stage (Fig. 7.7 c, d). Suh and Shin (2000) corroborated these findings by documenting similar hyphal structures and reproductive behaviours in *Monascus* sp. J101, highlighting the multifaceted morphological characteristics across different strains.

7.2.2.7 Morphology of *M. purpureus* C322 Cultures on Day-6 of Submerged Fermentation

On Day-6 of fermentation, a notable increase in mycelial density was observed, suggesting availability of nutrients, particularly carbohydrates, which serve as critical energy sources for fungal cells (Lin and Demain., 1991; Omamor et al., 2008). At this phase, the culture predominantly focuses on proceeding to pellet formation, although there is a continuation of some conidia production and the maturation of cleistothecia (Fig. 7.8 a-d).



Fig. 7.8 (a-d). Microscopic images of *M. purpureus* C322 morphology on Day 6 at 4x and 10x objective magnification, showing the condensation of mycelium into pellets, accompanied by simultaneous sporulation.

Similar results were observed by Lyu et al. (2017) and Silbir and Goksungur (2019) in *Monascus* sp. sjs-6 and CMU001 respectively, and suggested that the simultaneous occurrence of these processes underlines the adaptive strategies of *Monascus* culture for survival and propagation, contributing to the potential for secondary metabolite production. Suh and Shin (2000) also noted increased mycelial elongation with a relatively decreased conidia formation in *Monascus* sp. J101 cultures around 120 h. The simultaneous development of mycelial pellets, conidia, and cleistothecia not only reflects the complexity of the fungal life cycle but also its implications for industrial fermentation processes.

7.2.2.8 Morphology of *M. purpureus* C322 Cultures on Day-7 of Submerged Fermentation

By Day-7 of submerged fermentation, the *M. purpureus* C322 cultures showcased interconnection between condensed mycelia (Fig. 7.9 b), facilitating efficient utilisation of space within the fermentation medium (Fig. 7.9 a). This phenomenon, where mycelial networks form complex, interconnected structures is a characteristic feature of filamentous fungi under fermentation conditions. Similar patterns of mycelial interconnection and network formation have been documented by Bebber et al. (2007) and Zhang et al. (2023), indicating a common adaptive strategy among filamentous fungi to optimise their growth and survival in submerged fermentation environments. Sporulation continued and aleurioconidia was observed at hyphal tips. Moreover, the newly formed spores were dispersed into the medium (Fig. 7.9 a-d). The continuation of sporulation at this stage highlights propagation and the ongoing reproductive activity within the culture. These observations draw parallels with the findings of Mayer et al. (2021), who described the formation of a network-like structure between the mycelia of *A. niger* during fermentation.



Fig. 7.9. Microscopic images depicting the growth of *M. purpureus* C322 on Day-7. The images display the interconnection between two individual mycelia, with objective magnifications of 4x (a) and 10x (b-d) respectively using different imaging filters.

7.2.2.9 Morphology of *M. purpureus* C322 Cultures on Day-8 of Submerged Fermentation

By the eighth day of fermentation, the mycelial growth in *M. purpureus* C322 cultures had formed small, uniformly sized pellets, approximately 0.5 cm in diameter, as depicted in Figure 7.10 (a-c). This pelletisation process is characterised by compact and densely interwoven hyphae, which not only enhances the structural integrity of the pellets but also facilitates the uniform distribution of nutrients and metabolites within the fungal mass (Veiter et al., 2018). Chen et al. (2017) and Shu et al. (2022), documented similar mycelial interconnectivity and pellet formation in submerged fermentation settings.

Concurrently, sporulation was actively proceeding, with the dispersal and subsequent germination of spores indicating a vigorous reproductive cycle within the culture (Fig. 7.10 ac). This phase also showcased cleistothecia with spores, alongside observable conidia formation (Fig. 7.10 d, e). The simultaneous engagement in both asexual and sexual reproduction mechanisms is a phenomenon substantiated by Carels and Shepherd (1975), Rasheva et al. (1997, 1998), Vasilyeva et al. (2012), Pordel et al. (2015), and Husakova et al. (2021) in their studies on *Monascus* sp. The presence of mature cleistothecia with spores is a critical indicator of the sexual reproductive cycle reaching fruition, contributing to the genetic diversity and resilience of the fungal population.





Fig. 7.10. Microscopic images of *M. purpureus* C322 growth on Day-8; where a), b), c) Mycelium at 4x objective magnification, d) ascogenous hyphae at 100x, e) highlighting different aspects of mycelium using different imaging filters at 40x objective magnification.

Furthermore, the enhanced pigmentation observed at this stage is indicative of the accumulation of secondary metabolites, both within the mycelial cells and in the extracellular environment surrounding the pellets, suggesting elevated production of bioactive compounds, such as pigments and other metabolites, having applications in food, pharmaceuticals, and other industries.

7.2.2.10 Morphology of *M. purpureus* C322 Cultures on Day-9 of Submerged Fermentation

Similar patterns of sporulation and dispersion of spores as observed on Day-8 were noted in *M. purpureus* C322 cultures on Day-9 of fermentation, with some improvement in pigmentation and pellet expansion (Fig. 7.11 a-f). Research conducted by Chai et al. (2020) and Zhang et al. (2023) noted a significant impact of pellet morphology on the biosynthesis and accumulation of pigments and other valuable secondary metabolites in *Monascus* sp. LQ-

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6 and RP2, respectively. Similarly, in *Rhizopus oryzae*, pellet formation has been shown to enhance the production of lactic acid and fumaric acid (Liao et al., 2007).



Fig. 7.11. Microscopic images of *M. purpureus* C322 mycelia on Day-9 of submerged fermentation; where a), b), c) depicts pellets at 10x objective magnification and d), e), f) displays longer hyphae with terminated sporulation at 40x and 100x objective magnification using different imaging filters.

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Kim and Song (2009) discovered that the pellet size of the white rot fungus *Pleurotus ostreatus* directly influences its biodegradation capabilities, with biodegradation rates being directly linked to the activity of laccase and esterase enzymes, where cultures with smaller pellets were found to be the most conducive for the optimal functioning of these degradative enzymes. Furthermore, research has consistently shown that compact agglomerates and pellets are advantageous for the production of secondary metabolites, e.g. citric acid production in *A. niger* is enhanced when the pellet diameter is less than 0.5 mm in diameter (Papagianni & Mattey, 2006; Papagianni, 2007; Zhang & Zhang, 2016). These studies emphasise the importance of the specific structure and composition of the pellets in enhancing metabolic efficiency, leading to an enriched production of compounds of interest.

7.3 Summary

Microscopic analysis of *M. purpureus* C322 cultures from submerged fermentation revealed a well-organised and steady growth pattern, with germination of spores and hyphal development occurring within 24 hours of inoculation. *M. purpureus* C322 displayed both sexual and asexual reproduction at several stages throughout fermentation via formation of cleistothecia and aleurioconidia respectively. Although the development of cleistothecia occurred at early stages; the spore formation within the cleistothecia occurred during the later stages of fermentation. Sporulation was observed throughout the course of fermentation, with pigmentation becoming visible from Day-3. By Day- 5, the mycelium started condensing to initiate pellet formation, and by Day-7, dense and mature pellets (~0.5 mm diameter) were formed. These findings are consistent with previous studies on *Monascus* growth and development of other *Monascus* strains, which have reported similar patterns of mycelium and pellet formation under submerged fermentation conditions (Seenivasan, 2016; Silbir et al., 2019).

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Section

Conclusion

Concluding Experimental Results

This study elucidates the optimal conditions for the influence of elicitors and quorum sensing molecules on the cultivation and metabolic productivity of *M. purpureus* C322, highlighting potential avenues for enhancing biotechnological applications. The findings demonstrate that specific culture conditions and the strategies for the supplementation of the specified molecules can substantially impact the production of valuable metabolites such as pigments and lovastatin in both shaken flasks and bioreactor setups.

Optimisation experiments revealed that for shake flask cultures of *M. purpureus* C322, optimal growth, and productivity were achieved at a temperature of 25°C, a rotational speed of 120 rpm, and a pH of 6.5. In contrast, the bioreactor cultures performed best under the original settings without the need for further optimisation, maintaining a temperature of 32°C, a rotational speed of 300 rpm, pH 6.5, and an inoculum volume representing 15% of the working volume. These findings underscore the adaptability of *M. purpureus* C322 to different fermentation environments and highlight the importance of tailored cultural conditions for maximising metabolic outputs.

The investigation into the effects of elicitation and quorum sensing on *M. purpureus* C322, conducted in both shaken flasks and 2.5 L stirred tank fermenters, revealed notable enhancements in the production of pigments and lovastatin. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test (*p*-value<0.01) confirmed that elicitor treatments led to significant increases in metabolite yields compared to the control across all tested conditions. The addition of elicitors (oligoguluronate, oligomannuronate, mannan oligosaccharides) significantly enhanced the yields of yellow, orange, and red pigments, as well as lovastatin, across both experimental setups, demonstrating both the efficacy and scalability of this strategy (*p*-value<0.01). The results suggest potential for application in

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larger-scale production environments. Notably, OM emerged as the most effective elicitor, yielding the highest production of yellow and red pigments as well as lovastatin, while OG was particularly successful in increasing orange pigment output.

The quorum sensing experiments revealed the role of specific quorum sensing molecules in enhancing metabolite production. The QSMs—farnesol, tyrosol, and linoleic acid—resulted in statistically significant enhancements in the production of all pigment types and lovastatin compared to the control (*p*-value<0.01) in both shaken flasks and 2.5 L stirred thank fermenters. Among these, farnesol at 0.2 mM yielded the highest overall production. Conversely, Butyrolactone-I, despite being tested at various concentrations, did not demonstrate any notable impact on pigment or lovastatin production, performing comparably to the control groups, suggesting that, it may require further adjustments or combination with other treatments to maximise their yield. Thus, the findings of this study, highlight the crucial roles of specific quorum sensing molecules (F, T, LA) in enhancing the metabolic outputs of *M. purpureus* C322.

Furthermore, genomic studies provided a foundation for understanding the genetic basis of these responses. The whole-genome sequencing of *M. purpureus* C322, carried out for the first time, not only furnished a comprehensive map of its genetic architecture but also identified specific genes associated with quorum sensing pathways. These genetic insights are crucial for future manipulations aimed at enhancing metabolite production.

This comprehensive investigation has advanced our understanding of the optimal cultivation conditions and metabolic modulation of *M. purpureus* C322 and has also set the stage for innovative applications in the biotechnology industry. The combination of genomic insights with the proven efficacy of elicitation and quorum sensing strategies provides a solid foundation for future research focused on improving the commercial production of pigments

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and lovastatin. The scalability and reproducibility observed during these experiments further affirm their industrial relevance, particularly in meeting the growing demand for natural pigments and pharmaceutical compounds. Moving forward, these insights could guide the development of refined biotechnological methods tailored to harness further the potential of microbial fermentation processes.





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Future Work

Future Directions for *M. purpureus* C322 Research

The results from the optimisation experiments in shaken flasks suggest that a growth temperature of 25°C is optimal. Therefore, this temperature setting can be explored in 2.5 L stirred tank bioreactors to determine if these conditions would further enhance the pigment and lovastatin production.

The current study has demonstrated that elicitors (OG, OM, MO) and quorum sensing molecules (QSMs) like farnesol, tyrosol, and linoleic acid (LA) positively impact lovastatin and pigment production. Future studies could focus on further optimising the concentrations of these compounds to potentially boost the already enhanced production yields further. Additionally, scaling up these experiments to pilot and industrial fermenters could validate the reproducibility and scalability of the observed effects. It would also be worthwhile to extend these experiments to other microorganisms to assess the general applicability and effectiveness of elicitation and quorum sensing strategies across different species.

Despite the lack of impact from Butyrolactone-I at concentrations between 100-300 nM, literature suggests that gamma-butyrolactone has successfully enhanced lovastatin production in other studies. A revaluation of the concentration range for Butyrolactone-I, possibly increasing it as per literature suggestions, or testing gamma-butyrolactone itself could provide new insights into its efficacy in enhancing lovastatin and pigment production in *M. purpureus* C322.

Additionally, during the quorum sensing experiments, the supplementation of linoleic acid produced a noticeable sweet fragrance, suggesting the formation of volatile aromatic compounds. Future work could include Gas Chromatography (GC) analysis to identify these aromatic compounds, enhancing understanding of the metabolic pathways activated during these experiments.

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Future Work

This study demonstrated the effectiveness of quorum sensing molecules and elicitors in enhancing the production of *Monascus* pigments and lovastatin, both known for their antimicrobial activity. Future work could focus on evaluating the antimicrobial efficacy of these enhanced metabolites against a wider range of pathogens, including multidrug-resistant strains. In addition, efforts toward process scale-up, formulation into stable antimicrobial preparations, and investigation of potential synergistic effects with existing antibiotics or preservatives could support the development of novel therapeutic or food-grade antimicrobial agents.

Furthermore, gene knockout studies targeting the annotated QS genes could be instrumental in understanding the specific roles these genes play in the metabolic pathways of *M. purpureus* C322. Such studies would provide a deeper insight into the genetic basis of lovastatin and pigment production and could lead to more targeted approaches in strain improvement for industrial applications.

Appendix



Supplementary Information on Elicitation Methods

This section of appendix provides detailed information on elicitation methods using physical, hormonal and chemical elicitors. While the main focus of the thesis centres on carbohydrate elicitors and their effects on *M. purpureus* C322, the content here provides supplementary information on biotic and abiotic elicitors, that play a significant role in enhancing metabolic production in various microbial and plant systems. The information is included to offer a broader context and deeper understanding of the scope of elicitation techniques, supporting the methodologies and discussions presented in the main sections of this thesis.

A) Abiotic Elicitation: Utilising Non-Biological Stimuli to Boost Metabolite Synthesis

The abiotic elicitors can be classified into two categories: physical and chemical stresses.

I) **Physical Elicitors**

Physical elicitors encompass factors such as light, osmotic stress, salinity, drought, and thermal stress.

Light Stress

Light exposure can significantly influences the biosynthesis of secondary metabolites in fungi and microalgae, acting as a key factor in metabolic regulation (Hagimori et al., 1982; Rozema et al., 2002; Kumari et al., 2009). Light-dependent regulators, such as *VeA*, are crucial for growth, development, and secondary metabolite production in several organisms, including filamentous fungi like *Aspergillus nidulans*. *VeA* is particularly expressed in the dark, and its absence in mutant *A. nidulans* cells disrupts both sexual fruiting-body formation and the production of secondary metabolites, highlighting its pivotal role in linking environmental light conditions to fungal secondary metabolism. (Bayram et al., 2008; Pandit et al., 2018). In *Monascus* sp., red and blue light affect mycelial growth, spore formation, and secondary

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metabolite production, with red light enhancing gamma-aminobutyric acid, red pigments, monacolin K, and citrinin synthesis, while blue light suppresses citrinin biosynthesis (Miyake et al., 2005; Zhang et al., 2017; Yue et al., 2020; Farawahida et al., 2022). In *F. verticillioides* and *A. niger* cultures red and blue light exposure influences fumonisin B2 synthesis, highlighting the regulatory role of light on gene expression and metabolite production (Fanelli et al., 2012; Wang et al., 2012).

Osmotic Stress

Osmotic stresses induces changes in gene expression, impacting the transcription and translation processes and subsequently influencing metabolite production (Sevcikova and Kormanec, 2004; Takagi et al., 2006; Duan et al., 2012). For example, studies have shown that osmotic stress enhances the production of enzymes such as glucose oxidase in *A. niger* and increase glutathione levels in *Candida utilis* (Fiedurek, 1998; Liang et al., 2009). Similarly, osmotic stress was also found to affect the polar growth in *Streptomyces* sp. and stimulates antibiotic production through redD gene expression in *S. coelicolor* under high salt conditions, highlighting the intricate relationship between environmental stress and secondary metabolism (Gray et al., 1990; Koch et al., 1994; Sevcikova and Kormanec, 2004). Changes in osmolarity is found to influence mycotoxin synthesis in fungi like *Penicillium* and *Aspergillus*, indicating osmotic stress as an effective elicitor of secondary metabolites (Ochiai et al., 2007). For instance, *A. flavus* increases aflatoxin production under non-ionic solute stress, and *Penicillium nordicum* produces ochratoxin A under high-salt conditions, directly linking osmotic stress to metabolite production (Medina et al., 2015; Rodriguez et al., 2014).

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I Thermal Stress

Temperature fluctuations represent a notable stressor for microorganisms, markedly impacting microbial growth, development, and virulence. Such that thermal stress acts as an abiotic elicitor, enhancing the production of microbial metabolites by influencing protein synthesis, including the generation of heat shock proteins (HSPs), which are essential for various cellular processes (Garcia et al., 2010; Nicolaou et al., 2010; Gao et al., 2016; Chen et al 2022). For instance, heat shock treatment increased glycerol and jadomycin B production in *S. cerevisiae* and *Streptomyces venezuelae* cultures, respectively (Doull et al., 1993, 1994; Omori et al., 1996, 1997). The heat shock response, inducing proteins like *Hsf1*, is crucial for fungal adaptation and maintaining virulence in species such as *S. cerevisiae* and *C. albicans* (Morano et al., 2012; Leach et al., 2012; Veri et al., 2018). Similarly, the regulatory Velvet protein complex, responsible for virulence and mycotoxin production in fungi such as *A. fumigatus*, is influenced by temperature-dependent pathways (Lind et al., 2016; Wang et al., 2019; Zhang et al., 2019). Interestingly, a temperature-dependent dimorphic switching (*areA* gene) is observed in *Penicillium marneffei*, highlighting its role in adapting to environmental stress (Todd et al., 2003; Bugeja et al., 2012).

D pH Stress

pH impacts microbial metabolism, development, and secondary metabolite production. In fungi, the Pal/Rim pathway is crucial for adapting to environmental pH variations, ensuring survival under diverse conditions. Alkaline pH challenges fungal growth and pathogenicity by altering nutrient transport and ATP synthesis, leading to changes in gene expression. To maintain pH homeostasis in acidic environments, fungi ,limit proton permeation, boost proton pumps, and consume intracellular protons (Macalady and Banfield, 2003; Guan et al., 2013 and 2020; Xiong et al., 2014). Adjusting pH enhances the production of antibiotics, pigments, and mycotoxins in fungi. For instance, pH stress significantly increases red pigment production
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in *M. purpureus* and influences mycotoxin biosynthesis in *Aspergillus* and *Fusarium* sp. (Cotty, 1988; Orosco and Kilikian, 2008; Gardiner et al., 2009). Changes in culture pH from acidic to neutral boost siderophore production in *A. nidulans* (Eisendle et al., 2004). In *Penicillium expansum*, extreme pH levels inhibit spore germination by altering intracellular pH and ATP levels, with proteomic analysis showing changes in proteins related to synthesis and folding, indicating that ambient pH can modulate germination through protein expression alterations (Li et al., 2010).

Starvation Stress

Starvation stress, such as phosphate or nitrogen deficiency, modulates metabolite production in various organisms (Tavares et al., 1999; Hailei et al., 2009). For example, *M. purpureus* YY-1 shows increased pigmentation under carbon starvation and acetyl CoA elicitation (Yang et al., 2015). Similarly, *Debaryomyces hansenii* exhibits enhanced enzyme synthesis and increased xylitol levels under glucose starvation, highlighting the potential of environmental stresses in bioengineering and industrial biotechnology (Tavares et al., 1999; Hailei et al., 2009). In plants, nutrient deficiency leads to the accumulation of phenolic compounds, indicating a universal stress-induced metabolic reprogramming. For instance, long-term nitrogen deficiency in yarrow (*Achillea collina*) results in higher levels of phenolic acids in leaves and roots compared to control plants (Bongue-Bartelsman and Phillips, 1995; Kováčik et al., 2007; Giorgi et al., 2009).

Oxidative Stress

Oxidative stress regulates secondary metabolism in microbes and plants through specific transcription factors. For example, the transcription factor *AtfB* coordinates the activation of antioxidant genes with the synthesis of secondary metabolites like aflatoxins, establishing a link between stress responses and metabolite production (Hong et al., 2013). For instance, in

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P. aeruginosa, H₂O₂ treatment upregulates the *rhlAB* promoter, essential for rhamnolipid biosynthesis, demonstrating the complex regulatory networks involved in oxidative stress responses (Santamaria et al., 2022). The HOG (high osmolarity glycerol) pathway is crucial for managing oxidative stress and modulating morphology, virulence, and secondary metabolite production (Alonso et al., 2003; Bilsland et al., 2004; Enjalbert et al., 2006). In *C. albicans* and *S. cerevisiae*, it induces filamentous growth in response to oxidative stress, demonstrating resilience. For instance, deletion of the HOG1 gene in *C. albicans* mutants results in altered colony morphology and increased susceptibility to compounds like nikkomycin Z and Congo red, which disrupt cell wall functionality (Monge et al., 1999; Correia et al., 2020; Yaakoub et al., 2022).

I Nitrosative Stress

Microorganisms are also susceptible to nitrosative stress from high levels of reactive nitrogen species (RNS) like nitric oxide (NO) and its derivatives, impacting their pathogenicity and infection mechanisms in both human and plant hosts. This type of stress damages proteins through reactions with thiols and metal centres. Fungi counteract RNS through antioxidants like glutathione. This process involves converting nitric oxide (NO) into S-nitrosoglutathione (GSNO), which is then recycled back to glutathione by the enzymes GSNO reductase (GSNOR) and glutathione reductase. This highlights an evolutionarily conserved detoxification mechanism (Liu et al., 2001; Tillmann et al., 2015). The response to RNS varies among fungi, affecting gene expression profiles related to RNS detoxification and repair mechanisms. In *S. cerevisiae* and *C. albicans*, for example, exposure to RNS leads to altered expression of genes like *Yhb1* and those involved in glutathione synthesis, mediated by different transcription factors across species (De Hesus et al., 2003; Ullmann et al., 2004; Hromatka et al., 2005; Chiranand et al., 2008). Additionally, nitrosative stress also induces upregulation of transaldolase in these species, suggesting an increased production of NADPH

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via the pentose phosphate pathway to combat stress (Steen et al., 2003; Missall et al., 2006; Larochelle et al., 2006).

II) Chemical Elicitors

Heavy metals are increasingly present in the environment due to industrial and agricultural activities, posing a threat to living organisms (Cai et al., 2013). While the effects of heavy metals on plant growth are well-documented, their impact on secondary metabolite production is less understood. Heavy metals can alter metabolic activity in plants, affecting the production of pigments, sugars, proteins, and nonprotein thiols, possibly by inhibiting enzymes involved in natural product synthesis (Nasim et al., 2010). The regulation of secondary metabolite production by heavy metals primarily occurs at the transcriptional level (Weinberg, 1990; Zhao et al., 2001). For instance, *Brassica juncea* exhibited increased oil content in response to metal (Cr, Fe, Zn, Mn) accumulation (Singh et al., 2005). Treatment with Cu²⁺ and Cd²⁺ led to the highest accumulation of secondary metabolites such as shikonin and digitalin (Mizukami et al., 1977; Ohlsson et al., 1989). Similarly, Mn²⁺, Fe³⁺, and Zn²⁺ have been identified as key metals influencing secondary metabolism in microorganisms (Paranagama et al., 2007). Furthermore, Cu^{2+} , Cd^{2+} , and Cr^{3+} have been shown to increase the production of polyketide monocillin I by the fungus Paraphaeosphaeria quadriseptata (Paranagama et al., 2007). Interestingly, Tseng (2001) discovered that even low concentrations of sodium nitrite (<0.2 g/L) act as elicitors, enhancing mycelial growth and pigment production.

Organic compounds like ethanol and dimethylsulfoxide (DMSO) have also been used to stimulate secondary metabolite biosynthesis. Although their exact mechanisms remain unclear, they are favoured for their ease of use and cost-effectiveness. In the presence of 3% DMSO, *Streptomyces venezuelae* and *Streptomyces glaucescens* showed a threefold increase in chloramphenicol and tetracenomycin C production, while *Staphylococcus aureus* exhibited

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two-fold higher thiostrepton production (Chen et al., 2000). Similarly, ethanol (1%) induced the synthesis of a new chlorinated benzophenone antibiotic called pestalone in the marine fungus *Pestalotia* sp. (Cueto et al., 2001). Furthermore, compounds like goadsporin and 7ae have also shown to stimulate the production of secondary metabolites like antibiotics in various streptomyces strains (Onka et al., 2001, 2009; Foley et al., 2009).

Furthermore, enzyme inhibitors can also impact secondary metabolite production. The P450 inducer, phenobarbital, for instance, enhances ganoderic acid levels, leading to increased production of anti-tumour ganoderic acids (Liang et al., 2010). Similarly, chemical inhibitors like pyridine and imidazole stimulated lycopene formation in *Blakeslea trispora* by inhibiting enzymes responsible for lycopene cyclisation (Bode and Zeeck, 2000). Interestingly, the epigenetic manipulations using small-molecule inhibitors targeting histone deacetylase and DNA methyltransferase activities have led to the discovery and enhanced production of novel secondary metabolites, such as lunalides A and B polyketides, demonstrating the potential of these strategies in unlocking microbial biosynthetic capabilities (Shwab et al., 2007; Fox and Howlett, 2008; Williams et al., 2008; Henrikson et al., 2009).

B) Biotic Elicitation: The Influence of Hormones on Metabolic Activation

Hormonal elicitors, including jasmonates (JA and MeJA), salicylic acid (SA), and gibberellin (GA), enhance secondary metabolite production in various organisms; though they are predominantly applied for elicitation purposes in plant systems. (Creelman et al., 1997; Sembdner et al., 1993; Pauwels et al., 2009). They have been found to play a crucial role in modulating the production of secondary metabolites and offer valuable tools for biotechnological applications in various organisms (Halder et al., 2019). Jasmonic acid (JA) and salicylic acid (SA), along with their derivatives, are among the most extensively researched elicitors due to their critical functions in plant defence mechanisms. For instance, in

microalgae, the addition of phytohormones like Indole-3-acetic acid (IAA) and diethyl aminoethyl hexanoate (DAH) has been shown to significantly increase lipid content (15 g/L IAA, 10⁻⁵ M DAH) and affect biofilm formation (10 g/L IAA, 10⁻⁵ M DAH), demonstrating the potential of hormonal elicitors in microorganisms (Salama et al., 2014; Stirk and Staden, 2020; Hakim et al., 2023).

The following sections further detail the commonly utilised hormonal elicitors; namely jasmonic acid, salicylic acid and gibberellic acid.

Jasmonic Acid

Jasmonates, like jasmonic acid (JA) and methyl jasmonate (MeJA), belong to the cyclopentanone family of compounds and play a crucial role in regulating diverse plant physiological responses (Creelman et al., 1997; Sembdner et al., 1993). They are recognised as potent elicitors that significantly boost the production of secondary metabolites within in-vitro plant cultures. As key elicitors, jasmonates activate numerous plant secondary metabolic pathways, especially under specific environmental stress conditions, leading to enhanced synthesis of secondary metabolites (Pauwels et al., 2009). For instance, jasmonates (100-200 µM) have been shown to stimulate the biosynthesis of secondary metabolites such as rosmarinic acid (1.5-fold), terpenoid indole alkaloids (6.95-fold) and plumbagin (5.59-fold) in various plant cell cultures, demonstrating their effectiveness in promoting secondary metabolite production (Krzyzanowska et al., 2012; Almagro et al., 2014; Silja et al., 2014).

Gao et al. (2015) found that jasmonic acid (JA) and salicylic acid (SA) notably enhance astaxanthin production in *Haematococcus pluvialis* when supplemented at 25 or 50 mg/L. The impact is dose-dependent, with 25 mg/L of JA affecting *pds*, *crtR-B*, and *lyc* genes, while 50 mg/L of JA influences *ipi-1*, *ipi-2*, *psy*, *crtR-B*, and *crtO* genes. Additionally, 5 mg/L fulvic acid induces an 86.89% increase in astaxanthin content, upregulating phytoene desaturase

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(PDS), lycopene cyclase gene (LCY), and β -carotene hydroxylase (CHY) genes (Zhao et al., 2015). Similarly, Kim et al. (2009) observed that Methyl Jasmonate (MJ) induces farnesyl pyrophosphate synthase (FPS) expression, leading to the precursor for squalene biosynthesis. MJ treatment was also found to upregulates squalene synthase (SS) and squalene epoxidase (SE) genes in *P. notoginseng* and *Panax ginseng* (Rahimi et al., 2015; Kim et al., 2009). Some studies have explored the impact of MJ in enhancing pigment production within plant species and found that the addition of MJ affected silymarin pigment accumulation in *Silybum marimum* (100 μ M MJ), and increases solasodine content in *Solanum trilobatum* hairy root cultures by 6.5-fold (4 μ M MJ) (Sampedro et al., 2005; Baek et al., 2020; Xing et al., 2018).

G Salicylic Acid

Salicylic acid (SA), recognised for its role in mediating plant defence responses, also acts as a potent elicitor of secondary metabolites across various organisms (Hayat et al., 2010; Pieterse et al., 1999; Vlot et al., 2009; Huijsduijnen et al., 1989). Its efficacy extends beyond plants; for example, a study by Farag et al. (2017) reported elevated diterpenoid levels in the coral *Sarcophyton ehrenbergi* following SA treatment. In *Salvia miltiorrhiza*, SA supplementation was found to significantly increase tanshinone production, underscoring its utility in enhancing the synthesis of valuable secondary compounds (Xiaolong et al., 2015). Moreover, recent research by Hu et al. (2022) utilised metabolomic and transcriptomic analyses to demonstrate SA's capability to enhance antibiotic and polyketone production in *Pleurotus ostreatus*, highlighting as a critical elicitor in the production of secondary metabolites, offering promising avenues for its application in enhancing the yield of bioactive compounds in both plant and microbial systems.

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Gibberellic Acid

Gibberellin (GA), a plant hormone, plays a pivotal role in regulating a multitude of developmental stages, including germination, dormancy, flowering, and the expansion of stems, leaves, and fruits. (Hedden and Sponsel, 2015). Notably, gibberellic acid (GA) is acclaimed for its growth-promoting attributes, which have been demonstrated to significantly boost biomass in diverse organisms such as E. purpurea, Datura, and Artemisia hairy roots, corroborated by findings from Liang et al. (2013), Abbasi et al. (2012), Ohkawa et al. (1989), and Ali et al. (2015). Moreover, GA's influence extends to the stimulation of secondary metabolites, showcasing its dual functionality. This is evidenced by research indicating GA's ability to markedly increase artemisinin content in Artemisia annua hairy root cultures, as documented by Smith et al. (1997) and Banyai et al. (2011). Further illustrating GA's versatility, studies have shown its efficacy in enhancing the biosynthesis of caffeic acid derivatives in S. miltiorrhiza and tanshinone in Echinacea purpurea hairy root cultures (Abbasi et al., 2012; Yuan et al., 2008), underscoring the hormone's broad applicability in promoting both growth and secondary metabolite production across a spectrum of species. This comprehensive role of GA in both developmental regulation and metabolite elicitation underscores its potential as a key agent in the optimisation of biomass and valuable compound production within biotechnological applications.

Section II: Troubleshooting

Issue I: Precipitation

It was observed that salts from the growth media precipitated when autoclaved together (Fig.

- 1 a). To resolve this issue, four separate solutions were prepared and sterilised individually:
 - 1. MgSO4.7H2O solution: Autoclaved at 120°C for 15 minutes.
 - 2. Other salts solution: Autoclaved at 120°C for 15 minutes.
 - 3. Glucose solution: Autoclaved at 110°C for 10 minutes.
 - 4. Trace elements solution: Not autoclaved due to potential oxidation of FeSO4.7H2O at high temperatures, which causes a colour change (not precipitate) Instead, it was filtersterilised using a 0.22 µm filter (Millec GV Filter unit). This method effectively removes any microbial contaminants without altering the chemical composition of the trace elements.

Note: The trace element solution was prepared using sterilised DI water.

Once all the solutions were autoclaved (where applicable) and cooled to room temperature, they were combined and stored for future use (Fig. 1 b).







Fig. 1. Defined medium; a) Before (b) After troubleshooting precipitation issues.

Issue II: Mycelial fragments in Spore Suspension

Small fragments of mycelium in the spore suspension led to nonuniform growth during shaken flask experiments, as the mycelium began to form pellets before spore germination, which potentially causing clump formation and repeatability issues. To address this problem, a few sterile glass beads were carefully added on top of the pellet (Chapter II, Section 2.2.4) to trap any mycelia prior to resuspension with saline solution. Once saline solution was added, the pellet was gently flicked and the falcon tube was gently shaken to release spores into saline solution whilst trapping the mycelial fragments (Fig. 2). The spore solution above the glass beads was then pipetted into a fresh falcon tube and stored at 4°C for future use.



Fig 2: Spore suspension depicting trapped hyphae under sterile glass beads

Issue 3: Foaming and Sampling

Two problems were encountered during the test round of fermentation

- The sampling port was too narrow to extract *Monascus* pellets
- At the early stage of fermentation, foaming was detected (Fig. 3 a, b).

To overcome these problems, the following steps were taken:

- The sampling port was replaced by a 10 mm diameter tube (316 stainless steel)- 1 mm thickness (Fig. 3 e), fixed manually into the headplate using precisely cut septum (Fig. 3 d). The efficiency of the new inoculating tube was tested by sampling water and no leaks were detected in the new assembly.
- To reduce foaming, the rotational speed was gradually increased by 100 rpm every hour, starting from 100 till it reached 300 rpm at the start of fermentation. This gradual increase solved the foaming problem at the beginning of fermentation (Fig. 3 c).



Fig. 3. Fermentation run; a) Foam formation, b) Close up of foam formation, c) After optimisation, d) New sampling tube assembly, e) Sampling tube 10 mm diameter.

Section II: Standard Curves and HPLC Chromatograms

This section presents the standard curves for carbohydrate and lovastatin quantification, alongside HPLC chromatograms for lovastatin standard and experimental samples. Detailed analyses of lovastatin production are provided, encompassing results from elicitation and quorum sensing experiments. These data are essential for understanding the impact of various experimental conditions on lovastatin yield.



Fig. 4. Standard Glucose Curve.



Fig. 5. Chromatogram displaying lovastatin standards.



Fig. 6. Standard Lovastatin Curve.



Fig. 7. The HPLC chromatograms depict the concentration of lovastatin within each flask group (C, OG, OM, MO) on Day-18 of Shake Flask fermentation. Each elicitor was added at a concentration of 150 mg/L to the respective flask group at 48 h of fermentation. The distinct peaks represent the lovastatin levels in the respective groups, revealing variations in production across different fermentation conditions.



Fig. 8. The HPLC chromatograms depict the concentration of lovastatin within each bioreactor group (C, OG, OM, MO) during fermentation in 2.5 L Stirred Tank Bioreactors. Each elicitor was added at a concentration of 150 mg/L to the respective flask group at 24 h of fermentation. The distinct peaks represent the lovastatin levels in the respective groups, revealing variations in production across different fermentation conditions.



Fig. 9. The HPLC chromatograms depict the concentration of lovastatin within each flask group (C, T, F, LA) on Day-18 of Shake Flask fermentation. Each QSM was added to the respective flask group at specified concentrations 48 h into fermentation. The distinct peaks represent the lovastatin levels in the respective groups, revealing variations in production across different fermentation conditions.



Fig. 10. The HPLC chromatograms depict the concentration of lovastatin within each bioreactor group (C, T, F, LA) during fermentation in 2.5 L Stirred Tank Bioreactors. Each QSM was added to the respective flask group at the specified concentration 24 h into fermentation. The distinct peaks represent the lovastatin levels in the respective groups, revealing variations in production across different fermentation conditions.

Section III: ANNOVA

This section presents detailed ANOVA tables derived from optimisation, elicitation, and quorum sensing experiments conducted in this study. ANOVA, or Analysis of Variance, is utilised to assess the statistical significance of differences among group means. With an alpha factor of 0.05, significance levels are determined, shedding light on the impact of various factors studied in these experiments. The ANOVA tables offer insights into the relationships between experimental variables and outcomes, contributing to a comprehensive understanding of the experimental findings.

Chapter 3- Optimisation

Table 1: Analysis of variance (ANOVA) for biomass concentration and pigment production in samples cultured at different pH.

Source of Variation between Groups	P-value
Total CDW (g/L)	1.50E ⁻¹¹
Yellow Pigment Conc. at 400nm	2.62E ⁻¹⁵
Orange Pigment Conc. at 470nm	4.77E ⁻¹⁵
Orange Pigment Conc. at 470nm	4.48E ⁻¹⁷

Source of Variation- Between Experiments			P-value	
pH	CDW	Yellow	Orange	Red
рН 3.0	0.184	0.112	0.320	0.477
pH 4.0	0.853	0.928	0.128	0.340
рН 5.0	1.000	1.000	1.000	1.000
рН 6.0	0.006	0.022	0.036	0.385
рН 6.5	0.374	0.101	0.288	0.768

Below is a comprehensive analysis of ANOVA.

ANOVA							
Source of Variation	b/w						
Different pH Group	os SS	5	dj	f MS	F	P-value	F crit
Cell Dry Weight	39.3150)1333	4	9.828/53	49.77676	1.5E-11	2.75871
Yellow Pigment	45.9420)8	4	11.48552	106.2235	2.62E-15	2.75871
Orange Pigment	48.9882	21333	4	12.24705	100.9256	4.77E-15	2.75871
Red Pigment	56.3884	16667	4	14.09712	149.6668	4.48E-17	2.75871
ANOVA							
Source of Variation							
<i>b/w Different</i>							
Experiments pH 3.0	SS	df		MS	F	P-value	F crit
Cell Dry Weight	0.02535		1	0.0253	5 2.573604	0.183927	21.19769
Yellow Pigment	0.004267		1	0.00426666	7 4.129032	0.111957	21.19769
Orange Pigment	0.000817		1	0.000816667	1.289474	0.31957	21.19769
Red Pigment	0.000267		1	0.000266667	0.615385	0.476621	21.19769
ANOVA							
Source of Variation							
b/w Different							
Experiments pH 4.0	SS	df		MS	F	P-value	F crit
Cell Dry Weight	0.006667		1	0.00666	7 0.038835	0.853384	21.19769
Yellow Pigment	0.00015		1	0.0001	5 0.009288	0.927859	21.19769
Orange Pigment	0.019267		1	0.01926	7 3.658228	0.128353	21.19769
Red Pigment	0.030817		1	0.030817	1.170994	0.340073	21.19769

ANOVA						
Source of Variation						
<i>b/w Different</i>				_		
Experiments pH 5.0	SS	df	MS	F	P-value	F crit
Cell Dry Weight	0	1	0	0	1	4052.181
Yellow Pigment	0	1	0	0	1	4052.181
Orange Pigment	0	1	0	0	1	4052.181
Red Pigment	0	1	0	0	1	4052.181
ANOVA						
Source of Variation						
b/w Different						
Experiments pH 6.0	SS	df	MS	F	P-value	F crit
Cell Dry Weight	0.24	1	0.24	28.8	0.00582	21.1977
Yellow Pigment	1	0.481667	13.13636	0.0223	21.1977	1
Orange Pigment	0.24	1	0.24	9.6	0.03628	21.1977
Red Pigment	0.06	1	0.06	0.94737	0.38550	21.1977
ΔΝΟΥΔ						
Source of Variation						
h/w Different						
Experiments pH 6.5	SS	df	MS	F	P-value	F crit
Cell Dry Weight	0.006667	1	0.006667	1	0.373901	21.19769
Yellow Pigment	0.015	1	0.015	4.5	0.101192	21.19769
Orange Pigment	0.015	1	0.015	1.5	0.287864	21.19769
Red Pigment	0.001667	1	0.001667	0.1	0.767644	21.19769

Table 2. Effect of Rotation Speed on Biomass and Pigment Concentration: One-Way ANOVA Results

Source of Variation between Groups	P-value
Total CDW (g/L)	3.51E ⁻¹⁰
Yellow Pigment Conc. at 400nm	3.12E ⁻¹²
Orange Pigment Conc. at 470nm	8.73E ⁻¹²
Orange Pigment Conc. at 470nm	1.17E ⁻¹⁴

	P-value						
Source of Variation between Experiments	CDW	Yellow	Orange	Red			
120rpm	0.226	0.468	0.539	0.21			
180rpm	0.482	0.036	0.08	0.399			
250rpm	0.467	0.07	0.715	0.768			

Below is a comprehensive analysis of ANOVA.

ANOVA							
Source of Variation b	o/w						
Different RPM Grou	ps SS		df	MS	F	P-value	F crit
Cell Dry Weight	26.55444		2	13.27722	129.1838	3.51E-10	3.68232
Yellow Pigment	26.01444		2	13.00722	249.0745	3.12E-12	3.68232
Orange Pigment	36.36333		2	18.18167	216.1625	8.73E-12	3.68232
Red Pigment	52.93778		2	26.46889	532.9306	1.17E-14	3.68232
ANOVA							
Source of Variation							
b/w Different	CC	đf		MS	$m{F}$	Dyalua	E avit
Call Dry Weight	0.166667	иј	1	0 16666	<u> </u>	<i>I -value</i>	21 10760
Cell Dry weight	0.100007		1	0.10000	5 0 6 4 2 8 5	0.220325	21.19769
Yellow Pigment	0.015		1	0.01	5 0.642857	0.467605	21.19769
Orange Pigment	0.06		1	0.0	0.45	0.53908	21.19769
Red Pigment	0.081667		1	0.08166	2.227273	0.209875	21.19769
ANOVA							
Source of Variation							
b/w Different							
Experiments 180 rpm	SS	df		MS	F	P-value	F crit
Cell Dry Weight	0.06		1	0.0	06 0.6	0.481817	21.19769
Yellow Pigment	0.24		1	0.2	.4 9.6	0.036278	21.19769
Orange Pigment	0.281667		1	0.28166	5.451613	0.079825	21.19769
Red Pigment	0.081667		1	0.08166	0.890909	0.398676	21.19769
Source of Variation							
h/w Different							
Experiments 250 rnm	SS	df		MS	F	P-value	F crit
Cell Dry Weight	0.081667	- <u>J</u>	1	0.08166	0.644737	0.467	21.19769
Yellow Pigment	0.201667		1	0.20166	6.05	0.069716	21.19769
Orange Pigment	0.006667		1	0.00666	0.153846	0.714889	21.19769
	0.000007		-	0.00000			

Table 3. One-way analysis of variance (ANOVA) to analyse the effect of temperature on biomass concentration and pigment production.

ANOVA Source of Variation between Groups P-value Total CDW (g/L) 1.16E⁻¹⁵ Yellow Pigment Conc. at 400nm 3.87E⁻²¹ Orange Pigment Conc. at 470nm 1.04E⁻¹⁷ 6.06E⁻¹⁷ Orange Pigment Conc. at 470nm P-value Source of Variation CWD Yellow Orange Red between Experiments 25°C 0.224 0.251 1.000 0.548 30°C 0.074 0.329 0.053 0.834 32°C 0.152 0.696 0.670 0.302 37°C 0.561 0.670 0.025 0.072

Below is a comprehensive analysis of ANOVA.

ANOVA							
Source of Variation b	/w						
Different Temp. Grou	ps S	S	df	MS	F	P-value	F crit
Cell Dry Weight	31.	81667	3	10.60556	229.7232	1.16E-15	3.098391
Yellow Pigment	50.	95185	3	16.98395	828.4516	3.87E-21	3.098391
Orange Pigment	40.	86072	3	13.62024	372.3072	1.04E-17	3.098391
Red Pigment	61.	04995	3	20.34998	311.0627	6.06E-17	3.098391
-							
ANOVA							
Source of Variation							
b/w Different							
Experiments 25 °C	SS	df		MS	F	P-value	F crit
Cell Dry Weight	0.106667		1	0.10666	7 2.064516	0.224122	21.19769
Yellow Pigment	0.015		1	0.01	5 0.428571	0.548424	21.19769
Orange Pigment	0.06		1	0.0	6 1.8	0.250815	21.19769
Red Pigment	2.78E-17		1	2.78E-1	7 8.33E-16	1	21.19769

ANOVA						
Source of Variation						
b/w Different						
Experiments 30 °C	SS	df	MS	F	P-value	F crit
Cell Dry Weight	0.135	1	0.135	5.785714	0.073926	21.19769
Yellow Pigment	0.026667	1	0.026667	1.230769	0.32946	21.19769
Orange Pigment	0.135	1	0.135	7.363636	0.053338	21.19769
Red Pigment	0.001667	1	0.001667	0.05	0.834019	21.19769
ANOVA						
Source of Variation						
b/w Different						
Experiments 32 °C	SS	df	MS	F	P-value	F crit
Cell Dry Weight	0.135	1	0.135	3.115385	0.152317	21.19769
Yellow Pigment	0.006667	1	0.006667	0.210526	0.67018	21.19769
Orange Pigment	0.081667	1	0.081667	1.4	0.302242	21.19769
Red Pigment	0.041667	1	0.041667	0.176056	0.69634	21.19769
ANOVA						
Source of Variation						
b/w Different						
Experiments 27 °C	SS	$d\!f$	MS	F	P-value	F crit
Cell Dry Weight	0.006667	1	0.006667	7 0.4	0.56144	21.1977
Yellow Pigment	0.000417	1	0.000417	0.21008	0.6705	21.1977
Orange Pigment	0.011267	1	0.011267	7 12.0714	0.02548	21.1977
Red Pigment	0.030817	1	0.030817	5.88853	0.07224	21.1977

Table 4. Analysis of Variance in Pigment Concentration (yellow, orange, red) between Test Fermentation Experiments in 2.5L Stirred Tank Bioreactors. The results display the source of variation and *p*-value.

ANOVA- Yellow Pigment (400 nm)

Source of Variation	SS	$d\!f$	MS	F	P-value	F crit
Between Groups	81.41089	5	16.28218	4264.831	8.06E-42	2.533555
Within Groups	0.114533	30	0.003818			
-						
Total	81.52542	35				

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	100.7756	5	20.15513	9893.437	2.69E-47	2.533555
Within Groups	0.061117	30	0.002037			
Total	100.8368	35				
ANOVA-Red Pigment ((510 nm)					
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	114.1881	5	22.83763	4739.189	1.66E-42	2.533555
Within Groups	0.144567	30	0.004819			
Total	114.3327	35				

ANOVA- Orange Pigment (470 nm)

Table 5: Analysis of Variance in pH between the Test Fermentation Experiments in 2.5 L Stirred Tank Bioreactors.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.009445	1	0.009445	2.027468	0.213775	6.607891
Within Groups	0.023294	5	0.004659			
Total	0.032739	6				

Chapter 4- Elicitation

Table 6. Analysis of pH variation in different flask groups (C, OG, OM, MO) throughout fermentation in Shake flasks

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.067463	3	0.022488	2.315295	0.086769	2.786229
Within Groups	0.495349	51	0.009713			
Total	0.562812	71				

Table 7. Analysis of variation in biomass concentration in different flask groups (C, OG, OM, MO) throughout fermentation in Shake flasks

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.776667	3	0.258889	0.993074	0.408547	2.90112
Within Groups	8.342222	32	0.260694			
-						
Total	9.118889	35				

Table 8. Analysis of variation in yellow pigment concentration in different flask groups (C, OG, OM, MO) throughout fermentation in Shake flasks

ANOVA								
Source of Variation	SS	$d\!f$	MS	F	P-value	F	r crit	
Between Groups	90.0084	48 3	30.00283	523.6906	2.91E-27	2	.90112	2
Within Groups	1.833316 32		0.057291					
Total	91.841	79 35						
Number of families	1							
No. of comparisons per								
family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of dif	f. Below threshold?	,	Adjusted P Value			
Control vs. OG	-3.043	-3.518 to -2.568	Yes		< 0.0001	A-B		
Control vs. OM	-4.270	-4.745 to -3.795	Yes		< 0.0001	A-C		
Control vs. MO	-1.770	-2.245 to -1.295	Yes		< 0.0001	A-D		
OG vs. OM	-1.227	-1.702 to -0.7518	Yes		0.0002	B-C		
OG vs. MO	1.273	0.7985 to 1.748	Yes		0.0001	B-D		
OM vs. MO	2.500	2.025 to 2.975	Yes		< 0.0001	C-D		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. OG	3.863	6.907	-3.043	0.1483	3	3	29.02	8
Control vs. OM	3.863	8.133	-4.270	0.1483	3	3	40.72	8

Section

Control vs. MO	3.863	5.633	-1.770	0.1483	3	3	16.88 8
OG vs. OM	6.907	8.133	-1.227	0.1483	3	3	11.70 8
OG vs. MO	6.907	5.633	1.273	0.1483	3	3	12.14 8
OM vs. MO	8.133	5.633	2.500	0.1483	3	3	23.84 8
Compact letter display							
OM	А						
OG	В						
МО	С						
Control	D						

Table. 9. Analysis of Variance (ANOVA) in Orange Pigment Production in different flask groups (C, OG, OM, MO) during Shake Flask Fermentation.

ANOVA							
Source of Variation	n SS		df	MS	F	P-value	F crit
Between Groups	125.0	157	3	41.67189	3343.783	5.04E-40	2.90112
Within Groups	0.39	988	32	0.012463			
Total	n SS df 125.0157 125.0157 0.3988 3 125.4145 3 125.4145 3 1 6 0.05 Mean Diff. 95.00% CI of d -4.767 -4.928 to -4.600 -4.217 -4.378 to -4.050 -2.373 -2.534 to -2.212 0.5500 0.3891 to 0.710		35				
Number of families	1						
No. of comparisons							
per family	6						
Alpha	0.05						
Tukey's multiple						Adjusted	
comparisons test	Mean Diff.	95.00% C	CI of diff.	Below threshold	1?	P Value	
Control vs. OG	-4.767	-4.928 to	-4.606	Yes		< 0.0001	A-B
Control vs. OM	-4.217	-4.378 to	-4.056	Yes		< 0.0001	A-C
Control vs. MO	-2.373	-2.534 to	-2.212	Yes		< 0.0001	A-D
OG vs. OM	0.5500	0.3891 to	0.7109	Yes		< 0.0001	B-C
OG vs. MO	2.393	2.232 to 2	2.554	Yes		< 0.0001	B-D
OM vs. MO	1.843	1.682 to 2	2.004	Yes		< 0.0001	C-D

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. OG	3.433	8.200	-4.767	0.05026	3	3	134.1	8
Control vs. OM	3.433	7.650	-4.217	0.05026	3	3	118.7	8
Control vs. MO	3.433	5.807	-2.373	0.05026	3	3	66.78	8
OG vs. OM	8.200	7.650	0.5500	0.05026	3	3	15.48	8
OG vs. MO	8.200	5.807	2.393	0.05026	3	3	67.35	8
OM vs. MO	7.650	5.807	1.843	0.05026	3	3	51.87	8
Compact letter displa	У							
OG	А							
OM	В							
МО	С							
Control	D							

Table. 10. Analysis of Variation in Red Pigment Production in Different Flask Groups (C, OG, OM, MO) Throughout Fermentation in Shake Flasks.

ANOVA							
Source of Variation	SS	df		MS	F	P-value	F crit
Between Groups	153.024	46	3	51.0082	830.8276	2.04E-30	2.90112
Within Groups	1.964622		32	0.061394			
Total	154.98	92	35				
Number of families	1						
No. of comparisons							
per family	6						
Alpha	0.05						
Tukey's multiple		95.00% CI	Belo	W	Adjusted	b	
comparisons test	Mean Diff.	of diff.	thres	hold?	P Value		
		-4.521 to					
Control vs. OG	-4.250	-3.979	Yes		< 0.0001	A-B	
		-5.846 to					
Control vs. OM	-5.574	-5.303	Yes		< 0.0001	A-C	
		-3.318 to					
Control vs. MO	-3.047	-2.775	Yes		< 0.0001	A-D	

		-1.596 to						
OG vs. OM	-1.324	-1.053	Yes		< 0.0001	B-C		
		0.9319 to						
OG vs. MO	1.203	1.475	Yes		< 0.0001	B-D		
		2.256 to						
OM vs. MO	2.528	2.799	Yes		< 0.0001	C-D		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. OG	3.300	7.550	-4.250	0.08475	3	3	70.92	8
Control vs. OM	3.300	8.874	-5.574	0.08475	3	3	93.02	8
Control vs. MO	3.300	6.347	-3.047	0.08475	3	3	50.84	8
OG vs. OM	7.550	8.874	-1.324	0.08475	3	3	22.10	8
OG vs. MO	7.550	6.347	1.203	0.08475	3	3	20.08	8
OM vs. MO	8.874	6.347	2.528	0.08475	3	3	42.18	8
Compact letter								
display								
ОМ	А							
OG	В							
МО	С							
Control	D							

Table 11. Analysis of Variation in Carbohydrate Consumption in Different Flask Groups (C, OG, OM, MO) Throughout Fermentation in Shake Flasks.

ANOVA						
Source of Variation	SS	$d\!f$	MS	F	P-value	F crit
Between Groups	910.4444	3	303.4815	1.521899	0.227671	2.90112
Within Groups	6381.111	32	199.4097			
Total	7291.556	35				

Table 12. Analysis of Variation in Lovastatin Production in Different Flask Groups (C, OG, OM, MO) Throughout Fermentation in Shake Flasks.

ANOVA

Source of Variatio	n	SS	df	MS	F		P-value	F crit
Between Groups	-	74416.917	3 2	24805.639	42.86	7	2.826E-	⁰⁵ 7.591
Within Groups		4629.333	8	578.667				
Total		79046.25	11					
Number of families	1							
No. of comparisons								
per family	6							
Alpha	0.05							
Tukey's multiple	Mean	95.00% CI of	Below		Adjusted			
comparisons test	Diff.	diff.	threshold?		P Value			
		-94.01 to						
C vs. OG	-76.52	-59.02	Yes		< 0.0001	A-B		
		-112.9 to						
C vs. OM	-95.45	-77.96	Yes		< 0.0001	A-C		
		-70.52 to						
C vs. MO	-53.03	-35.54	Yes		< 0.0001	A-D		
		-36.43 to						
OG vs. OM	-18.94	-1.445	Yes		0.0345	B-C		
		5.991 to						
OG vs. MO	23.48	40.98	Yes		0.0112	B-D		
		24.93 to						
OM vs. MO	42.42	59.92	Yes		0.0002	C-D		
Test details	Mean 1	Mean 2	Mean Diff	. SE of diff	.n1	n2	q DF	
C vs. OG	63.64	140.2	-76.52	5.463	3	3	19.818	
C vs. OM	63.64	159.1	-95.45	5.463	3	3	24.718	
C vs. MO	63.64	116.7	-53.03	5.463	3	3	13.738	
OG vs. OM	140.2	159.1	-18.94	5.463	3	3	4.9038	
OG vs. MO	140.2	116.7	23.48	5.463	3	3	6.0808	
OM vs. MO	159.1	116.7	42.42	5.463	3	3	10.988	
Compact letter								
display								
ОМ	А							

OG	В
МО	С
С	D

Table 13. Analysis of Variation in pH among Different Bioreactor Groups (C, OG, OM, MO) Throughout Fermentation.

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.073139	3	0.02438	2.550462	0.104663	3.490295
Within Groups	0.114708	12	0.009559			
Total	0.187847	15				

Table 14. ANOVA Results for Variance in Biomass Concentration in 2.5 L Stirred Tank Fermenters

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.153333	3	0.717778	3.287532	0.079192	4.066181
Within Groups	1.746667	8	0.218333			
Total	3.9	11				
Within Groups Total	1.746667 <u>3.9</u>	8	0.218333			

Table 15. ANOVA Results for Variance in Yellow Pigment Production in 2.5 L Stirred Tank Fermenters

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	101.0129	3	33.67098	763.8029	3.6E-10	4.066181
Within Groups	0.352667	8	0.044083			
	0.002000	0	01011000			
Total	101.3656	11				

Number of families	1						
Number of comparisons							
per family	6						
Alpha	0.05						
Tukey's multiple	Mean	95.00% CI	Below		Adjusted		
comparisons test	Diff.	of diff.	threshold?		P Value		
C vs. OG	-5.193 -	-5.742 to -4.644	Yes		<0.0001 A	4- В	
C vs. OM	-7.660 -	-8.209 to -7.111	Yes		<0.0001 A	A-C	
C vs. MO	-2.253 -	2.802 to -1.704	Yes		<0.0001 A	A-D	
OG vs. OM	-2.467 -	-3.016 to -1.918	Yes		<0.0001]	3-C	
OG vs. MO	2.940	2.391 to 3.489	Yes		<0.0001 I	3-D	
OM vs. MO	5.407	4.858 to 5.956	Yes		<0.0001 0	C-D	
Test details	Mean 1	Mean 2	Mean Diff. S	E of diff.	n1	n2 q	DF
C vs. OG	5.013	10.21	-5.193	0.1714	3	3 42.84	8
C vs. OG C vs. OM	5.013 5.013	10.21 12.67	-5.193 -7.660	0.1714 0.1714	3 3	3 42.84 3 63.19	8 8
C vs. OG C vs. OM C vs. MO	5.013 5.013 5.013	10.21 12.67 7.267	-5.193 -7.660 -2.253	0.1714 0.1714 0.1714	3 3 3	3 42.84 3 63.19 3 18.59	8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM	5.013 5.013 5.013 10.21	10.21 12.67 7.267 12.67	-5.193 -7.660 -2.253 -2.467	0.1714 0.1714 0.1714 0.1714	3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35	8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO	5.013 5.013 5.013 10.21 10.21	10.21 12.67 7.267 12.67 7.267	-5.193 -7.660 -2.253 -2.467 2.940	0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25	8 8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO OM vs. MO	5.013 5.013 5.013 10.21 10.21 12.67	10.21 12.67 7.267 12.67 7.267 7.267	-5.193 -7.660 -2.253 -2.467 2.940 5.407	0.1714 0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25 3 44.60	8 8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO OM vs. MO Compact letter display	5.013 5.013 5.013 10.21 10.21 12.67	10.21 12.67 7.267 12.67 7.267 7.267	-5.193 -7.660 -2.253 -2.467 2.940 5.407	0.1714 0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25 3 44.60	8 8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO OM vs. MO Compact letter display OM	5.013 5.013 5.013 10.21 10.21 12.67 A	10.21 12.67 7.267 12.67 7.267 7.267	-5.193 -7.660 -2.253 -2.467 2.940 5.407	0.1714 0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25 3 44.60	8 8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO OM vs. MO Compact letter display OM OG	5.013 5.013 5.013 10.21 10.21 12.67 A B	10.21 12.67 7.267 12.67 7.267 7.267	-5.193 -7.660 -2.253 -2.467 2.940 5.407	0.1714 0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25 3 44.60	8 8 8 8 8
C vs. OG C vs. OM C vs. MO OG vs. OM OG vs. MO OM vs. MO Compact letter display OM OG MO	5.013 5.013 5.013 10.21 10.21 12.67 A B C	10.21 12.67 7.267 12.67 7.267 7.267	-5.193 -7.660 -2.253 -2.467 2.940 5.407	0.1714 0.1714 0.1714 0.1714 0.1714 0.1714	3 3 3 3 3 3	3 42.84 3 63.19 3 18.59 3 20.35 3 24.25 3 44.60	8 8 8 8

Table 16. ANOVA Results for Variance in Orange Pigment Production in 2.5 L Stirred Tank Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	127.7766	3	42.59221	259.7552	2.61E-08	4.066181
Within Groups	1.311765	8	0.163971			
Total	129.0884	11				

Number of families	1							
Number of comparisons								
per family	6							
Alpha	0.05							
Tukey's multiple	Mean	95.00% CI	Below		Adjusted			
comparisons test	Diff.	of diff.	threshold?		P Value			
		-9.235 to						
C vs. OG	-8.176	-7.118	Yes		< 0.0001	A-B		
		-8.592 to						
C vs. OM	-7.533	-6.474	Yes		< 0.0001	A-C		
		-5.002 to						
C vs. MO	-3.943	-2.884	Yes		< 0.0001	A-D		
		-0.4154 to						
OG vs. OM	0.6433	1.702	No		0.2831	B-C		
		3.175 to						
OG vs. MO	4.233	5.292	Yes		< 0.0001	B-D		
		2.531 to						
OM vs. MO	3.590	4.649	Yes		< 0.0001	C-D		
				SE of				
Test details	Mean 1	Mean 2	Mean Diff.	diff.	n1	n2	q	DF
C vs. OG	3.857	12.03	-8.176	0.3306	3	3	34.97	8
C vs. OM	3.857	11.39	-7.533	0.3306	3	3	32.22	8
C vs. MO	3.857	7.800	-3.943	0.3306	3	3	16.87	8
OG vs. OM	12.03	11.39	0.6433	0.3306	3	3	2.752	8
OG vs. MO	12.03	7.800	4.233	0.3306	3	3	18.11	8
OM vs. MO	11.39	7.800	3.590	0.3306	3	3	15.36	8
Compact letter display								
OG	А							
OM	А							
МО	В							
С	С							

Appendix

Table 17.	ANOVA	Results	for	Variance	in	Red	Pigment	Production	in	2.5	L	Stirred	Tank
Fermenter	S												

ANOVA

Source of Variation	SS	df	MS F		<i>P</i> -1	value	F crit
Between Groups	181.7242	3	60.57474	694.5	981 5.2	6E-10	4.06618
Within Groups	0.697667	8	0.087208				
Total	182.4219	11					
Number of families	1						
Number of comparisons							
per family	6						
Alpha	0.05						
Tukey's multiple	Mean Dif	_f 95.00% CI	Below		Adjusted		
comparisons test		of diff.	threshold?		P Value		
		-8.599 to					
C vs. OG	-7.827	-7.055	Yes		< 0.0001	A-B	
		-11.01 to					
C vs. OM	-10.24	-9.465	Yes		< 0.0001	A-C	
		-4.699 to					
C vs. MO	-3.927	-3.155	Yes		< 0.0001	A-D	
		-3.182 to					
OG vs. OM	-2.410	-1.638	Yes		< 0.0001	B-C	
		3.128 to					
OG vs. MO	3.900	4.672	Yes		< 0.0001	B-D	
		5.538 to					
OM vs. MO	6.310	7.082	Yes		< 0.0001	C-D	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff	f. n1	n2 (] DF
C vs. OG	4.177	12.00	-7.827	0.2411	3	3 4	45.908
C vs. OM	4.177	14.41	-10.24	0.2411	3	3	50.048
C vs. MO	4.177	8.103	-3.927	0.2411	3	3	23.038
OG vs. OM	12.00	14.41	-2.410	0.2411	3	3	14.148
OG vs. MO	12.00	8.103	3.900	0.2411	3	3	22.878
OM vs. MO	14.41	8.103	6.310	0.2411	3	3	37.018
Compact letter display							
OM	А						
OG	В						

MO	C
С	D

Table 18. ANOVA Results for Variance in Carbohydrate Consumption in 2.5 L Stirred Tank Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	216.6667	3	72.22222	0.439041	0.731316	4.066181
Within Groups	1316	8	164.5			
Total	1532.667	11				

Table. 19. ANOVA Results for Variance in Lovastatin Concentration in 2.5 L Stirred Tank Fermenters

ANOVA						
Source of	aa	10		F		
Variation	22	df	MS	F	P-value	F crit
Between Groups	81534.38	3	27178.13	280.548	4.19E ⁻⁰⁵	16.694
Within Groups	387.5	4	96.875			
Total	81921.88	7				
Number of families	1					
No. of comparisons						
per family	6					
Alpha	0.05					
Tukey's multiple		95.00% CI	Below	Adjusted		
comparisons test	Mean Diff.	of diff.	threshold?	P Value	e	
		-113.7 to				
C vs. OG	-95.45	-77.24	Yes	0.0001	l A-B	
		-140.9 to				
C vs. OM	-122.7	-104.5	Yes	< 0.0001	I A-C	
		-78.44 to				
C vs. MO	-60.23	-42.01	Yes	0.0006	6 A-D	
		-45.49 to				
OG vs. OM	-27.27	-9.060	Yes	0.0125	5 B-C	
		17.01 to				
OG vs. MO	35.23	53.44	Yes	0.0049) B-D	

		44.29 to						
OM vs. MO	62.50	80.71	Yes		0.0005	C-D		
				SE of				
Test details	Mean 1	Mean 2	Mean Diff.	diff.	n1	n2	q	DF
C vs. OG	73.86	169.3	-95.45	4.474	2	2	30.17	4
C vs. OM	73.86	196.6	-122.7	4.474	2	2	38.79	4
C vs. MO	73.86	134.1	-60.23	4.474	2	2	19.04	4
OG vs. OM	169.3	196.6	-27.27	4.474	2	2	8.621	4
OG vs. MO	169.3	134.1	35.23	4.474	2	2	11.14	4
OM vs. MO	196.6	134.1	62.50	4.474	2	2	19.76	4
Compact letter display								
OM	А							
OG	В							
МО	С							
С	D							

Chapter 5- Quorum Sensing

Table. 20. Shaken Flasks Run I- ANOVA Results for Variance in pH Concentration across C, CD and B flasks during Shaken Flask Fermenters

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.027324	2	0.013662	0.146062	0.864487	3.190727
Within Groups	4.489736	48	0.093536			
Total	4.51706	50				

Table. 21. Shaken Flasks Run I - ANOVA Results for Variance in pH Concentration between C and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.950745	1	0.950745	10.98402	0.002191	4.130018
Within Groups	2.942944	34	0.086557			
Total	3.893689	35				
Total	3.893689	35				

Table. 22. Shaken Flasks Run II- ANOVA Results for Variance in pH Concentration between C, B and T flask groups during Shaken Flask Fermenters

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.020839	2	0.01042	0.12947	0.878849	3.178799
Within Groups	4.104442	51	0.080479			
Total	4.125281	53				

Table. 23. Shaken Flasks Run III- ANOVA Results for Variance in pH Concentration between all flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.044083	5	0.008817	1.576924	0.175308	2.321812
Within Groups	0.475241	85	0.005591			
Total	0.519324	90				

Table. 24. Shaken Flasks Run I- ANOVA Results for Variance in Biomass Concentration across C, CD and B flasks during Shaken Flask Fermenters

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.004267	2	0.002133	0.243655	0.788765	4.256495
Within Groups	0.0788	9	0.008756			
Total	0.083067	11				

Appendix

Table. 25. Shaken Flasks Run I - ANOVA Results for Variance in Biomass Concentration between C and T flask groups during Shaken Flask Fermenters

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.6728	1	0.6728	55.29863	0.000304	5.987378
Within Groups	0.073	6	0.012167			
Total	0.7458	7				

Table. 26. Shaken Flasks Run II- ANOVA Results for Variance in Biomass Concentration between C, B and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.606667	2	0.303333	20.55723	0.000441	4.256495
Within Groups	0.1328	9	0.014756			
Total	0.739467	11				

Table. 27. Shaken Flasks Run III- ANOVA Results for Variance in Biomass Concentration between all flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.074987	5	0.014997	1.839441	0.122946	2.408514
Within Groups	0.391356	48	0.008153			
Total	0.466343	53				

Table. 28. Shaken Flasks Run I- ANOVA Results for Variance in Yellow Pigment	
Production across C, and CD flasks during Shaken Flask Fermenters	

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.061633	1	0.061633	2.430976	0.150018	4.964603
Within Groups	0.253533	10	0.025353			
Total	0.315167	11				

Table. 29. Shaken Flasks Run I - ANOVA Results for Variance in Yellow Pigment Production between C, B and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8.020133	2	4.010067	331.1064	3.89E-13	3.68232
Within Groups	0.181667	15	0.012111			
-						
Total	8.2018	17				

Table. 30. Shaken Flasks Run II- ANOVA Results for Variance in Yellow Pigment Production between C, B and T flask groups during Shaken Flask Fermenters

Source of Variation	55	df	MS	F	P value	F arit
variation	55	uj	MIS	ľ	I -vuiue	ГСЛИ
Between						
Groups	0.8337	2	0.41685	115.1519	7.9E-10	3.68232
_						
Within Groups	0.0543	15	0.00362			
-						
Total	0.888	17				

Table. 31. Shaken Flasks Run III- ANOVA Results for Variance in Yellow Pigment Production between all flask groups during Shaken Flask Fermenters

Source of Variation	SS	df	M		5	F	P-value		F crit	
Between Groups 88.374			5 17.6748		6748	3585.758	58 1.64E-60		2.408514	
Within Groups 0.2366		j	48 0.004929		4929					
Total	88.6106		53							
Number of families		1								
No. of comparisons										
per family		15								
Alpha		0.05								
		Mean	95.00	% CI	Below	A	djusted			
Tukey's multiple comparisons test		Diff.	of diff.		threshold?	Р	Value			
			0.152	2 to						
Control vs. Butyrolactone-I		0.2500	0.3478		Yes	<	0.0001	A-B		
			-2.09	8 to						
Control vs. Tyrosol (0.3mM)		-2.000	-1.902		Yes	<	0.0001	A-C		
			-0.19	78 to						
Control vs. Tyrosol (0.5mM)		-0.1000	-0.002	2249	Yes	0	.0439	A-D		
			-3.39	8 to						
Control vs. Farnesol (0.2mM)		-3.300	-3.20	2	Yes	<	0.0001	A-E		
Control vs. Lonoleic acid			-0.68	78 to						
(0.4mM)		-0.5900	-0.492	22	Yes	<	0.0001	A-F		
			-2.34	8 to						
Butyrolactone-I vs. Tyrosol (0.3mM	()	-2.250	-2.15	2	Yes	<	0.0001	B-C		
			-0.44	78 to						
Butyrolactone-I vs. Tyrosol (0.5ml	(M	-0.3500	-0.25	22	Yes	<	0.0001	B-D		
			-3.64	8 to						
Butyrolactone-I vs. Farnesol (0.2m	M)	-3.550	-3.45	2	Yes	<	0.0001	B-E		
			-0.93	78 to						
Butyrolactone-I vs. Lonoleic acid (0.4mM)		-0.8400	-0.74	22	Yes	<	0.0001	B-F		
Tyrosol (0.3mM) vs. Tyrosol			1.802	to						
(0.5mM)		1.900	1.998		Yes	<	0.0001	C-D		
Tyrosol (0.3mM) vs. Farnesol			-1.39	8 to						
(0.2mM)		-1.300	-1.202	2	Yes	<	0.0001	C-E		

		1.312 to						
Tyrosol (0.3mM) vs. Lonoleic acid (0.4mM	1.508	Yes		< 0.0001	C-F			
Tyrosol (0.5mM) vs. Farnesol		-3.298 to						
(0.2mM)	-3.200	-3.102	Yes		< 0.0001	D-E		
Tyrosol (0.5mM) vs. Lonoleic		-0.5878 to						
acid (0.4mM)	-0.4900	-0.3922	Yes		< 0.0001	D-F		
Farnesol (0.2mM) vs. Lonoleic		2.612 to						
acid (0.4mM)	2.710	2.808	Yes		< 0.0001	E-F		
			Mean					
Test details	Mean 1	Mean 2	Diff.	SE of dif	f. n1	n2	q	DF
Control vs. Butyrolactone-I	3.800	3.550	0.2500	0.02910	3	3	12.15	12
Control vs. Tyrosol (0.3mM)	3.800	5.800	-2.000	0.02910	3	3	97.19	12
Control vs. Tyrosol (0.5mM)	3.800	3.900	-0.1000	0.02910	3	3	4.860	12
Control vs. Farnesol (0.2mM)	3.800	7.100	-3.300	0.02910	3	3	160.4	12
Control vs. Lonoleic acid								
(0.4mM)	3.800	4.390	-0.5900	0.02910	3	3	28.67	12
Butyrolactone-I vs. Tyrosol (0.3mM)	3.550	5.800	-2.250	0.02910	3	3	109.3	12
Butyrolactone-I vs. Tyrosol (0.5mM)	3.550	3.900	-0.3500	0.02910	3	3	17.01	12
Butyrolactone-I vs. Farnesol (0.2mM)	3.550	7.100	-3.550	0.02910	3	3	172.5	12
Butyrolactone-I vs. Lonoleic acid (0.4mM)	3.550	4.390	-0.8400	0.02910	3	3	40.82	12
Tyrosol (0.3mM) vs. Tyrosol								
(0.5mM)	5.800	3.900	1.900	0.02910	3	3	92.33	12
Tyrosol (0.3mM) vs. Farnesol								
(0.2mM)	5.800	7.100	-1.300	0.02910	3	3	63.17	12
Tyrosol (0.3mM) vs. Lonoleic								
acid (0.4mM)	5.800	4.390	1.410	0.02910	3	3	68.52	12
Tyrosol (0.5mM) vs. Farnesol								
(0.2mM)	3.900	7.100	-3.200	0.02910	3	3	155.5	12
Tyrosol (0.5mM) vs. Lonoleic								
acid (0.4mM)	3.900	4.390	-0.4900	0.02910	3	3	23.81	12
Farnesol (0.2mM) vs. Lonoleic								
acid (0.4mM)	7.100	4.390	2.710	0.02910	3	3	131.7	12
Compact letter display								
Farnesol (0.2mM)	А							
Tyrosol (0.3mM)	В							
Lonoleic acid (0.4mM)	С							
Tyrosol (0.5mM)	D							
-----------------	---							
Control	E							
Butyrolactone-I	F							

Table. 32. Shaken Flasks Run I- ANOVA Results for Variance in Orange Pigment Production across C, and CD flasks during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.008533	1	0.008533	1.89911	0.198229	4.964603
Within Groups	0.044933	10	0.004493			
Total	0.053467	11				

Table. 33. Shaken Flasks Run I - ANOVA Results for Variance in Orange Pigment Production between C, B and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.991211	2	4.995606	1222.748	2.44E-17	3.68232
Within Groups	0.061283	15	0.004086			
Total	10.05249	17				

Table. 34. Shaken Flasks Run II- ANOVA Results for Variance in Orange Pigment Production between C, B and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1332	2	0.0666	20.47131	5.16E-05	3.68232
Within Groups	0.0488	15	0.003253			
	010100	10	01000200			
	0.400					
Total	0.182	17				

Table. 35. Shaken Flasks Run III- ANOVA Results for Variance in Orange Pigment Production between all flask groups during Shaken Flask Fermenters

Source of Variation	SS	dj	£	MS		F	P-valu	е	F crit
Between Groups	130.0854		5	26.017	708	7568.605	2.78E-	-68	2.408514
Within Groups	0.165		48 (0.0034	438				
-									
Total	130 2504		53						
Number of families	130.2304	1	55						
Number of comparisons									
per family		15							
Alpha		0.05							
1		Mean	95.00% CI		Below		Adjusted		
Tukey's multiple comparisons	test	Diff.	of diff.		threshold?		P Value		
		-	-0.2004 to -						
Control vs. Butyrolactone-I		0.1056	0.01075		Yes		0.0263	A-B	
Control vs. Tyrosol (0.3mM)		-2.326	-2.420 to -2	.231	Yes		< 0.0001	A-C	
		-	-0.8204 to -						
Control vs. Tyrosol (0.5mM)		0.7256	0.6307		Yes		< 0.0001	A-D	
Control vs. Farnesol (0.2mM		-4.226	-4.320 to -4	.131	Yes		< 0.0001	A-E	
Control vs. Lonoleic acid (0.4	4mM)	-1.146	-1.240 to -1	.051	Yes		< 0.0001	A-F	
Butyrolactone-I vs. Tyrosol ((0.3mM)	-2.220	-2.315 to -2	.125	Yes		< 0.0001	B-C	
	0.5	-	-0.7148 to -		V		0 0001		
Butyrolactone-I vs. Tyrosol ((0.5 mM)	0.6200	0.5252	025	Yes		<0.0001	B-D	
Butyrolactone-I vs. Farnesol	(0.2mN)	-4.120	-4.215 to -4	.025	Y es		<0.0001	B-E	
Butyrolactone-I vs. Lonoleic	acid (0.4mM)	-1.040	-1.135 to -0	.9452	Yes		<0.0001	B-F	
Tyrosol (0.3mM) vs. Tyrosol		1 (00	1 505 - 1 4		T 7		0.0001		
(0.5 mM)		1.600	1.505 to 1.6	195	Yes		<0.0001	C-D	
Tyrosol (0.3mM) vs. Farneso)l	1 000	1.005 / 1	005	37		0.0001		
(0.2 mM)	• • •	-1.900	-1.995 to -1	.805	Yes		<0.0001	C-E	
Tyrosol (0.3mM) vs. Lonolei	ic acid	1 100	1.005 . 1.0		T 7		0.0001	G F	
(0.4mM)		1.180	1.085 to 1.2	275	Yes		<0.0001	C-F	
Tyrosol (0.5mM) vs. Farneso)l	2 500	0.505	105	T 7		0.0001	DE	
(U.2mM)		-3.500	-3.595 to -3	.405	res		<0.0001	D-E	
Tyrosol (0.5mM) vs. Lonolei	ic acid	-	-0.5148 to -	i	X 7		0.0001		
(0.4mM)		0.4200	0.3252		Yes		<0.0001	D-F	

Farnesol (0.2mM) vs. Lonoleic acid (0.4mM) 3.080	2.985 to 3.175	Yes		< 0.0001	E-F		
Test details	Mean 1	l Mean 2	Mean Diff	.SE of diff	.n1	n2	q	DF
Control vs. Butyrolactone-I	3.774	3.880	-0.1056	0.02823	3	3	5.289	12
Control vs. Tyrosol (0.3mM)	3.774	6.100	-2.326	0.02823	3	3	116.5	12
Control vs. Tyrosol (0.5mM)	3.774	4.500	-0.7256	0.02823	3	3	36.35	12
Control vs. Farnesol (0.2mM)	3.774	8.000	-4.226	0.02823	3	3	211.7	12
Control vs. Lonoleic acid (0.4mM)	3.774	4.920	-1.146	0.02823	3	3	57.40	12
Butyrolactone-I vs. Tyrosol (0.3mM)	3.880	6.100	-2.220	0.02823	3	3	111.2	12
Butyrolactone-I vs. Tyrosol (0.5mM)	3.880	4.500	-0.6200	0.02823	3	3	31.06	12
Butyrolactone-I vs. Farnesol (0.2mM)	3.880	8.000	-4.120	0.02823	3	3	206.4	12
Butyrolactone-I vs. Lonoleic acid (0.4mM)	3.880	4.920	-1.040	0.02823	3	3	52.11	12
Tyrosol (0.3mM) vs. Tyrosol								
(0.5mM)	6.100	4.500	1.600	0.02823	3	3	80.17	12
Tyrosol (0.3mM) vs. Farnesol								
(0.2mM)	6.100	8.000	-1.900	0.02823	3	3	95.20	12
Tyrosol (0.3mM) vs. Lonoleic acid								
(0.4mM)	6.100	4.920	1.180	0.02823	3	3	59.12	12
Tyrosol (0.5mM) vs. Farnesol								
(0.2mM)	4.500	8.000	-3.500	0.02823	3	3	175.4	12
Tyrosol (0.5mM) vs. Lonoleic acid								
(0.4mM)	4.500	4.920	-0.4200	0.02823	3	3	21.04	12
Farnesol (0.2mM) vs. Lonoleic acid (0.4mM) 8.000	4.920	3.080	0.02823	3	3	154.3	12
Compact letter display								
Farnesol (0.2mM)	А							
Tyrosol (0.3mM)	В							
Lonoleic acid (0.4mM)	С							
Tyrosol (0.5mM)	D							
Butyrolactone-I	Е							
Control	F							

Total

,	2	,				
ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.012633	2	0.006317	1.518024	0.250971	3.68232
Within Groups	0.062417	15	0.004161			

Table. 36. Shaken Flasks Run I- ANOVA Results for Variance in Red Pigment Production across C, B and CD flasks during Shaken Flask Fermenters

Table. 37. Shaken Flasks Run I - ANOVA Results for Variance in Red Pigment Production between C and T flask groups during Shaken Flask Fermenters

17

0.07505

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.409408	1	6.409408	2250.231	4.18E-13	4.964603
Within Groups	0.028483	10	0.002848			
_						
Total	6.437892	11				

Table. 38. Shaken Flasks Run II- ANOVA Results for Variance in Red Pigment Production between C, B and T flask groups during Shaken Flask Fermenters

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.4188	2	0.2094	49.23197	2.57E-07	3.68232
Within Groups	0.0638	15	0.004253			
Total	0.4826	17				

Table. 39. Shaken Flasks Run III- ANOVA Results for Variance in Red Pigment Production between all flask groups during Shaken Flask Fermenters

Source of Variation	SS	df	MS	F	P-value	F crit	<u>.</u>
Between Groups	113.3976	5	22.67952	3213.155	2.26E-59	2.4085	514
Within Groups	0.3388	48	0.007058				
Total	113.7364	53					
Number of families		1					
Number of comparisons	per family	15					
Alpha		0.05					
		Mean	95.00% CI	Below		Adjusted	
Tukey's multiple compar	risons test	Diff.	of diff.	threshold?		P Value	
Control vs. Butyrolacto	one-I	0.04000	0-0.07761 to 0.157	6 No		0.8546	A-B
Control vs. Tyrosol (0.2	3mM)	-2.180	-2.298 to -2.062	Yes		< 0.0001	A-C
Control vs. Tyrosol (0.2	5mM)	-0.3500	-0.4676 to -0.2324	4 Yes		< 0.0001	A-D
Control vs. Farnesol (0	.2mM)	-4.050	-4.168 to -3.932	Yes		< 0.0001	A-E
Control vs. Lonoleic ac	eid (0.4mM)	-1.250	-1.368 to -1.132	Yes		< 0.0001	A-F
Butyrolactone-I vs. Tyr	cosol (0.3mM)	-2.220	-2.338 to -2.102	Yes		< 0.0001	B-C
Butyrolactone-I vs. Tyr	cosol (0.5mM)	-0.3900	-0.5076 to -0.2724	4 Yes		< 0.0001	B-D
Butyrolactone-I vs. Far	nesol (0.2mM)	-4.090	-4.208 to -3.972	Yes		< 0.0001	B-E
Butyrolactone-I vs. Lo	noleic acid (0.4mM)	-1.290	-1.408 to -1.172	Yes		< 0.0001	B-F
Tyrosol (0.3mM) vs. T	yrosol (0.5mM)	1.830	1.712 to 1.948	Yes		< 0.0001	C-D
Tyrosol (0.3mM) vs. Fa	arnesol (0.2mM)	-1.870	-1.988 to -1.752	Yes		< 0.0001	C-E
Tyrosol (0.3mM) vs. L	onoleic acid (0.4mM)	0.9300	0.8124 to 1.048	Yes		< 0.0001	C-F
Tyrosol (0.5mM) vs. Fa	arnesol (0.2mM)	-3.700	-3.818 to -3.582	Yes		< 0.0001	D-E

Tyrosol (0.5mM) vs. Lonoleic acid (0.4mM) -0.9000	0 -1.018 to -0.7824	Yes		< 0.0001	D-F		
Farnesol (0.2mM) vs. Lonoleic acid (0.4mM	1) 2.800	2.682 to 2.918	Yes		< 0.0001	E-F		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff	.n1	n2	q	DF
Control vs. Butyrolactone-I	3.750	3.710	0.04000	0.03501	3	3	1.616	12
Control vs. Tyrosol (0.3mM)	3.750	5.930	-2.180	0.03501	3	3	88.05	12
Control vs. Tyrosol (0.5mM)	3.750	4.100	-0.3500	0.03501	3	3	14.14	12
Control vs. Farnesol (0.2mM)	3.750	7.800	-4.050	0.03501	3	3	163.6	12
Control vs. Lonoleic acid (0.4mM)	3.750	5.000	-1.250	0.03501	3	3	50.49	12
Butyrolactone-I vs. Tyrosol (0.3mM)	3.710	5.930	-2.220	0.03501	3	3	89.67	12
Butyrolactone-I vs. Tyrosol (0.5mM)	3.710	4.100	-0.3900	0.03501	3	3	15.75	12
Butyrolactone-I vs. Farnesol (0.2mM)	3.710	7.800	-4.090	0.03501	3	3	165.2	12
Butyrolactone-I vs. Lonoleic acid (0.4mM)	3.710	5.000	-1.290	0.03501	3	3	52.10	12
Tyrosol (0.3mM) vs. Tyrosol (0.5mM)	5.930	4.100	1.830	0.03501	3	3	73.92	12
Tyrosol (0.3mM) vs. Farnesol (0.2mM)	5.930	7.800	-1.870	0.03501	3	3	75.53	12
Tyrosol (0.3mM) vs. Lonoleic acid (0.4mM) 5.930	5.000	0.9300	0.03501	3	3	37.56	12
Tyrosol (0.5mM) vs. Farnesol (0.2mM)	4.100	7.800	-3.700	0.03501	3	3	149.4	12
Tyrosol (0.5mM) vs. Lonoleic acid (0.4mM) 4.100	5.000	-0.9000	0.03501	3	3	36.35	12
Farnesol (0.2mM) vs. Lonoleic acid (0.4mM	1)7.800	5.000	2.800	0.03501	3	3	113.1	12
Compact letter display								
Farnesol (0.2mM)	А							
Tyrosol (0.3mM)	В							
Lonoleic acid (0.4mM)	С							
Tyrosol (0.5mM)	D							
Control	Е							

Butyrolactone-I

Е

Table. 40. Shaken Flasks Run I- ANOVA Results for Variance in Carbohydrate Consumption across C, CD and B flasks during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.053137	2	0.026568	1.171542	0.336695	3.68232
Within Groups	0.340172	15	0.022678			
Total	0.393309	17				

Table. 41. Shaken Flasks Run I - ANOVA Results for Variance in Carbohydrate Consumption between C and T flask groups during Shaken Flask Fermenters

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.679094	1	1.679094	52.56327	2.76E-05	4.964603
Within Groups	0.319442	10	0.031944			
Total	1.998536	11				

Table. 42. Shaken Flasks Run II- ANOVA Results for Variance in Carbohydrate Consumption between C, B and T flask groups during Shaken Flask Fermenters

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.010227	2	0.005113	0.117759	0.889724	3.68232
Within Groups	0.651323	15	0.043422			
_						
Total	0.661549	17				

Table. 43. Shaken Flasks Run III- ANOVA Results for Variance in Carbohydrate Consumption between all flask groups during Shaken Flask Fermenters

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.208493	5	0.041699	1.191641	0.327381	2.408514
	1 (70)(47	10	0.024002			
Within Groups	1.6/964/	48	0.034993			
Total	1.88814	53				

Table. 44. Shaken Flasks Run III- ANOVA Results for Variance in Lovastatin Production between all flask groups during Shaken Flask Fermenters

ANOVA							
Source of Variation	SS	df	MS	F	P-value	F crit	
Between Groups	88706.67	5	17741.33	47.54973	1.69E-07	3.1058	75
Within Groups	4477.333	12	373.1111				
Total	93184	17					
Number of families		1					
Number of comparisons							
per family		15					
Alpha		0.05					
		Mean	95.00% CI	Below	А	djusted	
Tukey's multiple compar	isons test	Diff.	of diff.	threshold?	Р	Value	
Control vs. Butyrolactor	ne-I	6.818	-5.222 to 18.8	6 No	0.	.4452	A-B
Control vs. Tyrosol (0.3	mM)	-31.82	-43.86 to -19.	78 Yes	<	0.0001	A-C
Control vs. Tyrosol (0.5	mM)	-17.88	-29.92 to -5.8	39 Yes	0.	.0033	A-D
Control vs. Farnasol (0.	2mM)	-38.26	-50.30 to -26.	22 Yes	<	0.0001	A-E

Control vs. Linoleic Acid (0.4mM)	-14.77	-26.81 to -2.733	3 Yes		0.0138	A-F	1
Butyrolactone-I vs. Tyrosol (0.3mM)	-38.64	-50.68 to -26.60)Yes		< 0.0001	B-C	
Butyrolactone-I vs. Tyrosol (0.5mM)	-24.70	-36.74 to -12.66	5 Yes		0.0002	B-D)
Butyrolactone-I vs. Farnasol (0.2mM)	-45.08	-57.12 to -33.04	4 Yes		< 0.0001	B-E	
Butyrolactone-I vs. Linoleic Acid (0.4mM)	-21.59	-33.63 to -9.551	l Yes		0.0007	B-F	
Tyrosol (0.3mM) vs. Tyrosol (0.5mM)	13.94	1.900 to 25.98	Yes		0.0205	C-D)
Tyrosol (0.3mM) vs. Farnasol (0.2mM)	-6.439	-18.48 to 5.600	No		0.5020	C-E	2
Tyrosol (0.3mM) vs. Linoleic Acid (0.4mM)	17.05	5.006 to 29.09	Yes		0.0048	C-F	
Tyrosol (0.5mM) vs. Farnasol (0.2mM)	-20.38	-32.42 to -8.339	9 Yes		0.0011	D-E	E
Tyrosol (0.5mM) vs. Linoleic Acid (0.4mM)	3.106	-8.934 to 15.15	No		0.9476	D-F	7
Farnasol (0.2mM) vs. Linoleic Acid (0.4mM)23.48	11.45 to 35.52	Yes		0.0003	E-F	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff	.n1	n2	q DF
Control vs. Butyrolactone-I	36.36	29.55	6.818	3.584	3	3	2.69012
Control vs. Tyrosol (0.3mM)	36.36	68.18	-31.82	3.584	3	3	12.55 12
Control vs. Tyrosol (0.5mM)	36.36	54.24	-17.88	3.584	3	3	7.05412
Control vs. Farnasol (0.2mM)	36.36	74.62	-38.26	3.584	3	3	15.09 12
Control vs. Linoleic Acid (0.4mM)	36.36	51.14	-14.77	3.584	3	3	5.82812
Butyrolactone-I vs. Tyrosol (0.3mM)	29.55	68.18	-38.64	3.584	3	3	15.2412
Butyrolactone-I vs. Tyrosol (0.5mM)	29.55	54.24	-24.70	3.584	3	3	9.744 12
Butyrolactone-I vs. Farnasol (0.2mM)	29.55	74.62	-45.08	3.584	3	3	17.7812
Butyrolactone-I vs. Linoleic Acid (0.4mM)	29.55	51.14	-21.59	3.584	3	3	8.51912
Tyrosol (0.3mM) vs. Tyrosol (0.5mM)	68.18	54.24	13.94	3.584	3	3	5.50012
Tyrosol (0.3mM) vs. Farnasol (0.2mM)	68.18	74.62	-6.439	3.584	3	3	2.541 12
Tyrosol (0.3mM) vs. Linoleic Acid (0.4mM)	68.18	51.14	17.05	3.584	3	3	6.725 12

Tyrosol (0.5mM) vs. Farnasol (0.2mM)	54.24	74.62	-20.38	3.584	3	3	8.04012
Tyrosol (0.5mM) vs. Linoleic Acid (0.4mM) 54.24	51.14	3.106	3.584	3	3	1.225 12
Farnasol (0.2mM) vs. Linoleic Acid (0.4mM	1)74.62	51.14	23.48	3.584	3	3	9.26612
Compact letter display							
Farnasol (0.2mM)	А						
Tyrosol (0.3mM)	А						
Tyrosol (0.5mM)	В						
Linoleic Acid (0.4mM)	В						
Control	С						
Butyrolactone-I	С						

Table. 45. ANOVA Results for Variance in pH during Fermentation in 2.5 L Stirred Tank Bioreactors

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.014809	3	0.004936	0.513272	0.680775	3.490295
Within Groups	0.115408	12	0.009617			
Total	0.130217	15				

Table. 46. ANOVA Results for Variance in Biomass Concentration during Fermentation in 2.5 L Stirred Tank Bioreactors

ANOVA						
Source of						
Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1522	3	0.050733	0.104447	0.95515	4.066181
Within Groups	3.885867	8	0.485733			
Total	4.038067	11				

Table. 47. ANOVA Results for Variance in Yellow Pigment Production during Fermentation in 2.5 L Stirred Tank Bioreactors

Source of Variation	SS	df		MS	F	P	value	Fc	erit
Between Groups	61.0737		3	20.357	79 706.2	.585 4.	92E-10	4	.066181
Within Groups	0.2306		8	0.02882	25				
Total	61.3043		11						
Number of families		1							
Number of comparisons per far	nily	6							
Alpha		0.05							
		Mean	95.0	0% CI	Below		Adjusted		
Tukey's multiple comparisons t	est	Diff.	of di	ff.	threshold?		P Value		
Control vs. Tyrosol (0.3mM)		-3.720	-4.13 3.28	54 to - 6	Yes		< 0.0001	A-B	
Control vs. Farnesol (0.2mM)		-6.153	-6.58 5.71	87 to - 9	Yes		<0.0001	A-C	
Control vs. Lonoleic acid (0.4	mM)	-1.360	-1.79 0.92	94 to - 59	Yes		< 0.0001	A-D	
Tyrosol (0.3mM) vs. Farnesol	(0.2mM)	-2.433	-2.80 1.99	57 to - 9	Yes		< 0.0001	B-C	
Tyrosol (0.3mM) vs. Lonoleic	acid (0.4mM)	2.360	1.92	6 to 2.794	Yes		< 0.0001	B-D	
Farnesol (0.2mM) vs. Lonolei	c acid (0.4mM)	4.793	4.35	9 to 5.227	Yes		< 0.0001	C-D	
Test details		Mean 1	Mea	n 2	Mean Diff.	SE of dif	f. n1	n2	q DF
Control vs. Tyrosol (0.3mM)		5.800	9.52	0	-3.720	0.1355	3	3	38.818
Control vs. Farnesol (0.2mM)		5.800	11.9	5	-6.153	0.1355	3	3	64.208
Control vs. Lonoleic acid (0.4	mM)	5.800	7.16	0	-1.360	0.1355	3	3	14.198
Tyrosol (0.3mM) vs. Farnesol	(0.2mM)	9.520	11.9	5	-2.433	0.1355	3	3	25.398
Tyrosol (0.3mM) vs. Lonoleic	acid (0.4mM)	9.520	7.16	0	2.360	0.1355	3	3	24.628
Farnesol (0.2mM) vs. Lonolei	c acid (0.4mM)	11.95	7.16	0	4.793	0.1355	3	3	50.018
Compact letter display									
Farnesol (0.2mM)		А							
Tyrosol (0.3mM)		В							
Lonoleic acid (0.4mM)		С							
Control		D							

Table. 48. ANOVA Results for Variance in Orange Pigment Production during Fermentation in 2.5 L Stirred Tank Bioreactors

Source of Variation	SS	df	MS	F	P-value	F crit		
Between Groups	148.2018	3	49.4006	1279.808	4.6E-1	1 4.0661	81	
Within Groups	0.3088	8	0.0386					
Total	148.5106	11						
Number of families		1						
Number of comparisons pe	r family	6						
Alpha		0.05						
Tukey's multiple compariso	ons test	Mean Diff.	95.00% CI of diff.	Below threshold?		Adjusted P Value		
Control vs. Tyrosol (0.3m	M)	-5.030	-5.544 to -4.510	5 Yes		< 0.0001	A-E	3
Control vs. Farnesol (0.2r	nM)	-9.520	-10.03 to -9.000	5 Yes		< 0.0001	A-C	2
Control vs. Lonoleic acid	(0.4mM)	-2.530	-3.044 to -2.010	5 Yes		< 0.0001	A-E)
Tyrosol (0.3mM) vs. Farm	uesol (0.2mM)	-4.490	-5.004 to -3.970	5 Yes		< 0.0001	B-C	
Tyrosol (0.3mM) vs. Lone	oleic acid (0.4mM)	2.500	1.986 to 3.014	Yes		< 0.0001	B-D)
Farnesol (0.2mM) vs. Lor	noleic acid (0.4mM)) 6.990	6.476 to 7.504	Yes		< 0.0001	C-D)
Test details		Mean 1	Mean 2	Mean Diff.	SE of diff.	.n1	n2	q DF
Control vs. Tyrosol (0.3m	M)	5.580	10.61	-5.030	0.1604	3	3	44.348
Control vs. Farnesol (0.2r	nM)	5.580	15.10	-9.520	0.1604	3	3	83.938
Control vs. Lonoleic acid	(0.4mM)	5.580	8.110	-2.530	0.1604	3	3	22.308
Tyrosol (0.3mM) vs. Farm	esol (0.2mM)	10.61	15.10	-4.490	0.1604	3	3	39.588
Tyrosol (0.3mM) vs. Lone	oleic acid (0.4mM)	10.61	8.110	2.500	0.1604	3	3	22.048
Farnesol (0.2mM) vs. Lor	noleic acid (0.4mM)) 15.10	8.110	6.990	0.1604	3	3	61.628
Compact letter display								

Farnesol (0.2mM) A

Tyrosol (0.3mM)	В
Lonoleic acid (0.4mM)	С
Control	D

Table. 49. ANOVA Results for Variance in Red Pigment Production during Fermentation in 2.5 L Stirred Tank Bioreactors

ANOVA									
Source of Variation	SS	df	MS	F	P	-value	Fa	rit	
Between Groups	102.362	3	34.12068	351.668	97.	87E-09	4.0	5618	1
Within Groups	0.7762	8	0.097025						
Total	103.1382	11							
Number of families		1							
Number of comparisons p	er family	6							
Alpha		0.05							
		Mean	95.00% CI	Below		Adjusted			
Tukey's multiple compari	sons test	Diff.	of diff.	threshold?		P Value			
Control vs. Tyrosol (0.3)	mM)	-4.320	-5.134 to -3.500	5 Yes		< 0.0001	A-B	5	
Control vs. Farnesol (0.2	2mM)	-8.150	-8.964 to -7.330	6 Yes		< 0.0001	A-C		
Control vs. Lonoleic acid	d (0.4mM)	-3.080	-3.894 to -2.266	6 Yes		< 0.0001	A-D)	
Tyrosol (0.3mM) vs. Far	mesol (0.2mM)	-3.830	-4.644 to -3.010	6 Yes		< 0.0001	B-C	2	
Tyrosol (0.3mM) vs. Lon	noleic acid (0.4mM)	1.240	0.4255 to 2.054	Yes		0.0054	B-D)	
Farnesol (0.2mM) vs. Lo	onoleic acid (0.4mM) 5.070	4.256 to 5.884	Yes		< 0.0001	C-D)	
					SE of				
Test details		Mean 1	Mean 2	Mean Diff.	diff.	n1	n2	q	DF
Control vs. Tyrosol (0.3)	mM)	5.550	9.870	-4.320	0.2543	3	3	24.0	28
Control vs. Farnesol (0.2	2mM)	5.550	13.70	-8.150	0.2543	3	3	45.3	28
Control vs. Lonoleic acid	d (0.4mM)	5.550	8.630	-3.080	0.2543	3	3	17.1	38
Tyrosol (0.3mM) vs. Far	mesol (0.2mM)	9.870	13.70	-3.830	0.2543	3	3	21.3	08
Tyrosol (0.3mM) vs. Los	noleic acid (0.4mM)	9.870	8.630	1.240	0.2543	3	3	6.89	58

Farnesol (0.2mM) vs. Lonoleic acid	(0.4mM) 13.70	8.630	5.070	0.2543 3	3	28.198
Compact letter display						
Farnesol (0.2mM)	А					
Tyrosol (0.3mM)	В					
Lonoleic acid (0.4mM)	С					
Control	D					

Table. 50. ANOVA Results for Variance in Carbohydrate Consumption during Fermentation in 2.5 L Stirred Tank Bioreactors

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	125.7778	5	25.15556	0.147204	0.977078	3.105875
Within Groups	2050.667	12	170.8889			
Total	2176.444	17				

Table. 51. ANOVA Results for Variance in Lovastatin Production during Fermentation in 2.5 L Stirred Tank Bioreactors

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	75075	3	25025	160.16	0.000127	6.591382
Within Groups	625	4	156.25			
Total	75700	7				
Number of families		1				
Number of comparisons pe	er family	6				
Alpha		0.05				
		Mean	95.00% CI	Below	Adjuste	ed
Tukey's multiple comparis	ons test	Diff.	of diff.	threshold?	P Value	e
Control vs. Tyrosol (0.3n	nM)	-39.20	-50.77 to -27.0	64 Yes	0.0006	A-B

Control vs. Farnesol (0.2mM)	-57.95	-69.52 to -46.39	9 Yes		0.0001	A-C		
Control vs. Lonoleic acid (0.4mM)	-16.48	-28.04 to -4.912	2 Yes		0.0150	A-D)	
Tyrosol (0.3mM) vs. Farnesol (0.2mM)	-18.75	-30.31 to -7.185	5 Yes		0.0094	B-C		
Tyrosol (0.3mM) vs. Lonoleic acid (0.4mM)	22.73	11.16 to 34.29	Yes		0.0046	B-D		
Farnesol (0.2mM) vs. Lonoleic acid (0.4mM)	41.48	29.91 to 53.04	Yes		0.0004	C-D		
				SE of				
Test details	Mean 1	Mean 2	Mean Diff.	diff.	n1	n2	q	DF
Control vs. Tyrosol (0.3mM)	39.20	78.41	-39.20	2.841	2	2	19.52	4
Control vs. Farnesol (0.2mM)	39.20	97.16	-57.95	2.841	2	2	28.85	4
Control vs. Lonoleic acid (0.4mM)	39.20	55.68	-16.48	2.841	2	2	8.202	4
Tyrosol (0.3mM) vs. Farnesol (0.2mM)	78.41	97.16	-18.75	2.841	2	2	9.334	4
Tyrosol (0.3mM) vs. Lonoleic acid (0.4mM)	78.41	55.68	22.73	2.841	2	2	11.31	4
Farnesol (0.2mM) vs. Lonoleic acid (0.4mM)	97.16	55.68	41.48	2.841	2	2	20.65	4
Compact letter display								
Farnesol (0.2mM)	А							
Tyrosol (0.3mM)	В							
Lonoleic acid (0.4mM)	С							
Control	D							

Section IV: Genome Assembly, Annotation and Transcriptomics

Genomic and Transcriptomic Analysis

This section investigates the genomic assembly, annotations, and comparison between *M. purpureus* C322 and other fungal species through pairwise alignments, revealing shared traits and evolutionary connections. Additionally, it explores the presence and functionality of QSM genes within *M. purpureus* C322, validated by both pairwise alignment and RT-PCR analyses. Utilising tools such as NCBI databases, BLAST, and Geneious Prime software, these experiments aim to elucidate fundamental genetic associations and signalling pathways influencing the behaviour and functions of this fungal species.

	Name ^	Description	% GC	Max Sequen	Min Sequen	Read Techn	# Sequences
4	NG-21878_Mona	Paired reads created from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and NG-21878_Monascus_purpure	48.1%	151	151	Illumina	11,852,314
₽	NG-21878_Mona	Couldn't be merged 4,375,314 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and N	48.1%	151	151	Illumina	8,750,628
Þ	NG-21878_Mona	Couldn't be merged 4,375,314 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and N	48.1%	151	151	Illumina	7,861,152
4	NG-21878_Mona	Couldn't be merged 4,375,314 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and N	49.1%	151	151	Illumina	6,815,454
4	NG-21878_Mona	Merged 1,550,843 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and NG-21878_Mo	48.2%	293	35	Illumina	1,550,843
Þ	NG-21878_Mona	Merged 1,550,843 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and NG-21878_Mo	48.8%	293	43	Illumina	717,723
₽	NG-21878_Mona	Merged 1,550,843 of 5,926,157 pairs from NG-21878_Monascus_purpureus_lib353627_6573_1_1 and NG-21878_Mo	49.1%	293	43	Illumina	577,825

Fig. 11. Summarising the process of assembling raw data through various stages. Each record in the table is identified by a description that details the specific assembly step and sequencing conditions, indicating whether the reads were merged or remained unmerged. Additionally, it presents the percentage of guanine and cytosine bases (% GC), the maximum and minimum sequence lengths, the type of sequencing technology employed (Illumina), and the total count of sequences.

	Name 🔨	Description	% HQ	Sequence L	Post-Trim Le	% Pairwise I	% GC	% Identical
Ø	[MP C322 Chr 1]	1,721,050 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009898.1 using Geneious	92.3%	4,110,925	4,110,925	98.0%	49.3%	81.3%
ø	[MP C322 Chr 2]	1,571,889 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009899.1 using Geneious	92.8%	3,845,046	3,845,046	99.0%	49.0%	81.4%
ø	[MP C322 Chr 3]	2,135,823 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009900.1 using Geneious	89.4%	3,436,821	3,436,821	98.7%	49.2%	80.5%
ø	[MP C322 Chr 4]	1,218,900 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009901.1 using Geneious	96.8%	2,822,336	2,822,336	99.2%	48.9%	81.5%
ø	[MP C322 Chr 5]	1,110,692 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009902.1 using Geneious	91.7%	2,740,423	2,740,423	98.9%	49.2%	81.5%
ø	[MP C322 Chr 6]	1,140,313 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009903.1 using Geneious	93.3%	2,685,662	2,685,662	98.2%	49.1%	81.1%
ø	[MP C322 Chr 7]	921,557 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009904.1 using Geneious	93.1%	2,250,131	2,250,131	99.0%	49.0%	81.5%
ø	[MP C322 Chr 8]	758,377 reads from NG-21878_Monascus_purpureus_lib353627_6573_1 mapped to CM009905.1 using Geneious	90.1%	1,927,652	1,927,652	99.2%	49.2%	81.6%

Fig. 12. Summarising the results from assembling raw data and contigs using the reference genome M. purpureus YY1, with detailed entries categorised by chromosome. Each entry includes a unique identifier, the number of reads, mapping details, and the specific reference used. Raw data was also utilised to fill any gaps in the contigs and for confirmation of sequence

integrity. Key metrics presented include the percentage of high-quality reads (% HQ), the total sequence length before and after trimming, the percentage of pairwise identity between reads, the GC content, and the percentage of sequences that exactly match the reference. This format effectively showcases the accuracy and quality of the genomic assembly and alignment processes applied to the data.

	Name	% Pairwise Identity
⊨	Candica albicans GCA_000182965.3_ASM18296v3_genomic Vs Monascus purpureus C322 Chrom	72.5%
⊨	Saccharomyces cerevisiae GCA_000146045.2_R64_genomic Vs Monascus purpureus C322 Chrom	72.3%
⊨	Neurospora crassa GCA_000182925.2_NC12_genomic Vs Monascus purpureus C322 Chromosom	73.6%
⊨	Fusarium oxysporum GCA_013085055.1_ASM1308505v1_genomic Vs Monascus purpureus C322	70.6%
⊨	Penicillium chrysogenum GCA_028827035.1_ASM2882703v1 Vs Monascus purpureus C322 Chro	74.5%
F	Aspergillus Terreus NT_165947.1 - NT_165944.1 - NT_165942.1 (+24) Vs Monascus purpureus C32	75.1%
⊨	Aspergillus niger NT_166525.1 - NT_166529.1 - NT_166518.1 (+17) Vs Monascus purpureus C322	75.2%
⊨	Monascus purpureus YY1 Vs Monascus purpureus C322 Chromosome 1 - 8 Concatenated	93.8%
⊨	Monascus pilosus GCA_018806995.1_ASM1880699v1_genomic Vs Monascus purpureus C322 Chr	94.0%
⊨	Monascus ruber GCA_002976275.1_ASM297627v1 Vs Monascus purpureus C322 Chromosome 1	94.4%

Fig. 13. Comparative genomic analysis via Geneious Prime software, showing the percentage of pairwise identity between the genome of *M. purpureus* C322 and various other fungal genomes. Each row lists a different fungal species along with the specific genomic accession number, compared against *M. purpureus* C322. The pairwise identity percentages indicate the degree of genetic similarity with *M. purpureus* C322.

Minimum v	Maximum	Sequence (with extension)	Length (with extensio	%GC	Hairpin Tm	Self Dimer Tm	Tm
1	20	GACAGAATTGGGCCAGCTCT	20	55.0	None	10.1	60.0
1	20	TGTTGTCCTGGATGTCGGTG	20	55.0	None	None	60.0
1	20	CCACATGATCGGCTGCATTG	20	55.0	None	None	60.0
1	20	GATCCATCGCGTAGTGCAGA	20	55.0	47.0	5.8	60.0
1	20	AATTCTGAGCCCGCATCCAA	20	50.0	None	None	60.0
1	20	TCCCGAATCGATCATGCTGG	20	55.0	None	3.4	60.0
1	20	ACTCGCATTCCCATAGCTCG	20	55.0	None	None	60.0
1	20	TGGTGCAGAAAACCCACTGT	20	50.0	35.8	None	60.0
1	20	CCAAGGATGTTCCCCTGGAC	20	60.0	40.7	None	60.0
1	20	CACCTGCAGGCAGACCAATA	20	55.0	46.3	33.1	60.0
1	20	GAAGGTTCCGTCGACAGTGT	20	55.0	None	17.2	60.0
1	20	AATCTCTTTGCCCCGATGCA	20	50.0	40.1	None	60.0
1	20	AGAGAGGTTTTCGGGGGATCG	20	55.0	40.9	None	59.2
1	22	GGGTAGGAATCAAATGGGTGTC	22	50.0	None	None	58.7
1	20	AACCTCTCAGTGGAACGCAG	20	55.0	45.9	None	60.0
1	20	TGTCTTCTGCCAGAACGACC	20	55.0	34.5	22.7	60.0
1	20	GCAGCATCTCCCTGTATCCC	20	60.0	32.9	None	60.0
1	20	GGGGTCTTGGGAATTGGGAG	20	60.0	None	None	60.0
1	20	GTGTATCTTCCGCCGGCATA	20	55.0	None	29.0	60.0
1	20	ACGCAGGAGATTGTTCGTGT	20	50.0	35.8	None	60.0
1	20	TCGGCTGTGAAGCTGTGAAT	20	50.0	41.6	6.5	60.0
1	20	TCGTTCAAGAGACTTGGCCC	20	55.0	None	None	60.0
1	20	CTATTCGCCTCTCCCAGCTG	20	60.0	None	6.8	60.0
1	20	GGATGATCTCGAGCAAGCCA	20	55.0	None	7.8	59.9
1	20	GACGGGTGCCTATGTGAACA	20	55.0	None	None	60.0
1	20	AGCGTACCCGAAGAAAAGCA	20	50.0	35.6	None	60.0

Fig. 14. Details of RT-qPCR primers designed using Geneious prime software for the analysis of the quorum sensing genes (QSMs).

18S rRNA Gene Sequence Alignment with M. purpureus C322 Genome

Given below are the results from NCBI BLAST alignment for the 18S rRNA sequence from *M. pilosus* IFO 4480 with *M. purpureus* C322. These results reveal the sequence and precise location of the 18S rRNA gene within Chromosome-3 of *M. purpureus* C322.

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Query ID	NG_064816.1 (nucleic ac	id)											
Query Descr	Monascus pilosus IFO 44	80 18S rRNA gene	e, partial seque	r							Filter	Rese	t
Query Length	1730												
Subject ID	lcl Query_4832553 (dna)												
Subject Descr	None												
Subject Length	982938												
Other reports	MSA viewer 🕜												
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Sequence ID: Query_4832553 Length: 982938 Number of Matches: 1

Range 1: 39729 to 41458 Graphics

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Score 3190 b	oits(1727	Expect	Identities 1729/1730(99%)	Gaps 0/1730(0%)	Strand Plus/Plus	_
Query	1	AAAGATTAAGCCAT	GCATGTCTAAGTGTAAGC	AATTTATACTGTGAAACTGC	GAATGGCT 60	
Sbjct	39729	AAAGATTAAGCCAT	GCATGTCTAAGTGTAAGC	AATTTATACTGTGAAACTGC	GAATGGCT 397	88
Query	61	CATTAAATCAGTTA	TCGTTTATTTGATAGTAC	CTTACTACATGGATACCTGT	GGTAATTC 120)
Sbjct	39789	CATTAAATCAGTTA	TCGTTTATTTGATAGTAC	CTTACTACATGGATACCTGT	GGTAATTC 398	48
Query	121	TAGAGCTAATACAT	GCTAAAAACCCCGACTTC	GGAAGGGGTGTATTTATTAG	ATAAAAAA 180)
Sbjct	39849	TAGAGCTAATACAT	GCTAAAAACCCCGACTTC	GGAAGGGGTGTATTTATTAG	ATAAAAAA 399	08
Query	181	CCAACGCCCTTCGG	GGCTCCTTGGTGAATCAT	AATAACTAAACGAATCGCAT	GGCCTTGC 240)
Sbjct	39909	CCAACGCCCTTCGG	GGCTCCTTGGTGAATCAT	AATAACTAAACGAATCGCAT	GGCCTTGC 399	68
Query	241	GCCGGCGATGGTTC/	ATTCAAATTTCTGCCCTA	TCAACTTTCGATGGTAGGAT	AGTGGCCT 300)
Sbjct	39969	GCCGGCGATGGTTC	ATTCAAATTTCTGCCCTA	TCAACTTTCGATGGTAGGAT	AGTGGCCT 400	28
Query	301	ACCATGGTGGCAAC	GGGTAACGGGGAATTAGG	GTTCGATTCCGGAGAGGGAG	CCTGAGAA 360)
Sbjct	40029	ACCATGGTGGCAAC	GGGTAACGGGGAATTAGG	GTTCGATTCCGGAGAGGGAG	CCTGAGAA 400	88
Query	361	ACGGCTACCACATC	CAAGGAAGGCAGCAGGCG	CGCAAATTACCCAATCCCGA	CACGGGGA 420)
Sbjct	40089	ACGGCTACCACATC	CAAGGAAGGCAGCAGGCG	CGCAAATTACCCAATCCCGA	CACGGGGA 401	.48
Query	421	GGTAGTGACAATAA	ATACTGATACGGGGCTCT	TTCGGGTCTCGTAATCGGAA	TGAGAACG 480)
Sbjct	40149	GGTAGTGACAATAA	ATACTGATACGGGGCTCT	TTCGGGTCTCGTAATCGGAA	TGAGAACG 402	08
Query	481	АССТАААТААССТА	ACGAGGAACAATTGGAGG	GCAAGTCTGGTGCCAGCAGC	CGCGGTAA 540)
Sbjct	40209	ACCTAAATAACCTA	ACGAGGAACAATTGGAGG	GCAAGTCTGGTGCCAGCAGC	CGCGGTAA 402	168
Query	541	TTCCAGCTCCAATA	GCGTATATTAAAGTTGTT	GCAGTTAAAAAGCTCGTAGT	TGAACCTT 600)
Sbjct	40269	TTCCAGCTCCAATA	GCGTATATTAAAGTTGTT	GCAGTTAAAAAGCTCGTAGT	TGAACCTT 403	28
Query	601	GGGTCTGGCTGGCC	GGTCCGCCTCACCGCGAG	TACTGGTCCGGCCGGACCTT	тссттста 660)
Sbjct	40329	GGGTCTGGCTGGCC	GGTCCGCCTCATCGCGAG	TACTGGTCCGGCCGGACCTT	TCCTTCTG 403	88
Query	661	GGGAACCTCATGGC	CTTCACTGGCTGTGGGGG	GAACCAGGACTTTTACTGTG	AAAAAATT 720)
Sbjct	40389	GGGAACCTCATGGC	CTTCACTGGCTGTGGGGG	GAACCAGGACTTTTACTGTG	AAAAAATT 404	48
Query	721	AGAGTGTTCAAAGC	AGGCCTTTGCTCGAATAC	ATTAGCATGGAATAATAGAA	TAGGACGT 780)
Sbjct	40449	AGAGTGTTCAAAGC	AGGCCTTTGCTCGAATAC	ATTAGCATGGAATAATAGAA	TAGGACGT 405	608
Query	781	GCGGTTCTATTTTG	TTGGTTTCTAGGACCGCC	GTAATGATTAATAGGGATAG	TCGGGGGC 840)
Sbjct	40509	GCGGTTCTATTTG	TTGGTTTCTAGGACCGCC	GTAATGATTAATAGGGATAG	TCGGGGGC 405	68
Query	841	GTCAGTATTCAGCT	GTCAGAGGTGAAATTCTT	GGATTTGCTGAAGACTAACT	ACTGCGAA 900)
Sbjct	40569	GTCAGTATTCAGCT	GTCAGAGGTGAAATTCTT	GGATTTGCTGAAGACTAACT	ACTGCGAA 406	528
Query	901	AGCATTCGCCAAGG	ATGTTTTCATTAATCAGG	GAACGAAAGTTAGGGGATCG	AAGACGAT 960)
Sbjct	40629	AGCATTCGCCAAGG	ATGTTTTCATTAATCAGG	GAACGAAAGTTAGGGGATCG	AAGACGAT 406	88
Query	961	CAGATACCGTCGTA	GTCTTAACCATAAACTAT	GCCGACTAGGGATCGGACGG	GTTTCTAT 102	0
Sbjct	40689	CAGATACCGTCGTA	GTCTTAACCATAAACTAT	GCCGACTAGGGATCGGACGG	GTTTCTAT 407	48

Query	1021	GATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGGAGTATGGT	1080
Sbjct	40749	GATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGGAGTATGGT	40808
Query	1081	CGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGC	1140
Sbjct	40809	CGCAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGC	40868
Query	1141	TTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAATAAGGATTGACAGATT	1200
Sbjct	40869	TTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAATAAGGATTGACAGATT	40928
Query	1201	GAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCCTAGTTGGTGGAGTGA	1260
Sbjct	40929	GAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCCTAGTTGGTGGAGTGA	40988
Query	1261	TTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCAT	1320
Sbjct	40989	TTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCAT	41048
Query	1321	TTGCGGGCCGCTGGCTTCTTAAGGGGACTATCGGCTCAAGCCGATGGAAGTGCGCGGCAA	1380
Sbjct	41049	TTGCGGGCCGCTGGCTTCTTAAGGGGACTATCGGCTCAAGCCGATGGAAGTGCGCGGCAA	41108
Query	1381	TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACAGGGCC	1440
Sbjct	41109	TAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACAGGGCC	41168
Query	1441	AGCGAGTACATCACCTTGGCCGAGAGGCCTGGGTAATCTTGTTAAACCCTGTCGTGCTGG	1500
Sbjct	41169	AGCGAGTACATCACCTTGGCCGAGAGGCCTGGGTAATCTTGTTAAACCCTGTCGTGCTGG	41228
Query	1501	GGATAGAGCATTGCAATTATTGCTCTTCAACGAGGAATGCCTAGTAGGCACGAGTCATCA	1560
Sbjct	41229	GGATAGAGCATTGCAATTATTGCTCTTCAACGAGGAATGCCTAGTAGGCACGAGTCATCA	41288
Query	1561	GCTCGTGCCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAA	1620
Sbjct	41289	GCTCGTGCCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAA	41348
Query	1621	TGGCTCAGTGAGGCCTCCGGACTGGCCCAGGGAGGTTGGCAACGAcccccccGGGCCGGA	1680
Sbjct	41349	TGGCTCAGTGAGGCCTCCGGACTGGCCCAGGGAGGTTGGCAACGACCCCCCGGGCCGGA	41408
Query	1681	AAGCTGGTCAAACTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTT 1730	
Sbjct	41409	AAGCTGGTCAAACTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTT 41458	

The NCBI BLAST results for the 18S rRNA gene were corroborated using Geneious Prime

software. The alignment with chromosome 3 of *M. purpureus* C322 is displayed below:

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	37,500	38,000	38,500	39,000		39,688	40,000	40,500	41,000	41,460	42,000) 42.5	00	43,000	43,500	44,000	44,500
Consensus Identity																	
1. 185 RNA gene 2. Chr 3 extract						1											

28S rRNA Sequence Alignment with M. purpureus C322 genome

Below are the outcomes of the NCBI BLAST alignment between the 28S rRNA sequence from *M. pilosus* IFO 4480 and *M. purpureus* C322. These findings elucidate not only the sequence but also the precise location of the 28S rRNA gene within Chromosome-3 of *M. purpureus* C322.

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Query	1	GCATATCAATAAGCG	GAGGAAAAGAAGCCGACC	GGGATTGCCTCAGTAACG	SCGAGTGAA	60
Sbjct	42018	GCATATCAATAAGCG	GAGGAAAAGAAACCAACC	GGGATTGCCTCAGTAACG	GCGAGTGAA	42077
Query	61	GCGGCAAGAGCTCAA	ATTTGAAAGCTGGCCCCT	CCGGGGTCCGCATTGTAA	TTTGCAGAG	120
Sbjct	42078	ĠĊĠĠĊĂĂĠĂĠĊŤĊĂĂ	ATTTGAAAGCTGGCCCCT	CCGGGGTCCGCGTTGTAA	TTTGCÁGÁG	42137
Query	121	GATGCTTCGGGCTCA	GCCCCCGTCTAAGTGCCC	TGGAACGGGCCGTCGGAG	AGGGTGAGA	180
Sbjct	42138	GATGCTTCGGGCTCA	GCCCCCGTCTAAGTGCCC	TGGAACGGGCCGTCGGAG	AGGGTGAGA	42197
Query	181	ATCCCGTCTGGGACG	GGGTGCCTGGGTCCATGT	GAAGCTCCTTCGACGAGT	CGAGTTGTT	240
Sbjct	42198	ATCCCGTCTGGGACG	GGGTGCCTGGGTCCATGT	GAAGCTCCTTCGACGAGT	CGAGTTGTT	42257
Query	241	TGGGAATGCAGCTCT	AAATGGGTGGTAAATTTC	ATCTAAAGCTAAATACTG	SCCGGAGAC	300
Sbjct	42258	TGGGAATGCAGCTCT	AAATGGGTGGTAAATTTC	ATCTAAAGCTAAATACTG	SCCGGAGAC	42317
Query	301	CGATAGCGCACAAGT	AGAGTGATCGAAAGATGA	AAAGCACTTTGAAAAGAG	AGTTAAACA	360
Sbjct	42318	CGATAGCGCACAAGT	AGAGTGATCGAAAGATGA	AAAGCACTTTGAAAAGAG	AGTTAAACA	42377
Query	361	GCACGTGAAATTGTT	GAAAGGGAAGCGCTTGCG	ATCAGACTCGCCTGCGGG	STTCAGCCG	420
Sbjct	42378	GCACGTGAAATTGTT	GAAAGGGAAGCGCTTGCG	ATCAGACTCGCCTGCGGG	GTTCAGCCG	42437
Query	421	GCATTCGTGCCGGTG	TACTTCCCCGTGGGCGGG	CCAGCGTCGGTTCGGGTG	SCCGGTCAA	480
Sbjct	42438	GCATTCGTGCCGGTG	TACTTCCCCGTGGGCGGG	CCAGCGTCGGTTCGGGTG	SCCGGTCAA	42497
Query	481	AGGCCCCGGGAATGT	GTCGCCCTCCGGGGCGTC	TTATAGCCCGGGGTGCCA	IIIIIIIIII	540
Sbjct	42498	AGGCCCCGGGAATGT	GTCGCCCTCCGGGGCGTC	TTATAGCCCGGGGTGCCA	TGCGGCCTA	42557
Query	541	CCTGGACCGAGGAAC	GCGCTTCGGCTCGGACGC	TGGCGTAATGGTCGTAAG	CGACCCGTC	600
Sbjct	42558	CCTGGACCGAGGAAC	GCGCTTCGGCTCGGACGC	TGGCGTAATGGTCGTAAG	CGACCCGTC	42617
Query	601	TTGAAACACGGACCA	AGGAGTCTAACATCTACG	CGAGTGTTTGGGTGGCAA	ACCCATACG	660
Sbjct	42618	TTGAAACACGGACCA	AGGAGTCTAACATCTACG	CGAGTGTTTGGGTGGCAA	ACCCATACG	42677
Query	661	CGCAGTGAAAGCGAA	CGGAGGTGGGAACCCTTC	GGGGCGCACCATCGACCG	ATCCTGATG	720
Sbjct	42678	CGCAGTGAAAGCGAA	CGGAGGTGGGAACCCTTC	GGGGCGCACCATCGACCG	ATCCTGATG	42737
Query	721	TCTTCGGATGGATTT	GAGTAAGAGCGTAGCTGT	GGGGACCCGAAAGATGGT	SAACTATGC	780
Sbjct	42738	TCTTCGGATGGATTT	GAGTAAGAGCGTAGCTGT	GGGGACCCGAAAGATGGT	SAACTATGC	42797
Query	781	CTGAATAGGGCGAAG	CCAGAGGAAACTCTGGTG	GAGGCTCGCAGCGGTTCT	SACGTGCAA	840
Sbjct	42798	CTGAATAGGGCGAAG	CCAGAGGAAACTCTGGTG	GAGGCTCGCÁGCGGTTCT	SACGTĠĊĂĂ	42857
Query	841	ATCGATCGTCAAATT	TGGGTATAGGGGCGAAAG	ACTAATCGAACCATCTGG	TAGCTGGGT	900
Sbjct	42858	ATCGATCGTCAAATT	TGGGTATAGGGGCGAAAG	ACTAATCGAACCATCTGG	TAGCTĠĠTŤ	42917
Query	901	C-TGC 904				
Sbjct	42918	CCTGC 42922				

The NCBI BLAST results for the 28S rRNA gene were confirmed using Geneious Prime

software. The alignment with chromosome 3 of *M. purpureus* C322 is displayed below:



Table 52 presents all the QS genes aligned with *M. purpureus* C322 genome. Notably, genes

exhibiting similarity are highlighted in bold for ease of reference. Furthermore, Figure 15

complements this analysis by depicting the RT-PCR results via an amplification plot,

confirming the presence of QS genes within *M. purpureus* C322 genome.

Table 52: List of all QS gene sequences	aligned pairwise	with <i>M</i> .	purpureus	C322	genome
using Geneious Prime Software					

Gene	Reference	Organism	Length (bp)
Farnesyl	CJI97_003197 -	Candida auris	1053bp
pyrophosphate	40028343		
synthase			
tRNA	CJI97_003267 -	Candida auris	1456bp
dimethylallyltransferase	40028413		
SARP family	HMPREF1085_RS27110	Clostridium bolteae	1236bp
transcriptional regulator	- 23116519	90A9	
	(discontinued)		
UDP-	AT5G03490 - 831823	Arabidopsis thaliana	1869bp
Glycosyltransferase			
superfamily protein			
UGT85A1: UDP-	UGT85A1 - 838846	Arabidopsis thaliana	3310bp
Glycosyltransferase			
superfamily protein			
tyrosine decarboxylase 1	LOC109840915	Asparagus officinalis	12789bp
Putative tyrosine	BO66DRAFT_379788 -	Aspergillus	1900bp
decarboxylase	37148948	aculeatinus CBS	
		121060	
Sphingosine-1-	NFIA_072940 - 4586038	Aspergillus fischeri	1808bp
phosphate		NRRL 181	
phosphohydrolase			
Polyketide synthase,	AFUA_1G02740 -	Aspergillus	1447bp
putative	3507885	fumigatus Af293	
Copper amine oxidase,	AFUA_3G14590 -	Aspergillus	2177bp
putative [ty oxi]	3512134	fumigatus Af293	
Farnesyl-	AFUA_5G02450 -	Aspergillus	1119bp
pyrophosphate	3505656	fumigatus Af293	
synthetase			
PAP2 domain protein	AFUA_6G04240 -	Aspergillus	830bp
	3505155	fumigatus Af293	
Isopentenyldiphosphate	BO70DRAFT_320148 -	Aspergillus	1094bp
isomerase	37062702	heteromorphus CBS	
		117.55	
AMO1_ASPNG	AN2532.2 - 2875160	Aspergillus nidulans	2,185bp
Copper amine oxidase		FGSC A4	
1 [ty oxi]			
Hypothetical protein	AN4991.2 - 2872788	Aspergillus nidulans	1376bp
Accession no: 2872788		FGSC A4	
Hypothetical protein	AN7316.2 - 2869849	Aspergillus nidulans	1016bp
Accession no: 2869849		FGSC A4	

ADH1 EMENI Alcohol	A N18070 2 2868277	Asparaillus nidulans	1168bp
debudrogenese I (A DH	AN0979.2 - 2008277	ECSC AA	11000p
I)		TUSC A4	
Lynothetical protein	<u> </u>	Aspanaillus nigan	1069hp
(discontinued) Accessio	All08g08900 - 4983208	CDS 512 99	100800
n no: 4083208		CDS 515.00	
11 110. 4983208	DO06DDAET 417170	A an anaillua nia an	2622hn
Aromatic ammo aciu	27101895	CDS 101992	20230p
Matalla hata lastamasa	ANOM 002802		1022hm
superfemily protein	ANOM_002803 -	NDDI 12127	10520p
	20804007	NKKL 1515/	14501-
IKINA Iganantanyityanafayaga	ANOM_005522 -	Aspergilius nomiae	14580p
Dutative C 8 stand	D174DDAET 442147	Amanaillua	1000hm
Futative C-8 steroi	P1/4DRAF1_442147 -	Asperguius	10990p
Isomerase (Erg-1)	30334834	novojumigatus IB I	
	A DUI5 54220272		10451
ADH5: NAD/NADP	ADH5 - 54529505	Aspergilius tanneri	18450p
debudrogenees			
uenyurogenase			90.4h-r
yisp: squalene/phytoene	yisr - 14352947	DACHIUS SUDTILIS	8040p
forD: formagy1	forD 026252	$\frac{\text{DES1}/013}{\text{Dasillus subt}^{11}}$	925hp
dinhoonhoto nhoonhotooo	Tarr - 950555	Ductitus subtilia atr	8230p
dipnosphate phosphatase		subsp. subuns str.	
	CLAC19.2 105755965	108	
Milk conjugated linoleic	CLAC18:2 - 105755865	Bos taurus	
acid percentage	A DUO 2640751		10471
ADH2: alconol	ADH2 - 3640/51	Canaiaa albicans	1047bp
Delichyl nymenhoanhote	CD26 02100 - 9044419	Candida dublinionaia	711hn
phosphatase	CD30_02100 - 8044418	Cunatua aublimensis	/110p
Inositol phosphoryl	CD36, 08590 - 8045048	Candida dubliniansis	1617bn
transferase putative	CD30_00370 - 0045040	CD36	10170p
fdft1: farnesyl-	fdft1 - 113572100	Flectrophorus	8 273hn
diphosphate		electricus	0,2750p
farnesyltransferase 1		ciccinicus	
vhiG· undecanrenvl	vbiG - 6301165	Frwinia	585hn
nvronhosnhate		tasmaniensis Ft1/99	50500
phosphatase		iasmanichistis Etti/99	
Probable C-8 sterol	FFUJ 02631 - 35396113	Fusarium fuiikuroi	760bp
isomerase erg-1		IMI 58289	r
Alcohol dehvdrogenase	FOYG 00855 -	Fusarium oxysporum	2.722bp
	42008387	NRRL 32931	
tRNA	FOXG 09080 -	Fusarium oxvsporum	1,817 bp
dimethylallyltransferase	28950664	f. sp. lycopersici	
, . ,		4287	
ADH5-alcohol	FPRO 06554 -	Fusarium	1,183bp
dehydrogenase V	42051433	proliferatum ET1	· •
N-3-oxododecanoyl-L-	SCV20265_3994 -	Pseudomonas	375bp
homoserine lactone	18073945 (discontinued)	aeruginosa	
quorum-sensing		SCV20265	
transcriptional activator			
LasR			
ADH1: alcohol	ADH1 - 854068	Saccharomyces	1,047bp
dehydrogenase ADH1		cerevisiae S288C	_

ADH2: alcohol	ADH2 - 855349	Saccharomyces	1,047bp
dehydrogenase ADH2		cerevisiae S288C	
ADH3: alcohol	ADH3 - 855107	Saccharomyces	1,128bp
dehydrogenase ADH3		cerevisiae S288C	
ADH4: alcohol	ADH4 - 852636	Saccharomyces	1,149bp
dehydrogenase ADH4		cerevisiae S288C	
ADH5: alcohol	ADH5 - 852442	Saccharomyces	1,056bp
dehydrogenase ADH5		cerevisiae S288C	
PHO8: alkaline	PHO8 - 852092	Saccharomyces	1,701bp
phosphatase PHO8		cerevisiae S288C	
AfsR/SARP family	ON12_RS10615 -	Streptomyces	1,290bp
transcriptional regulator	33088280 (discontinued)	acidiscabies 84-104	
AfsR-like regulator	ON12_RS22210 -	Streptomyces	930bp
	33085093 (discontinued)	acidiscabies 84-104	_
avaA: gamma-	avaA - 1210386	Streptomyces	1,038bp
butyrolactone	(discontinued)	avermitilis MA-4680	
biosynthesis protein		= NBRC 14893	
A-factor biosynthesis	I3J06_RS30410 -	Streptomyces	891bp
protein AfsA	61474228	clavuligerus	
bprA: butenolide	bprA - 6209752	Streptomyces griseus	849bp
phosphate reductase		subsp. griseus NBRC	
BprA		13350	
aldo-keto oxidoreductase	THAPS_28998 -	Thalassiosira	1,261bp
	7447146	pseudonana	
		CCMP1335	



Fig 15. The amplification curves obtained from RT-PCR analysis of various gene targets in *M. purpureus* C322. Each curve represents the real-time quantification of a specific gene as it undergoes amplification over successive PCR cycles. Relative Fluorescence Units (RFU) on Y-axis, indicating the level of fluorescent signal detected, which correlates with the amount of DNA being amplified. X-axis indicates the number of PCR cycles, showing the progression of the amplification process. Early Take-off (Low Cq value) indicates a higher initial amount of target DNA, while late Take-off (High Cq value) indicates a comparatively lower initial amount of target DNA.

Sequence Alignment of QS Genes with *M. purpureus* C322 Genome Using Geneious Prime Software

Below information provides an in-depth analysis of the sequence alignment of multiple quorum sensing (QS) genes with *M. purpureus* C322 genome. Geneious Prime software was utilised to identify and annotate several regions of genomic DNA exhibiting similarity to the QS genes. The alignments highlight key attributes such as nucleotide sequences, alignment intervals, and similarity percentages, facilitating a comprehensive understanding of the genetic correspondence between these QS genes and the *M. purpureus* C322 genome.











3) Sphingosine-1-phosphate phosphohydrolase [NFIA_072940 - 4586038] from *Aspergillus fischeri* NRRL 181; L- 1808 bp



160,000	1,465,000	1,470,000	1,475,000	1,480,000
	-			



4) Copper amine oxidase, putative [tyrosine oxidase type] [AFUA_3G14590 - 3512134] from *A. fumigatus* Af293; L- 2177 bp



Section

Name: copper amine oxidase, putative CDS Type: CDS Total Length: 2016 (over 4 intervals) Interval 1 Length: 332 Interval 1: 3,726,667 -> 3,726,998 Interval 1 Bases: ATGCCCCCCATCCTCTCGCC.. Intervals: <u>3,726,667 -> 3,726,998</u>, <u>3,727,061 -> 3,727,637</u>, <u>3,727,707 -> 3,728,677</u>... Bases: ATGCCCCCCATCCTCTCGCC. locus_tag: AFUA_3G14590 old_locus_tag: Afu3g14590 note: encoded by transcript AFUA_3G14590A codon_start: 1 transl table: 1 product: copper amine oxidase, putative protein_id: XP_754167.1 db_xref: GI:70998891 db_xref: GeneID:3512134 Transferred Translation: MAPHPLAILSEEETNLARDVVIAEHPNTVIRFREIYLLEPPKDQLREFLALEHAGRLSPTTPRPP RLATCOYDVIGADHIPSFHESVVDVVARKRVKHHIVGKOHHAPLTMSEFENLVERCFSSPLFKKALEDFDLPPGFEVTIEPWPYGGLDNT EENRRYFQGLCFATDKSKNNPDANFYSYPLPLIPVMDALTQKIIRVDRPATGGKGDGLTEQTFKRDIIGHCKASDYVPELLPDGTRRDLK PLNVVQPEGPSFRITNESLVEWQKWSFRVGFNPREGATIHDVWYDGRSVMHRLSISEMTVPYADPRPPYHRKQAFDFGDGGGGNMANNLS IGCDCLGVIKYFDAIVTGPDGSAKKLPNAICLHEQDNGIGWKHSNWRTGRAVVTRNRELVVQFIITLANYEYIFAYKFDQSGGITVESRA TGILNVVNIDPGKVSDYGNVVSGGVLAQNHQHIFCVRMDPAIDGPNNSVVVEESHPVPMNDVTNPKGNFYKVTQQTLERACYLDAAPQLN RTIKMINPHKKNPISGHPVGYKFIPLATQLLLADPNSVQAKRAQFAQHHVWVTKHRDGELYAGGRYTLQSQQEVDGVADAVKRGESVVDT DVVVWSTFGITHNPRVEDWPVMPVEIFQLMIKPADFFTANPSLDVPSSRNAASRVVQKECCRDAQL NCBI Feature Key: CDS NCBI Join Type: join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AFUA_3G14590 - 3512134 Transferred Similarity: 70.88% Primary Match: 70.88%; 3,726,667 -> 3,726,998, 3,727,061 -> 3,727,637, 3,727,707 -> 3,728,677, 3,728,741 -> 3,728,876; copper amine oxidase, putative CDS (AFUA 3G14590 - 3512134 Automatic Translation: MPPHPLAILSEQETTRARDVVLASYTDKVIDFREIYLLEPPKAQLKEYLALEHSARLSPTSPRPPRL ALCQYDVIGTDRLPEYHESVVDVELGKCVKHQVVGKPHHASIIQKEFDILVERCETSSLFKQALSDFDLPEGFEVVIEPWPYGPVDYPEE NRRYVQALCFARDTRSGNPDANFYSYPLPVIPVMDAVTQEIVRVDRPATGGKGEGLREQTFNRDIIGHCKASDYIPELLPEGTRKDLKQL NVVQPEGPSFRITDESLVEWQKWRFRVGFNPREGATIHDVWYDGRSVMYRLSVSEMTVPYADPRPPYHRKQAFDFGDGGGGNMANNLSLG CDCLGLIKYFDAVATEADGTAKKLPNVICLHEQDNGIGWKHSNWRTGRAVVTRHRELVVQFIITLANYEYIFAYKFDQSGGITLEARATG ILNVVNIDAGKTSEYGNVVSEGVLAQNHQHVFNVRIDPAIDGHENSVIVEEAHRVPMNQETNPNGNFYKIVRTPVERAGWLDAAPELNRT VKIINPHKINPISKNPVAYKFQPLPTQLLLADPDSIQARRAQFAQHHVWVTKYKDYELYAGGRYTLQSREEIGGVADAVQRGESVTDTDL WWTSFGITHIPRVEDWPVMPVETFQLMIRPYDFFTENPSLDVPSNKNLSSTLAPANCCRHSHI* >>> [?> ? Find ORFs (3.484 new) % Minimum size: 400 🔿 Name: copper amine oxidase, putative CDS Type: CDS Total Length: 2016 (over 4 intervals) Interval 2 Length: 577 Interval 2: 3,727,061 -> 3,727,637 Interval 2 Bases: GAAGGAGTTTGATATTCTCGT.. Intervals: <u>3,726,667 -> 3,726,998</u>, <u>3,727,061 -> 3,727,637</u>, <u>3,727,707 -> 3,728,677</u>, <u>3,728,741 -> 3,728,876</u> Bases: ATGCCCCCCATCCTCTCGCC... locus_tag: AFUA_3G14590 old_locus_tag: Afu3g14590 note: encoded by transcript AFUA_3G14590A codon_start: 1 transl table: 1 product: copper amine oxidase, putative protein_id: XP_754167.1 db xref: GI:70998891 db_xref: GeneID:3512134 Transferred Translation: MAPHPLAILSEEETNLARDVVIAEHPNTVIRFREIYLLEPPKDQLREFLALEHAGRLSPTTPRPP RLATCQYDVIGADHIPSFHESVVDVVARKRVKHHIVGKQHHAPLTMSEFENLVERCFSSPLFKKALEDFDLPPGFEVTIEPWPYGGLDNT EENRRYFQGLCFATDKSKNNPDANFYSYPLPLIPVMDALTQKIIRVDRPATGGKGDGLTEQTFKRDIIGHCKASDYVPELLPDGTRRDLK PLNVVOPEGPSERITNESI VEWOKWSERVGENPREGATIHDVWYDGRSVMHRI SISEMTVPYADPRPPYHRKOAEDEGDGGGGNMANNI S IGCDCLGVIKYFDAIVTGPDGSAKKLPNAICLHEQDNGIGWKHSNWRTGRAVVTRNRELVVQFIITLANYEYIFAYKFDQSGGITVESRA TGILNVVNIDPGKVSDYGNVVSGGVLAQNHQHIFCVRMDPAIDGPNNSVVVEESHPVPMNDVTNPKGNFYKVTQQTLERACYLDAAPQLN RTIKMINPHKKNPISGHPVGYKFIPLATOLLLADPNSVOAKRAOFAOHHVWVTKHRDGELYAGGRYTLOSOOEVDGVADAVKRGESVVDT DVVVWSTFGITHNPRVEDWPVMPVEIFQLMIKPADFFTANPSLDVPSSRNAASRVVQKECCRDAQL NCBI Feature Key: CDS NCBI Join Type: join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AFUA_3G14590 - 3512134 Transferred Similarity: 70.88%
Primary Match: 70.88%; 3,726,667 -> 3,726,998, 3,727,061 -> 3,727,637, 3,727,707 -> 3,728,677, 3,728,741 -> 3,728,876; copper amine oxidase, putative CDS (AFUA 3G14590 - 3512134) Automatic Translation: MPPHPLAILSEQETTRARDVVLASYTDKVIDFREIYLLEPPKAQLKEYLALEHSARLSPTSPRPPRL ALCQYDVIGTDRLPEYHESVVDVELGKCVKHQVVGKPHHASIIQKEFDILVERCETSSLFKQALSDFDLPEGFEVVIEPWPYGPVDYPEE NRRYVQALCFARDTRSGNPDANFYSYPLPVIPVMDAVTQEIVRVDRPATGGKGEGLREQTFNRDIIGHCKASDYIPELLPEGTRKDLKQL NVVQPEGPSFRITDESLVEWQKWRFRVGFNPREGATIHDVWYDGRSVMYRLSVSEMTVPYADPRPPYHRKQAFDFGDGGGGNMANNLSLG CDCLGLIKYFDAVATEADGTAKKLPNVICLHEODNGIGWKHSNWRTGRAVVTRHRELVVOFIITLANYEYIFAYKFDOSGGITLEARATG ILNVVNIDAGKTSEYGNVVSEGVLAQNHQHVFNVRIDPAIDGHENSVIVEEAHRVPMNQETNPNGNFYKIVRTPVERAGWLDAAPELNRT VKIINPHKINPISKNPVAYKFQPLPTQLLLADPDSIQARRAQFAQHHVWVTKYKDYELYAGGRYTLQSREEIGGVADAVQRGESVTDTDL VVWTSFGITHIPRVEDWPVMPVETFQLMIRPYDFFTENPSLDVPSNKNLSSTLAPANCCRHSHI* [?> Find ORFs (3,484 new) ? 28 Minimum size: 400 🗘



500,000 1,000,000 1,500,000 2,000,000 2,500,000 3,000,000 3,500,000 3,845,046 gene; 75.69% 280,000 220,000 240,000 260,000 320,000 340,000 chr_2_v1 P source Aspergillus fumigatus Af293; 75.69% 280,000 285,000 290,000 295,000 300,000 chr_2_v1 gene; 75.69% 3 43 source Aspergillus fumigatus Af293; 75.69% Name: source Aspergillus fumigatus Af293 Type: Source Length: 1114 Interval: <u>289,614 -> 290,727</u> Bases: ATGGCGACGACCACGAAGCGT.. organism: Aspergillus fumigatus Af293 mol_type: genomic DNA Sequence View Text View Lineage Info strain: Af293 \ominus Extract 🖉 R.C. 🛞 Translate 🖄 Add/Edit Anr db_xref: taxon:330879 chromosome: 5 ,500,00 500,00 ,000,000 NCBI Feature Key: source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AFUA_5G02450 - 3505656 Transferred Similarity: 75.69% Primary Match: 75.69%; <u>289,614 -> 290,727;</u> source Aspergillus fumigatus Af293 (AFUA <u>5G02450 - 3505656</u>) Primary Match Alignment: Open Alignment Source ATGGCAACTACCACCAGTCGTGCAGCCTTTGAGGCTGTTT...CGAGGCATTCCTTGGCAAGATCTACAAGCGCACCAAATAG Target ATGGCGACGACGACGAAGCGTGCTGATTTCGAGGCCGTGT...TGAATCTTTTCTGGCCAAGATTTACAAGCGGACCAAATAC \Box 220,000 240,000 260,000 280,000 300,000 320,000 340,000 Similarity: 40 % hr_2_v1 ₽> (?) Find: O Best match % source Aspergillus fumigatus Af293; 75.69% All matching annotations \$ Apply Advanced...

5) Farnesyl-pyrophosphate synthetase [AFUA_5G02450 - 3505656] from *A. fumigatus* Af293; L-1119 bp

6) PAP2 domain protein [AFUA_6G04240 - 3505155] from *Aspergillus fumigatus* Af293; L- 830 bp

chr_5_v2	134,000	2,136,000	2,138,000	2,140,000	2,142,000 gene; 60.36% Aspergillus fumigatu	2,144,000 us Af293; 60.36%
$ \begin{array}{c} < \\ \hline \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	Text View I Extract	Lineage Info anslate 🖒 Add/Edit Anr 0 1.000.000 1.250.000	Name: source Aspergillus Type: Source Length: 807 Interval: 2,140,044 -> 2,14 Bases: ATGGATCAACCAAC organism: Aspergillus fur mol_type: genomic DNA ot strain: Af293 db_xref: taxon:330879 chromosome: 6 NCBI Feature Key: source	s fumigatus Af293 10,850 GACTGGCA nigatus Af293		×
chr_5_v2	2.136.000	2.138,000	note: Derived Using Gene Transferred From: AFUA, Transferred Similarity: 6 Primary Match: 60.36%; j Primary Match Alignmer Source ATGGARGATACA Target ATGGATCAACCA rarget aTGGATCAACCA	GG04240 - 3505155 0.36% 2,140,044 -> 2,140,850; source nt: <u>Open Alignment</u> CCACTGGCATCGCTGTCGTTAAC AGACTGGCATCATTATCCCTAAC	Acard Goga Acard	AFUA 6G04240 - 3505155) GTTSAAGGGCGAGGTCACAGTGA GTCGAGGCGTCATGGTTGCAGTGA (d) % (ch ?) % (ch ?) % (d)

7) AMO1_ASPNG Copper amine oxidase 1 [tyrosine oxidase type] [AN2532.2 - 2875160] from *A. nidulans* FGSC A4; L-2,185 bp



Name: source Aspergillus nidulans FGSC A4 Type: Source Length: 2208 Interval: 3,726,667 → 3,728,874 Bases: ATGCCCCCCCCCCATCCTCGCC organism: Aspergillus nidulans FGSC A4 mol_type: genomic DNA strain: FGSC A4 bype: genomic DNA strain: FGSC A4 db.xref: taxon:227321 none: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred Similarity: 65.18% Primary Match: 65.18%; 3,726,667 -> 3,728,874; source Aspergillus nidulans FGSC A4 (AN2532.2 - 2875160)				X
Type: Source Length: 2208 Interval: 3,726,667 >> 3,728,874 Bases: ATGCCCCCCATCCTCTCGCC organism: Aspergillus nidulans FGSC A4 mol_type: genomic DNA strain: FGSC A4 db_xref: taxon:227321 chromosome: VII map: unlocalized NCB: Feature Key: source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From AN2532.2 - 2875160)	Name: source Aspergi		Name: source Aspergillus nidulans FGSC A4	
Length: 2208 Sequence View Text View Interval: 3,726,667 → 3,728,874 Bases: ATGCCCCCCATCCTCTCGCC organism: Aspergillus nidulans FGSC A4 mo_type: genomic DNA strain: FGSC A4 db_xref: taxon:227321 1 500,000 1.000,000 1.500,000 2.000,000 0 Transfered From: AN2532.2 - 2875160	Type: Source		Type: Source	
Interval: 3,726,667 >> 3,728,874 Bases: ATGCCCCCATCCTCTCCCCC organism: Aspergillus nidulans FGSC A4 mol_type: genomic DNA strain: FGSC A4 db_xref: taxon:227321 chores of the strain of	Length: 2208		Length: 2208	
Image: Sequence View Text View Lineage Info Sequence View Text View Lineage Info Image: Sequence View Text View View Lineage Image: Sequence View Text View View Lineage Image: Sequence View Text View View Lineage Image: Sequence View Text View	Interval: <u>3,726,667 -></u>		Interval: <u>3,726,667 -> 3,728,874</u>	
Sequence View Text View Lineage Info Sequence View Text View Lineage Info Strain: FSGC A4 strain: FSGC A4 db_xref: taxon:227321 db_xref: taxon:227321 1 500,000 1.000,000 2.000,000 VEB Feature Key: source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN2532.2 - 2875160 Transferred Similarity: 65.18% Primary Match: 65.18% 3,726,667 -> 3,728,874; source Aspergillus nidulans FGSC A4 (AN2532.2 - 2875160)	Bases: ATGCCCCCCA		Bases: ATGCCCCCCATCCTCTGCC	
Sequence View Text View Lineage Info mol type: genomic DNA strain: FGS CA4 ← → ← ←	organism: Aspergillus		organism: Aspergillus nidulans FGSC A4	
Kerrant	mol_type: genomic DN	equence View Text View Lineage Info	ew Lineage Info mol_type: genomic DNA	
 ← → → → Extract @ R.C. @ Translate → Add/Edit Annot, db,xref: taxon:227321 1 500,000 1.000,000 1.500,000 2.000,000 1.500,000 2.000,000 NCBI Feature Key: source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN2532.2 - 2875160 Primary Match: 65.18%; 3,726,667 -> 3,728,874; source Aspergillus nidulans FGSC A4 (AN2532.2 - 2875160) 	strain: FGSC A4		strain: FGSC A4	
1 500,000 1.000,000 1.500,000 2.000,000 map: unlocalized NCBI Feature Key: Source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN2532.2 - 2875160 Transferred Similarity: 65.18% Primary Match: 65.18% (3,726,667 -> 3,728,874; source Aspergillus nidulans FGSC A4 (AN2532.2 - 2875160)	dit Annota db_xref: taxon:227321	→ ⊖ Extract 🕼 R.C. 🕃 Translate 🖄 Add/Edi	. 🛞 Translate 🖆 Add/Edit Annota db_xref: taxon:227321	
map: unlocalized NCBI Feature Key: source note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN2532.2 - 2875160 Transferred Similarity: 65.18% Primary Match: 65.18%; <u>3,726,667 -> 3,728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)	2.000.000 chromosome: VII	500.000 1.000.000 1.500.000	1500,000 1,500,000 2,000,000 chromosome: VII	
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note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN2532.2 - 2875160 Transferred Similarity: 65.18% Primary Match: 65.18%; <u>3,726,667 -> 3,728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)			NCBI Feature Key: source	
Transferred From: AN2532.2 - 2875160 Transferred Similarity: 65.18% Primary Match: 65.18%; <u>3.726.667 -> 3.728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)	note: Derived using Ge		note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity	
Transferred Similarity: 65.18% Primary Match: 65.18%; <u>3,726,667 -> 3,728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)	Transferred From: AN		Transferred From: AN2532.2 - 2875160	
Primary Match: 65.18%; <u>3,726,667 -> 3,728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)	Transferred Similarit		Transferred Similarity: 65.18%	
	Primary Match: 65.18		Primary Match: 65.18%; <u>3,726,667 -> 3,728,874</u> ; source Aspergillus nidulans FGSC A4 (<u>AN2532.2 - 2875160</u>)	
Primary Match Alignment: Open Alignment	Primary Match Align		Primary Match Alignment: Open Alignment	
Source ATGGCCCCTCAACCCCCATCCCTCTGGCGATCACGCCTCTGGGGTCCGAGTGCCGAACTGCCCAACTTTAAGGCGGATTA	Source ATGGCCCCT		Source ATGGCCCCTCAACCCCATCCCCCCCCCCCCCCCTCTCTGAGGTCCGAGTGCTGTCGAACTGCCCAACTTTAAGGCGGAT	AA
Target ATGCCCCCCCATCCTCTCCCCCATCCTGTCTGAACACGTTGCCGAACTTCCCGCGAACTTCACATATT	Target ATGCCCCC-		Target ATGCCCCCCCATCCTCTCGCCATCCTGTCTGAACACGTTGCGCCCTGCGAATTGCTGCCGACATTCACATAT	тт
3,717,500 3,722,000 3,722,500 3,727,500 3,727,500 3,73,000 3,73,500 3,73,500 3,73,500 3,740,000 Similarity:	3,727,500 3,730,000 3,732	3,717,500 3,720,000 3,722,500 3,725,000	المراجع من مراجع من م	-
				>
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source 🛁 🗌 🖓 👋	sourc		sourc. All matching annotations	õ
				22
Anniv Advanced			Apply Advanced	15

8) ADH2_EMENI Alcohol dehydrogenase II (ADH II) [AN3741.2 - 2873163] from A. nidulans FGSC A4; L- 1320 bp



1	500,000 1,000,000	1,500	0,000 2,000,000	2,500,000	3,000,000	3,500,000	4,110,925
							1: chr_1_V2 ✓
chr_1_V2	3.000.000	3,200,00	Name: source Asp Type: Source Length: 1346 Interval: 3,140,653 Bases: TCACTCTTC/ organism: Aspergi mol_type: genomic strain: FGSC A4 db_xref: taxon:227: chromosome: II map: unlocalized NCBI Feature Key: note: Derived using Transferred From: Transferred From: Transferred Simila Primary Match Aii Source ATGGCTG	ergillus nidulans FG 3 -> 3,139,308 ACATGGCTGAA Ilus nidulans FGSC , c DNA 321 : source g Geneious Prime 2 : AN3741.2 - 287316 arity: 60.22% 0.22%; 3,140,653 -> : ignment: Open Alic crocrogaaarcccoaa	SC A4 V4 V21.2.2 'Annotate I 3 1,139,308; source A Inment GAAGGAAAAGGCTG3 TCAAATGCCAACGA	irom Database' spergillus nidul ICA GGGBAAG	based on nucleotide similarity lans FGSC A4 (AN3741.2 - 2873163)
Cursor befo	re base 3,470,646. Mouse over b	base 3,13	Target TCACTCT	TCAACATGGCTGAACO	TCAAATCCCAACCA	GCGGGAAAG	TTGCAGGGG <mark>A</mark> GAGTGGT CT T G GATCTTTCTTA
Intervals: 3.4 Bases: CGGCA locus_tag: AN codon_start: transl_table: product: ADH protein_id: Xf db_xref: Giene Transferred I TQPGQVGGHE ALAPPLICAGV HVKSLTTKGLG AHFREEKMEAL NCBI Feature NCBI Join Typ note: Derived NCBI Feature NCBI Join Typ note: Derived Primary Matc Automatic Tr DRLAAMKESEF	88.793 -> 3.488.668, 3.191.562 -> 3 GGTCGTGGCCATCCCC 3741.2 1 2_EMENI Alcohol dehydrogenase 1 2_661345.1 525567 1012873163 ranslation: MAAPEIPKKQKAVIYD IGVGKVVKLGAGAEASGLKIGDRVGV TVYASLKRSKAQPGQWIVISGAGGG AHAVIVCTASNIAYAQSLLFLRYNGTI .TEIFKEMEEGKLGGVVLDLS Key: CDS e: Join using Geneious Prime 2021.2.2 'A rom: AN3741.2 - 2873163 imilarity: 68.69% th: 68.69% 3.488.793 - 3.488.668 anslation: RQVVAIPKKMPLSCEDNT **SSSALEPMEPG*RLAIG*ESNGCPP 0*NAAKPNOASGL*FPAGAAAWA	II (ADH II NPGTVST NKWISSAC LGHLAVC MVCVGIP Annotate , <u>3,191,56</u> FGPVINH VHAGTVT T*PSK*P	3,140,321 -> 3,140,165 a,10 CRVVELDVPEPGDNEVLIN CGQCPPCQDGADGLCFN QLAAKGMGLRVIGVDHGS ENEPQAIASAYPGLFIQK from Database' based of 52 -> 3,191,519, 3,140,37 IGCRAAFDFTLLFEQAILSJ HATQAQTESASTRKSPA	1, 3,140,080 -> 3,139,30 NLTHSGVCHSDFGIMTN IQKVSGYYTPGTFQQYU KKELVKASGAEHFVDIT HVHVTGSAVGNRNEAII on nucleotide similarit 21 -> 3,140,165, 3,140, ALQRRLQLLEEEAEDGN TTPPAHSNNTRQAQLT TTPPAHSNNTRQAQLT TPPSLRAVPKSISTEPHSF	I TWKILPFP GPAQYVTPIPDGLPS (FPTGDKFEAISS TMEFAARGVIK W 80 -> 3,139,308; ADH: SPTQPSP *PRSPKTSLRPRP *PRSPKTSLRPRP KTTMALPSRTT*S	? EMENI Alcohol di	ehydrogenase II (ADH II) CDS (<u>AN3741.2 - 28731</u>
PSRQRSWGPT GWLSWRI*OI	PLSSARRQTRRTRRHFSSCASMVRWS	SVWGCR	NMSPRLLLRHIRLPSFSTR	LLLPVRQWGIG*RRLRC	ILPLGVLSNLML		
chr_	1_V2		50 S.F.F		5,000,000	-1, I T U, J Z J	Similarity: 40 % Find: 0 Best match ? All matching annotations
Name: ADH2 Type: CDS Total Length Interval 3: a. Interval 3: a. In	EMENI Alcohol dehydrogenase II 1100 (over 4 intervals) ingth: 157 140,321 -> 3,140,165 isse: TGGAAACTGCTCCCCTACCCA. 188,793 -> 3,488,668, 3,191,562 -> 3 AGGTCGTGGCCATCCCC 13741.2 1 1 1 2,EMENI Alcohol dehydrogenase P,661345.1 7526567 eID:2873163 Translation: MAAPEIPKKQKAVIYD EGVGKVVKLGAGAEASGLKIGDRVGA GAHAVIVCTASNIAYAQSLLFRYNGT LTEIFKAMEEGKLQGRVVLDLS e Key: CDS pe: join 1 using Genelous Prime 2021.2.2 '/ From: AN3741.2 - 2873163 Similarity: 68.69% ch: 68.69%; 3,488,793 -> 3,488,668 ranslation: RQVVAIPKMPLSCEDNI R*SSALEPMEPG*RLAIG*ESNGCP IQP*NAAKPNQASGL*FPAQAAAW/ PLSSARQTRRTRRHFSCASMKWIFL	(ADH II) 3,191,519 II (ADH II DNPGTVST /KWISSAG SLGHLAVG MVCVGIP Annotate 5,3,191,54 SLGHLAVG MVCVGIP Annotate 5,3,191,54 SUUCCON Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate 5,3,191,54 Annotate	CDS , 3,140,321 -> 3,140,165 I) TKVVELDVPEPGDNEVLIN CGQCPPCQDGADGLCFN QIAAKGMGLRVIGVDHG3 VENEPQAIASAYPGLFIQK from Database' based 52 -> 3,191,519, 3,140,33 HGCRAAFDFTLIFEQAILS HATQAQTESASTRKSPA PVEAWACA*SASTTAVKC NMSPRLLLRHIRLPSFSTR	xLTHSGVCHSDFGIMTN QKVSGVYTPGTFQQYVL SKEELVKASGAEHFVDITI HVHVTGSAVGNRNEAIE on nucleotide similarit 21 -> 3,140,165, 3,140,0 ALQRRLQLLEEAEDGNU TIPRAHSNNTRQAQLTI JPSLRNQVRSISSISPHSR LLLPVRQWGIG*RRLRC	WKILPFP SPAQVYTPIPDGLPS IFPTGDKFEAISS TMEFAARGVIK 9 80 -> 3,139,308; ADH2 SPTQPSP *PRSPKTSLRPRP KTTMALPSRHT*S ILPLGVLSNLML	EMENI Alcohol de	shydrogenase II (ADH II) CDS (<u>AN3741.2 - 287316</u>
chr_	_1_V2	(Find: Best match
							All matching annotations
Section

Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS		×
Type: CDS		
Total Length: 1100 (over 4 intervals)		
Interval 2 Length: 44		
Interval 2. 3, 191,302 -> 3, 191,319		
Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165, 3,140,080 -> 3,139,308		
Bases: CGGCAGGTCGTGGCCATCCCC		
locus_tag: AN3741.2		
codon_start: 1		
transl_table: 1		
product: ADH2_EMENI Alcohol dehydrogenase II (ADH II)		
protein_id: XP_obi345.1		
dD_xref: G_16/52056/		
Transferred Translation: MAAPEIPKKOKAVIYDNPGTVSTKVVELDVPEPGDNEVLINLTHSGVCHSDEGIMTNTWKILPEP		
TOPGQVGGHEGVGKVVKLGAGAEASGLKIGDRVGVKWISSACGQCPPCQDGADGLCFNQKVSGYYTPGTFQQYVLGPAQVVTPIPDGLPS		
AEAAPLLCAGVTVYASLKRSKAQPGQWIVISGAGGGLGHLAVQIAAKGMGLRVIGVDHGSKEELVKASGAEHFVDITKFPTGDKFEAISS		
HVKSLTTKGLGAHAVIVCTASNIAYAQSLLFLRYNGTMVCVGIPENEPQAIASAYPGLFIQKHVHVTGSAVGNRNEAIETMEFAARGVIK		
AHFREEKMEALTEIFKEMEEGKLQGRVVLDLS		
NCBI Feature Key: CDS		
NCBI Join Type: join		
Transformed From: AN3741 2 - 2873163		
Transferred Similarity: 68 69%		
Primary Match: 68.69%; 3.488.793 -> 3.488.668, 3.191.562 -> 3.191.519, 3.140.321 -> 3.140.165, 3.140.080 -> 3.139.308; ADH2 EMENI Alcohol	dehydrogenase II (ADH II) CDS (AN3741.2 - 2873	3163)
Automatic Translation: RQVVAIPKMPLSCEDNTFGPVINHGCRAAFDFTLLFEQAILSALQRRLQLLEEEAEDGNCSPTQPSP		
DRLAAMKESER*SSSALEPMEPG*RLAIG*ESNGCPVHAGTVTHATQAQTESASTRKSPATTPRAHSNNTRQAQLTT*PRSPKTSLRPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTERPRPARAMENTAMENTAMENTERPRPARAMENTAMENTAMENTAMENTAMENTAMENTAMENTAMENT		
RPFSAPASPSTQP*NAAKPNQASGL*FPAQAAAWAT*PSK*PVEAWACA*SASTTAVKQPSLRNQVRSISSISPHSRKTTMALPSRHT*S		
PSRQRSWGPTPLSSARRQTRRTRRHFSSCASMVRWSVWGCRNMSPRLLLRHIRLPSFSTRLLLPVRQWGIG*RRLRC*ILPLGVLSNLML		
GWLSWRI*QICLMRWQRESCRGEWSWIFL		
chr_1_V2	Similarity: 40 %	د?ع
	Find: O Best match	4
	All matching apportations	%
		Sar
	Apply Advanced	\$\$ \$
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS	Apply Advanced	(3) (3) (3) (3) (3) (3) (3) (3) (3) (3)
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS	Apply Advanced	×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals)	Apply Advanced	×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 3 488 768	Apply Advanced	× \$
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1: 3,488,793 -> 3,488,668 Interval 1: Bases: CGCAGGTCGTGGCCATCCCC	Apply Advanced	×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1: 3,488,793 -> 3,488,668 Interval 1: 3,488,793 -> 3,488,668 Interval: 3,488,793 -> 3,488,668 Interval: 3,488,793 -> 3,488,668	Apply Advanced	× 3
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 3,488,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGGCCATCCCC	Apply Advanced	×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Interval: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Iocus_tag: AN3741.2	Apply Advanced	(B) x
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Iocus, tag: AN3741.2 codon, start: 1	Apply Advanced	(A)
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668 Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGCAGGTCGTGGCCATCCCCC Iocus_tag: AN3741.2 codon_start: 1 transl_table: 1 served.ut ADH2 EMENI Alcohol dehydrogenegate II (ADH II)	Apply Advanced	(A)
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1: 3,488,793 -> 3,488,668 Interval 1: Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Iocus; tag: AN3741.2 codon, start: 1 transl_table: 1 product: ADH2_EMENI Alcohol dehydrogenase II (ADH II) product: XDH2_EMENI Alcohol dehydrogenase II (ADH II)	Apply Advanced	w ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 3,488,793 ~ 3,488,668 Interval 1 8,388; CGGCAGGTCGTGGCCATCCCC Interval 3,388,793 ~ 3,488,668 JINTERVAL 3,488,793 ~ 3,488,668 JINTERVAL 3,488,793 ~ 3,488,668 Interval 1,348,793 ~ 3,488,668 Interval 1,3	Apply Advanced	⊕ ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 3,488,793 -> 3,488,668 Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 4,487,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGTGGCCATCCCC Intervals: 4,487,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGTGGCCATCCCC Intervals: 4,487,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGTGGCCATCCCC Intervals: 4,487,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGTGGCCATCCCC Intervals: 4,487,793 -> 4,487,487,487,487,487,487,487,487,487,48	Apply Advanced	¢ ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: <u>3,488,793</u> -> <u>3,488,668</u> Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: <u>3,488,793</u> -> <u>3,488,668</u> , <u>3,191,562</u> -> <u>3,191,519</u> , <u>3,140,321</u> -> <u>3,140,165</u> Bases: CGCAGGTCGTGGCCATCCCCC Iocus tag: AN3741.2 codon, start: 1 transl table: 1 product: ADH2_EMENI Alcohol dehydrogenase II (ADH II) protein [d: XP_661345.1 db_xref: GeneID:2873163 Transferred Translation: MAAPEIPKKQKAVIYDNPGTVSTKVVELDVPEPGDNEVLINLTHSGVCHSDFGIMTNTWKILPFP	Apply Advanced	¢ ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCCC Iocus_tag: AN3741.2 codon_start: 1 transl_table: 1 product: ADH2_EMENI Alcohol dehydrogenase II (ADH II) protein_id: XP_661345.1 db_xref: Gir57526567 db_xref: GeneID:2873163 Transferred Translation: MAAPEIPKKQKAVIYDNPGTV5TK/VELDVPEPGDNEVLINLTHSGVCH5DFGIMTNTWKILPFP TQPGQVGGHEGVGKVVKLGAGAEASGLKIGDRVGVKWISSACGQCPPCQDGADGLCFNQKVSGYYTPGTFQQYVLGPAQYVTPIPDGLPS	Apply Advanced	@ ×
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Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Ength: 126 Interval 1 Bases: CGCAGGTCGTGGCCATCCCC Intervals: <u>3,488,793 -> 3,488,668</u> Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: <u>3,488,793 -> 3,488,668</u> J100us Lq3: AN3741.2 codon start: 1 transl table: 1 product: ADH2_EMENI Alcohol dehydrogenase II (ADH II) protein_id: XP_661345.1 db_xref: Gene[D:2873163 Transferred Translation: MAAPEIPKKQKAVIYDNPGTVSTKVVELDVPEPGDNEVLINLTHSGVCH5DFGIMTNTWKILPFP TQPGQVGGHEGVGKVVKLGAGAEASGLKIGDRVGKWISSACGQCPPCQDGADGLCFNQKYSGYVTPGTFQQVVLDPAQYVTPIPDGLPS AEAAPLICAGVTVVASLKRSKAQPGQWTVSGAGGGLGHLAVQIAAKGMGLRVIGVDHGSKEELVKASGAEHPVDTKFPFCDGKFEAISS HVKSLTTKGLGAHAVIVCTASNIAVAQSLLFLRYNGTMVCVGIPENEPQAIASAYPGLFQKHVHVTGSAVGNRNEAIETMEFAARGVIK AHFREEKMEALTEIFKEMEEGKLQGRVVLDLS NCBI Join Type: join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN3741.2 - 2873163 Transferred From: AN3741.2 - 2873163 Transferred Similarity: 68.69% Primary Match: 68.69%; <u>3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165, 3,140,080 -> 3,139,308; ADH2 EMENI Alcohol co Automatic Translation: RVVAIPKMPLSCEDNTFGPVINHGCGRAAFDFILLFEQALSALQRRLQLUEEEEAEDGNCSPTQPSP DRLAAMKESER*SSSALEPMEPG*RLAIG*ESNGCPVHAGTVTHATQAQTESASTKSPATTPRAHSNNTRQAQLT+*RSPKTSLRPRP RPF5APASPSTQP*NAAKRNQASGL *FPAQAAWAT*PSK*PVEAWACA*SASTTAVKQPSLRNQVRSISSISPHSRKTTMALPSRHT*S PSRQRSWGPTLSSARRQTRRTRRHFSCASMVRUSWWGCRNMSSPRLLLRHIRLPSFSTRLLLPVRQWGIG*RRLRC*1LPLGVLSNLML GWUSWRI*QICLMBWQRESCRGEWSWIEL</u>	Apply Advanced	© × ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Sa48,793 ~ 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 ~ 3,488,668, 3,191,562 ~ 3,191,519, 3,140,321 ~ 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC Iocus_tag: AN3741.2 codon_start: 1 transl_table: 1 product: ADH2_EMENI Alcohol dehydrogenase II (ADH II) protein_id: XP_661345.1 db_xref: Gfc7526567 db_xref: GeneID:2873163 Transferred Translation: MAAPEIPKKQKAVIYDNPGTVSTKVVELDVPEPGDNEVLINLTHSGVCHSDFGIMTNTWKILPFP TypCqVCsFH6GVKVVVLGGAGAEASGLKIGDRVGVKWISSACCQCPPCQDGADGLCENQKVSGVYTPGTPQQVU_GPAQVYTPIPDGLPS AEAAPLLCAGVTVYASLKRSKAQPGQWIVISGAGGGLGHLAVQIAAKGMGLRVIGVDHGSKEELVKASGAEHPVDITKFPTGDKFEAISS HVKSLTTKGLGAHAVUCTASNIAYAQSLLFLRYNGTNUCVGIPENPQAIASAYPGLFIQKHVHVTGSAVGNRNEAIETMEFAARGVIK AHFREEKMERGLGQGRVVLDLS NCBI Feature Key: CDS NCBI Join Type: join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN3741.2 · 2873163 Transferred Similarity: 68.69% Primary Match: 68.69%; 3,488,668, 3,191,562 ~ 3,191,519, 3,140,321 ~ 3,140,165, 3,140,080 ~ 3,139,308; ADH2 EMENI Alcohol of Automatic Translation: RQVAIPKMPLSCEDNTGPVINHGCRAAPDTLIFEQALSALQRRLQLUEEAEDGONCSPTQPSP DRLAAMKESER*SSSALEPMEPG*RLAIG*ESNGCPVIAGTVTHATQAQTESASTRKSPATTPRAHSNNTRQAQUT*PRSPKTSLRPPP RPSAPASPSTQP*NAAKPNQASGL*FPAQAAAWAT*PSK*PVEAWACA*SASTTAVKQPSLRNQVRSISSISPHSRKTTMALPSRH*S PSRQRSWGFTPLSSABRQTRRTRRHFSSCASMVRWSVWGCRNMSSPRLLLRHIRLPSFSTRLLLPVRQWGIG*RRLRC*LIPLGVLSNLML GWLSWRI*QICLIMWQRESCREWSWIFL	Apply Advanced	() ×
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 1 Length: 126 Interval 1 Sa48,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Intervals: 3,488,793 -> 3,488,668 .110:us tag: AN3741.2 codon start: 1 transl_table: 1 produc: ADH2_EMENI Alcohol dehydrogenase II (ADH II) protein_jid: XP_661345.1 db_xref: GineD22873163 Transferred Translation: MAAPEIPKKQKAVIYDNPGTV5TKVVELDVPEPGDNEVLINLTHSGVCHSDFGIMTNTWKILPFP TQPGQVGGHEGVGKVVKIKGAGAEASGLKIGDRVQVKWISSACGQCPPCQDGADGLCFNQKVSGVYTPGTPQVU.GPAQVYTPIPDGLPS AEAAPLLCAGVTVASLKRSKAQPGQWINISGAGGGLGHLAVQIAAKGMGLRVIGVDHGSKEELVKASGAEHFVDITKFPTGDKFEAISS HVKSLTTKGLGAHAVIVCTASNIAYAQSLLFLRYNGTNVCVGIPENEPQAIASAYPGLFIQKHVHVTGSAVGNRNEAIETMEFAARGVIK AHFREEKMEALTEIFKEMEGRLQGRVVLUS NCBI Feature Key: CDS NCBI Join Type: join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transferred From: AN3741.2 - 2873163 Transferred From: AN3741.2 - 2873163 Transferred Fin: RAN741.2 - 2873163 Transferred Similarity: 68.69% Primary Matt: 68.69% Primary 2021.2.2 'Annotate from Database' based on nucleotide similarity Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Primary Matt: 68.69% Pri	Apply Advanced dehydrogenase II (ADH II) CDS (AN3741.2 - 28731 Similarity: 40 %	(b) × 163
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 Intervals) Interval 1 2488,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Interval 3, 348,793 -> 3,488,668 Interval 1 Bases: CGGCAGGTCGTGGCCATCCCC Interval: 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165 Bases: CGGCAGGTCGTGGCCATCCCC locus tag: AN3741.2 codon, start: 1 transl, table: 1 product: ADH2, EMENI Alcohol dehydrogenase II (ADH II) protein_id: XP 661345.1 db xref: GeneID:2873163 Transfered Translation: MAAPEIPKKQKAVIYDNPGTVSTK/VELDVPEPGDNEVLINLTHSGVCHSDFGIMTNTWKILPFP TQFGQVGGHEGVGK/VKLGAGAEASGLKIGDRVGVKWISSACGQCPPCQDGADGLCFNQK/SGYTPGTFQQYVLGPAQYYTPIPDGLPS AEAAPLLCACYTYASLKRSKAQPGQWIVISGAGGGLGHLAVQIAAKGMGLRVIGVDHGSKEELVKASGAEHFVDITKFPTCDKFEAISS HVKSITTKGLGAHAVUCTASNIAVASLLFLRVNGTMVCVGIPENEPQAIASAYPGLFIQKHVHVTGSAVGNRNEAIETMEFAARGVIK AHFREEKMEALTEIFKEMEEGKLQGRVVLDLS NCBI Join Type: Join note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity Transfered From: AN3741.2 - 2873163 Transfered From: AN3741.2 - 2873163 Transfered Similarity: 68.69% Primary Match: 68.69%; 3,488,793 -> 3,488,668, 3,191,562 -> 3,191,519, 3,140,321 -> 3,140,165, 3,140,080 -> 3,139,308; ADH2 EMENI Alcohol of Automatic Translation: RPVWJPKMEMESCEDNTFGPVINHGCRAAPDFTLIFEQALISALQRRLQLIEEEAEDGNCSPTOPSP DRIAAMKESER*5SSALEPMEPG*RLAIG*ESNGCPVINAGTVTHATQAQTESASTRKSPATTPAHSNNTRQAQLTT*PRSPKTSLPRPP PPRSpRSWGPTLSSARROTRTRHFFSCSCASMWRVSVWGCRNMSPRLLLRHIRLPSFSTRLLLPVRQWSGG*RRLRC*ILPLGVLSNLML GWLSWRI*QICLMRWQRESCRGEWSWIFI.	Apply Advanced dehydrogenase II (ADH II) CDS (AN3741.2 - 28731 Similarity: 40 % Find: Best match 7 All matching appendictions 41	(中) * * * * * * * * * * * * * * * * * * *
Name: ADH2 EMENI Alcohol dehydrogenase II (ADH II) CDS Type: CDS Total Length: 1100 (over 4 intervals) Interval 7: 3488,793 - 3,488,668 Interval 7: 3488,793 - 3,488,668 Interval 7: 3488,793 - 3,488,668 Interval 7: 348,793 - 3,488,793 - 3,488,793 - 3,488,668 Interval 7: 348,793 - 3,488,793 - 3,488,668 Interval 7: 348,793 - 3,488,668 Interval 7: 348,793 - 3,488,668,3,191,562 - 3,191,519,3,140,321 - 3,140,165,3,140,080 - 3,139,308; ADH2 EMENI Alcohol de Automatic 7: 7: 348,793 - 3,488,668,3,191,562 - 3,191,519,3,140,321 - 3,140,165,3,140,080 - 3,139,308; ADH2 EMENI Alcohol de Automatic 7: 7: 348,793 - 3,488,668,3,191,562 - 3,191,519,3,140,321 - 3,140,165,3,140,080 - 3,139,308; ADH2 EMENI Alcohol de Automatic 7: 7: 348,793 - 3,488,668,3,191,562 - 3,191,519,3,140,321 - 3,140,165,3,140,080 - 3,139,308; ADH2 EMENI Alcohol de Automatic 7: 7: 348,793 - 3,488,668,3,191,562 - 3,191,519,3,140,321 - 3,140,165,3,140,080 - 3,139,308; ADH2 EMENI Alcohol de Automatic 7: 7: 7: 7: 7: 7: 7: 7: 7: 7: 7: 7: 7:	Apply Advanced dehydrogenase II (ADH II) CDS (AN3741.2 - 28731 Similarity: 40 % Find: Best match All matching annotations 3	(a) × 133 (b) × 4

9) Hypothetical protein [AN4991.2 - 2872788] from *A. nidulans* FGSC A4; - 1376 bp; Accession no: 2872788

	1,437,500	1,440,000	1,442,500	1,445,000	1,447,500	1,450,000	1,452,500	1,455,000	1,457,500	1,460,0
chr_3_v	/1				gene: 69.1	2%				
						270		_		
					- source /	Aspergillus r	idulans FGS	C A4; 69.12%	ó	
					+- hypothe	etical protein	CDS; 69.529	6		
				ł	hypothetical	protein mRN	VA; 69.52%			
			N	ame: source Aspr	ergillus nidulans i	EGSC A4		•••	70	K N X
			т	/pe: Source	crymus maaians i	OSC A4				
			Le	angth: 1383 hterval: 1 445 524	L-> 1 446 906					
	1.437.500 1.440.000	1.442.500 1.4	45.000 1.447 B	ases: ATGAATTCC	ATCCTTCCTTCC					
chr_3_v1 -		.,,	°!	rganism: Aspergil	llus nidulans FGS(C A4				
			st	rain: FGSC A4						
			-so di	o_xref: taxon:2273	321					
			m	ap: unlocalized						
			hypoth N	CBI Feature Key:	source	2021 2 2 'Annota	to from Database	bacad on nucley	otido cimilarity	
			T	ransferred From:	: AN4991.2 - 28723	2021.2.2 Annota 788	te irom Database	based on nucleo	Jude similarity	
			TI	ansferred Simila	arity: 69.12%	4 446 006				•
			PI	rimary Match: 69 rimary Match Ali	9.12%; <u>1,445,524 -</u> gnment: Open A	<u>> 1,446,906</u> ; sourc lignment	e Aspergillus nid	ulans FGSC A4 (<u>Al</u>	<u> 14991.2 - 2872788</u>	<u>8</u>)
<				Source ATGAATC	AAACACTTCCCAC	TGGAAGGACCGCA	CGCAGA TAC T	GATATTGAGTCCGG	CAGGCATACTTTCA	AGCCCTTGA
Mouse over l	base 1,446,059 (C), residue	161 (P/Pro/Proline	e) Same	larget ATGAATT	CCATCCTTCCTTCC	TGGAAGGA T CG T A	CACAGGCGAT	CACGAGCCGTCACG	JTAGACAGTG TTCA	ACCGTGGCT
			Type:	CDS	Stelli CDS					
			Total	Length: 1329 (ove	er 2 intervals)					
			Interv	/al 1: 1,445,524 ->	1,445,927					
			Interv	ral 1 Bases: ATGA	ATTCCATCCTTCCT	гтсс				
<			Bases	als: <u>1,445,524 -> 1</u>	<u>1,445,927</u> , <u>1,445,9</u> CTTCCTTCC	<u>982 -> 1,446,906</u>				
Sequence	e View Text View	Lineage In	fo locus	tag: AN4991.2						
$\leftarrow \rightarrow ($	→ Extract @ RC. S	Translate 🖆 A	dd/Edit A codon	similar to AF07669 • start: 1	91_1 aureobasidir	n-resistance prote	in; AurA			
1	500.000 1.	.000.000	1 B00.000 transl	table: 1						
-			produ	ct: hypothetical p in id: XP 662595.1	orotein 1					
			db_xre	ef: <u>GI:67537642</u>						
			db_xre Trans	≱f: <u>GeneID:287278</u> ferred Translatio	38 m: MNOTI PTWKD			ARRKI RSKI RSRAS	PTSSIASI OTSI SP.	AD
			TLRSL	QSHRWTVYDFQYL	LLLIVGIFSLTVIESP	GPLGKTAIFSMLLF	SLLIPMTRQFFLPF	LPIAGWLLPRAAVT	THICRFIPSDWRPA	AI
			WVRV		NILSAHQNVVLDVL SLARIDKI EGIDI VT	AWLPYGICHYGAF	PFVCSLIMFIFGPPG	TVPLFARTFGYISM	TAVTIQLFFPCSPP	WYE WYI SHHVA
chr 3 v1 =	1,437,500 1,440,000	1,442,500 1,4	VDLVA	GGLLAAIAFYFAKT	RFLPRVQLDKTFRV	WDYDYVEFGESALE	YGYGAAGYDGDF	NLDSDEWTVGSSS	SVSSGSLSPVDDH	IYSWETE
	_			HTDIESGRHTFSP						
			NCBI	Join Type: join						
			note:	Derived using Ger	neious Prime 202	1.2.2 'Annotate fre	om Database' bas	sed on nucleotide	similarity	
			Trans	ferred From: AN4	+991.2 - 2872788 : 69.52%					
			Prima	ry Match: 69.52%	6; <u>1,445,524 -> 1,4</u>	<u>45,927, 1,445,982</u>	<u>-> 1,446,906</u> ; hyp	othetical protein	CDS (<u>AN4991.2 - 7</u>	<u>2872788</u>)
			RALO	natic Translation	I: MNSILPSWKDR	I QDQLGKLQIQVP\ PLTKTAMATLLI ISI	/VKSFQLLVPHRMR _LMPITROFFLPFI F	RKIRSKLRSRVAPA	SSIAALQTSFSPLD	V V
			RILPA	LENILYGANISNILS	ANSNTVLDLLAWL	PYGICHYGAPFVV	SLILFIFGPPGTTPL	AQTFGYISITAVLI	QLAFPCSPPWYEN	IL
			YGLAP			GFKQSPVVFGAFP		ASYVEPKLKPLEVTY	TLWMWWATMYF	-SHHYAVD
Kouse over	base 1,445,692 (C), residue	e 57 (L/Leu/Leucin	e) SNSDI	HEPSR*TVFTVA	LI NCQI DIVITIWI	, o neighorf D h		00244AL00000000	SSSESI VEDITITIV	COLICI

	Name: hypothetical protein CDS	L
	Type: CDS	
	Total Length: 1329 (over 2 intervals)	İ.
	Interval 2 Length: 925	F
	Interval 2: 1,445,982 -> 1,446,906	
	Interval 2 Bases: GGTAGTTCTTCTGTCACTAAT	
	Intervals: <u>1,445,524 -> 1,445,927</u> , 1,445,982 -> 1,446,906	L
<	Bases: ATGAATTCCATCCTTCCTTCC	ĩ
Seguence View Lineage Infe	locus_tag: AN4991.2	1
Sequence view Text view Lineage Thio	note: similar to AF076691_1 aureobasidin-resistance protein; AurA	
\leftarrow \rightarrow \bigcirc Extract $@$ R.C. $@$ Translate $ riangle$ Add/Edit A	v codon_start: 1	I.
1 500.000 1.000.000 1.500.000	- transl_table: 1	ł.
1,000,000 1,000,000 1,000	product: hypothetical protein	
	protein_id: XP_662595.1	
	- db_xref: <u>GI:67537642</u>	
	db_xref: GeneID:2872788	£.
	Transferred Translation: MNQTLPTWKDRTQNQFGKLQIQVPWRSIQLLVPHRMRRKLRSKLRSRASPTSSIASLQTSLSPAD	1
	${\sf TLRSLQSHRWTVYDFQYLLLLVGIFSLTVIESPGPLGKTAIFSMLLFSLLIPMTRQFFLPFLPIAGWLLPRAAVTHICRFIPSDWRPAI}$	
	WVRVLPALENILYGANISNILSAHQNVVLDVLAWLPYGICHYGAPFVCSLIMFIFGPPGTVPLFARTFGYISMTAVTIQLFFPCSPPWYE	
1.437.500 1.440.000 1.442.500 1.445.000	NRYGLAPADYSIQGDPAGLARIDKLFGIDLYTSGFHQSPVVFGAFPSLHAADSTLAALFMSHVFPRMKPVFVTYTLWMWWATMYLSHHYA	5
chr 3 v1	VDLVAGGLLAAIAFYFAKTRFLPRVQLDKTFRWDYDYVEFGESALEYGYGAAGYDGDFNLDSDEWTVGSSSSVSSGSLSPVDDHYSWETE	£.
	ALTSPHTDIESGRHTFSP	Y
	S NCBI Feature Key: CDS	s
	NCBI Join Type: join	śν
	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity	
	Transferred From: AN4991.2 - 2872788	£.
h	Transferred Similarity: 69.52%	1
	Primary Match: 69.52%; <u>1,445,524 -> 1,445,927</u> , <u>1,445,982 -> 1,446,906</u> ; hypothetical protein CDS (<u>AN4991.2 - 2872788</u>)	
	Automatic Translation: MNSILPSWKDRTQDQLGKLQIQVPWRSFQLLVPHRMRRKIRSKLRSRVAPASSIAALQTSFSPLDTL	
	RALQKHQWTLYDFQYMFLLIIGIFSLSIIAVPGPLTKTAMATLLLISLLMPITRQFFLPFLPIAAWLTVVLLSLIPIRFIPSDWRPAIWV	£.
	RILPALENILYGANISNILSANSNTVLDLLAWLPYGICHYGAPFVVSLILFIFGPPGTTPLFAQTFGYISITAVLIQLAFPCSPPWYENL	72
	YGLAPADYSMQGNPAGLARIDKLFGVDLYSSGFKQSPVVFGAFPSLHAADSTLAALFMSYVFPKLKPLFVTYTLWMWWATMYFSHHYAVD	ŀ
<	LVCGGLLAAVAFYFAKTRFLPRCQPDKTFRWDYDYIEIGNSAPDYGYDLASLDGDYNLDDEWALGSSSVSSGSLSPVEDHYTWEGETLT	I.
Mouse over base 1,446,537 (A), residue 320 (T/Thr/Threonine)	SNSDHEPSK*TVFTVA	E

10) Hypothetical protein [AN7316.2 - 2869849] from *A. nidulans* FGSC A4; L- 1016 bp; Accession no: 2869849





11) ADH1_EMENI Alcohol dehydrogenase I (ADH I) [AN8979.2 - 2868277] from *A. nidulans* FGSC A4; L- 1168 bp



	Name: ADH1 EMENI A	ohol dehvdrogenase I (AD	H I) CDS			×
	Type: CDS	····· · · · · · · · · · · · · · · · ·	,			
	Total Longth: 1052 (ov	ar 2 inton(alc)				
	Interval 3 Length: 47	er 5 intervals/				
	Interval 3 Length: 47	2 0 4 2 4 0 4				
	Interval 3: 2,842,527 ->	2,642,461				
	Interval 3 Bases: ICCG	CGGAGGACIAGGGGGGGG				
	Intervals: <u>2,911,254 -></u>	<u>2,910,604</u> , <u>2,910,529 -> 2,91</u>	0,175, 2,842,527 -> 2,842, 4	<u>181</u>		
	Bases: ATGTCTCTTCCA	AGCTTCAA				
	locus_tag: AN8979.2					
	codon_start: 1					
	transl_table: 1					
	product: ADH1 EMENI	Alcohol dehvdrogenase I (A	DH I)			
	protein id: XP 682248.	, , , , ,				
	db yref: GI:67903984					
	db_xref: GapaID:286827	7				
	db_krei. deneid.280827				PLVC	
		n: MSIPTMQWAQVAEKVGG	PLVYKQIPVPKPGPDQILVK		PLVG	
	GHEGAGIVVAKGELVHEF	EIGDQAGIKWLNGSCGECEF	RQSDDPLCARAQLSGYTVL	GIFQQYALGKASHASKIPAGVPLDA	AAPVLCA	
<	GITVYKGLKEAGVRPGQT	VAIVGAGGGLGSLAQQYAKA	MGIRVVAVDGGDEKRAMCE	SLGTETYVDFTKSKDLVADVKAATPD	GLGAHAV	
Sequen	ILLAVSEKPFQQATEYVRS	RGTIVAIGLPPDAYLKAPVIN1	VVRMITIKGSYVGNRQDGV	EALDFFARGLIKAPFKTAPLKDLPKIYE	LM	
bequein	EQGRIAGRYVLEMPE					
$\leftarrow \rightarrow$	🕞 E NCBI Feature Key: CDS					
	NCBI Join Type: join					
1	⁵⁰ note: Derived using Ger	neious Prime 2021.2.2 'Ann	otate from Database' base	ed on nucleotide similarity		
	Transferred From: AN8	979.2 - 2868277				
	Transferred Similarity	69.42%				
	Primary Match: 69.42%	2 911 254 -> 2 910 604 2	910 529 -> 2 910 175 2 843	527 -> 2 842 481: ADH1 EMENI AL	cobol debydrogenase I (ADH I) CDS (At	18070 2 - 2868277)
	Automatic Translation	MSI BKI OWAOVA/EEAGGB				10575.2 - 2000277
	EGAGVVVAKGQLVNNVN	IIGDHAGVKWLNGSCLSCEFC	RISDESLCPDASLSGTIVDG	IFQQFCIAKAAHVSILPKDVPLDAVAI	PVLCAGI	
	TVYKGLKESGIQPGQTLA	VGAGGGLGSLAQQYAKAMO	LRVIAIDGGEEKKELCQQLG	AESYVDFTKSKDLVKDVQAATPDGLC	SPHAALL	
	LAVAEIPFQQAAAYVRPR	GVVVAIGLPAGAFLKAPVFST	/VRMIQIRGSYVGNRQDGIE	AIDFLRRGLIKAPFKTVDLSELPKVYEL	MVR	
	GGLGGGYVLFLLWG					
	2,000,000	2,000 2,00	2,550,000	3,000,000	Similarity:	40 %
cnr_1_v2			2		Similarity.	
					Find: 💽 Best match	(?)
						~ ~
		1			 All matching annotati 	ons
		1	-		A market A sharen and	<u></u>
	Name: ADH1 EMENI Ald	ohol dehydrogenase I (ADI	H I) CDS			ж
	Name: ADH1 EMENI Ald	ohol dehydrogenase I (ADI	H I) CDS			х
	Name: ADH1 EMENI Ald Type: CDS	ohol dehydrogenase I (ADI	H I) CDS			×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove	ohol dehydrogenase I (ADI rr 3 intervals)	H I) CDS			×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2 910 520 ->	ohol dehydrogenase I (ADI r 3 intervals) 2 910 175	H I) CDS			x
	Name: ADH1 EMENI Ald Type: CD5 Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 ->	ohol dehydrogenase I (ADI rr 3 intervals) 2,910,175	H I) CDS			×
	Name: ADH1 EMENI Ak Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2 Bases: TCCT/	ohol dehydrogenase I (ADI r 3 intervals) 2,910,175 XCGTCGACTTCACCAAG	4 I) CDS			x
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Bases: TCCT Interval 2 Bases: TCCT Intervals: <u>2,911,254 -> ;</u>	ohol dehydrogenase I (AD) er 3 intervals) 2,910,175 XGGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u>	<u>81</u>		x
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2: 2,910,529 -> Interval 2: 2,911,254 -> Bases: ATGTCTCTTCCAP	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 ACGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u>	<u>81</u>		×
	Name: ADH1 EMENI Ak Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2: Bases: TCT/ Interval: 2,911,254 -> ; Bases: ATGTCTCTCCAP locus_tag: AN8979.2	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u>	<u>81</u>		x
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2 Bases: TCCTI Intervals: <u>2,911,254 -></u> Bases: ATGTCCTTCCAP locus_tag: AN8979.2 codon_start: 1	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 XGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175, <u>2,842,527 → 2,842,</u> 4	<u>81</u>		×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Length: 355 Interval 2 Bases: TCCT/ Intervals: <u>2,911,254 ->;</u> Bases: ATGTCTCTTCCAA locus_tag: AN8979.2 codon_start: 1 trans_table: 1	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 ACGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175, 2,842,527 → 2,842,4	<u>81</u>		×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2 Bases: TCCT/ Intervals: 2,911,254 -> 3 Bases: ATGTCTCTCCAA locus_tag: AN8379.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI/	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 ACGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175 , <u>2,842,527 → 2,842,4</u> DH I)	<u>81</u>		x
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 2; 2,910,529 -> Interval 2 Bases: TCCTI Intervals: <u>2,911,254 -></u> Bases: ATGTCCTTCCAP locus_tag: AN8979.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI / protein id: XP 682248.1	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 XGGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u> DH I)	<u>81</u>		×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Length: 355 Interval 2 Bases: TCCT/ Intervals: 2,911,254->; Bases: ATGTCCTTCCAA locus_tag: AN8979.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI / protein_id: XP_682248.1 db xref: G167903894	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 ACGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0.175 , <u>2,842,527 -> 2,842,4</u> DH I)	<u>81</u>		×
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2: 2,910,529 -> Interval 2 Bases: TCCT/ Intervals: 2,911,254 -> 3 Bases: ATGTCTCTCCAA locus_tag: AN8379.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI / protein_id: XP_682248.1 db_xref: GeneD: 286827	ohol dehydrogenase I (ADI er 3 intervals) 2,910,175 ACGTCGACTTCACCAAG ,910,604, 2,910,529 -> 2,91 AGCTTCAA	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u> DH I)	81		x
	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 2, 2,910,529 -> Interval 2 Bases: TCCTI Intervals: 2,911,254 -> ; Bases: ATGTCCTTCCAA locus_tag: AN8979.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI / protein_id: XP_682248.1 db_xref: G:67903984 db_xref: Gene10:286827 Transferred Translatio	ohol dehydrogenase I (ADI rr 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA Ncohol dehydrogenase I (A Z	H I) CDS 0,175 , <u>2,842,527 -> 2,842,4</u> DH I)		PLVG	×
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✓ Sequence ← → (1)	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Larght: 355 Interval 2 Bases: TCCT/ Interval 2 Bases: TCCT/ Intervals: 2,911,254 -> ; Bases: ATGTCTTCCAA Bases: ATGTCTTCCAA locus_tag: AN8379.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI / protein_id: XP_682248.1 db_xref: Ghe2P03884 db_xref: Ghe2P03884 EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE Finary Match: 69.42% Automatic Translation EGGAVVVAKGQLVNVNX	iohol dehydrogenase I (AD) ir 3 intervals) 2,910,175 VGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA NICohol dehydrogenase I (A 7 m: MSIPTMQWAQVAEKVGG EIGDQAGIKWLNGSCGECEFG (AUVGAGGGLGSLAQQYAKA RGTIVAIGLPPDAYLKAPVINT helous Prime 2021.2.2 'Ann 979.2 - 2868277 69.42% ; 2,911.254-> 2,910,604, 2,1 : MSLPKLQWAQVVEEAGGP IGDHAGVKWLNGSCLSCEFG	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVKI RQSDDPLCARAQLSGYTVD MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI otate from Database' base 10,529 -> 2,910,175, 2,842 /IYKQIPVPTPGPDEVLVNIK RTSDESLCPDASLSGYTVDG LWM0FCFUVECCOF	181 IRYSGVCHTDLHAMMGHWPIPVKMI IGTFQQYALGKASHASKIPAGVPLDA SLGTETYVDFTKSKDLVADVKAATPD EALDFFARGLIKAPFKTAPLKDLPKIYE 2d on nucleotide similarity 1527 -> 2.842.481; ADH1 EMENI AI YTGVCHTDLHARKGDWPLPVKMPLJ TFQQFCIAKAAHVSILPKDVPLDAVAJ	PLVG AAPVLCA GLGAHAV ELM Icohol dehydrogenase I (ADH I) CDS (AI /GGH PVLCAGI	* <u>N8979.2 - 2868277</u>)
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Sequence € → (0) 1 chr_1_V2	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Langth: 355 Interval 2 Langth: 357 Bases: ATGTCTCTCAA Iocus tag: AN8379.2 codon_start: 1 transl_table: 1 protein_id: XP_682248.1 db_xref: Gls67903984 db_xref: Gls67903984 comparing table: Cls674 db_xref: Gls67903984 db_xref: Gls67904 db_xref: Gls67904 d	ohol dehydrogenase I (AD) r 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA NICohol dehydrogenase I (A 7 m: MSIPTMQWAQVAEKVGG EIGDQAGIKWLNGSCGECEFC AIVGAGGGLGSLAQQYAKA RGTIVAIGLPPDAYLKAPVINT belous Prime 2021.2.2 'Ann 979.2 - 2868277 69.42% ; 2,911,254 -> 2,910,604, 2,4 : MSLPKLQWAQVVEEAGGP IGDHAGVKWLNGSCLSCEFC IGDHAGVKWLNGSCLSCEFC SVVVAIGLPAGAFLKAPVFSTN	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVK RQ5DDPLCARAQLSGYTVDG MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI btate from Database' base 210,529 -> 2,910,175, 2,842 VTYKQIPVPTPGDEVLVNIK RTSDESLCPDASLSGYTVDG VRMIQIRGSYVGNRQDGIE	81 IRYSGVCHTDLHAMMGHWPIPVKMI GTFQQYALGKASHASKIPAGVPLDA SLGTETYVDFTKSKDLVADVKAATPD EALDFFARGLIKAPFKTAPLKDLPKIYE ed on nucleotide similarity 1527 -> 2,842,481; ADH1 EMENI AI YTGVCHTDLHARKGDWPLPVKMPLV TFQQFCIAKAAHVSILPKDVPLDAVAI AESYVDFTKSKDLVKDVQAATPDGLC ALDFLRRGLIKAPFKTVDLSELPKVYEL	PLVG AAPVLCA JGLGAHAV LM Icohol dehydrogenase I (ADH I) CDS (Af /GGH PVLCAGI SPHAALL MVR	× N8979.2 - 2868277)
Sequence € → (1) 1 chr_1_V2	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Langth: 355 Interval 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases	ohol dehydrogenase I (ADI ar 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA A	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVK RQSDDPLCARAQLSGYTVD MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI otate from Database' base 210,529 -> 2,910,175, 2,842 VYKQIPVPTPGPDEVLVNIK RTSDESLCPDASLSGYTVDG LRVIAIDGGEEKKELCQQLG VRMIQIRGSYVGNRQDGIE	181 IRYSGVCHTDLHAMMGHWPIPVKMI IGTFQQYALGKASHASKIPAGVPLDA SLGTETYVPFTKSKDLVADVKAATPD EALDFFARGLIKAPFKTAPLKDLPKIYE 2d on nucleotide similarity 2527 -> 2,842,481; ADH1 EMENI Al YTGVCHTDLHARKGDWPLPVKMPLV TFQQFCIAKAAHVSILPKDVPLDAVAI AESYVPTKSKDLVKDVQAATPDGLQ AIDFLRRGLIKAPFKTVDLSELPKVYEL	PLVG AAPVLCA GLGAHAV :LM Icohol dehydrogenase I (ADH I) CDS (AI /GGH PVLCAGI 3PHAALL MVR 	× N8979.2 - 2868277)
< Sequence Sequence	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Larght: 355 Interval 2 Bases: TCCT Intervals: 2,911,254->; Bases: ATGTCTTCCAA locus_tag: AN8979.2 codon_start: 1 transl_table: 1 product: ADH1_EMENI/ protein_id: XP_682248.1 db_xref: G:f67903984 db_xref: GeneID:288827 Transferred Translatio GHEGAGIVVAKGELVHEFI EQGRIAGRYVLEMPE EQGRIAGRYVLEMPE E NCBI Feature Key: CDS NCBI Join Type: Join note: Derived Vision: AN8 Transferred Similarity; Primary Match: 69.42% Automatic Translation EGAGVVAKGQLVNNVN TVYKGLKESGIQPGQTLAI LAVAEIPFQQAAAVVRPR GGLGGGYVLFLLWG	ohol dehydrogenase I (ADI sr 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA SICobal dehydrogenase I (A <u>7</u> m: MSIPTMQWAQVAEKVGG EIGDQAGIKWLNGSCGECEFG /AIVGAGGGLGSLAQQYAKA RGTIVAIGLPPDAYLKAPVINT eleious Prime 2021.2.2 'Ann 979.2 - 2868277 69.42% ; 2,911.254 -> 2,910,604, 2,' EIDHAGVKWLNGSCLSCEFC VGAGGGLGSLAQQYAKAMCG SVVVAIGLPAGAFLKAPVFSTN	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVKI RQ5DDPLCARAQLSGYTVD MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI otate from Database' base 210,529 -> 2,910,175, 2,842 VIXKQIPVPTPGPDEVLVNIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASLSGYTVAIK RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDASL RTSDESLCPDA	181 IRYSGVCHTDLHAMMGHWPIPVKMI IGTTQQYALGKASHASKIPAGVPLDA ISLGTETYVDFTKSKDLVADVKAATPD FALDFFARGLIKAPFKTAPLKDLPKIYE ed on nucleotide similarity 1.527 2.842,481; ADH1 EMENI AI 1.527 2.842,481; ADH1 EMENI AI TGVCHTDLHARKGDWPLPVKMPLV TFQQFCIAKAAHVSILPKDVPLDAVAI AESYVDFTKSKDLVKDVQAATPDGLC AIDFLRRGLIKAPFKTVDLSELPKVYEL	PLVG AAPVLCA JGLGAHAV LM Icohol dehydrogenase I (ADH I) CDS (Af /GGH PVLCAGI 3PHAALL MVR Similarity: Find: Best match	× N8979.2 - 2868277) () () () () () () () () () (
Sequence ← → (1) chr_1_V2	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Langth: 355 Interval 2 Langth: 355 Interval 2 Bases: TCCT/ Interval: 2 Jases: TCCT/ Jases: 1 Jases: 1	ohol dehydrogenase I (AD) r 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA NICOHOL dehydrogenase I (A 7 m: MSIPTMQWAQVAEKVGG EIGDQAGIKWLNGSCGECEFC /AIVGAGGGLGSLAQQYAKA RGTIVAIGLPPDAYLKAPVINT belous Prime 2021.2.2 'Ann 979.2 - 2868277 69.42% ; 2,911.254 -> 2,910,604, 2,4 : MSLPKLQWAQVYEEAGGP IGDHAGVKWLNGSCLSCEFC VGAGGGLGSLAQQYAKAMC SVVVAIGLPAGAFLKAPVFSTN	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVKI RQ5DDPLCARAQLSGYTVD MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI btate from Database' base 210,529 -> 2,910,175, 2,842 VIYKQIPVPTPGDEVLVNIK RTSDESLCPDASLSGYTVDG VRMIQIRGSVKGNRQDGIE	81 IRYSGVCHTDLHAMMGHWPIPVKMI GTFQQYALGKASHASKIPAGVPLDA SLGTETYVDFTKSKDLVADVKAATPD EALDFFARGLIKAPFKTAPLKDLPKIYE ed on nucleotide similarity 1527 -> 2,842,481; ADH1 EMENI Aİ YTGVCHTDLHARKGDWPLPVKMPLV TFQQFCIAKAAHVSILPKDVPLDAVAI AESYVDFTKSKDLVKDVQAATPDGLC AIDFLRRGLIKAPFKTVDLSELPKVYEL	PLVG AAPVLCA IGLGAHAV ILM Icohol dehydrogenase I (ADH I) CDS (AI /GGH PVLCAGI SPHAALL MVR Similarity: Find: Best match All matching annotati	× N8979.2 - 28682777) (2) (2) (2) (2) (2) (3) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40) (40)
<pre>< Sequence < → (1 chr_1_V2</pre>	Name: ADH1 EMENI Ald Type: CDS Total Length: 1053 (ove Interval 2 Length: 355 Interval 2 Langth: 355 Interval 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases: TCCT/ Interval: 2 Bases	ohol dehydrogenase I (ADI ar 3 intervals) 2,910,175 XCGTCGACTTCACCAAG 2,910,604, 2,910,529 -> 2,91 AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA AGCTTCAA A	H I) CDS 0,175, 2,842,527 -> 2,842,4 DH I) PLVYKQIPVPKPGPDQILVK RQSDDPLCARAQLSGYTVD MGIRVVAVDGGDEKRAMCE VVRMITIKGSYVGNRQDGVI otate from Database' base 210,529 -> 2,910,175, 2,842 VTXQIPVPTPGPDEVLVNIK RTSDESLCPDASLSGYTVDG LRVIAIDGGEEKKELCQQLG VRMIQIRGSYVGNRQDGIE	181 IRYSGVCHTDLHAMMGHWPIPVKMI IGTFQQYALGKASHASKIPAGVPLDA SLGTETYVDFTKSKDLVADVKAATPD EALDFFARGLIKAPFKTAPLKDLPKIYE 2d on nucleotide similarity 2527 -> 2,842,481; ADH1 EMENI Al YTGVCHTDLHARKGDWPLPVKMPLV TFQQFCIAKAAHVSILPKDVPLDAVAI AESYVDFTKSKDLVKDVQAATPDGLC AIDFLRRGLIKAPFKTVDLSELPKVYEL	PLVG AAPVLCA GLGAHAV H Icohol dehydrogenase I (ADH I) CDS (A GGH PVLCAGI SPHAALL MVR Similarity: Find: Best match All matching annotati	× N8979.2 - 2868277) () () () () () () () () () () () () ()

	Name: ADH1 EMENI Alcohol dehydrogenase I (ADH I) CDS	×
	Type: CDS	
	Total Length: 1053 (over 3 intervals)	
	Interval 1 Length: 651	
	Interval 1: 2,911,254 -> 2,910,604	
	Interval 1 Bases: ATGTCTCTTCCAAAGCTTCAA	
	Intervals: 2,911,254 -> 2,910,604, 2,910,529 -> 2,910,175, 2,842,527 -> 2,842,481	
	Bases: ATGTCTCTTCCAAAGCTTCAA	
	locus_tag: AN8979.2	
	codon_start: 1	
	transl_table: 1	
	product: ADH1_EMENI Alcohol dehydrogenase I (ADH I)	
	protein_id: XP_682248.1	
	db_xref: GI:67903984	
	db_xref: <u>GeneID:2868277</u>	
	Transferred Translation: MSIPTMQWAQVAEKVGGPLVYKQIPVPKPGPDQILVKIRYSGVCHTDLHAMMGHWPIPVKMPLVG	
	GHEGAGIVVAKGELVHEFEIGDQAGIKWLNGSCGECEFCRQSDDPLCARAQLSGYTVDGTFQQYALGKASHASKIPAGVPLDAAAPVLCA	
	GITVYKGLKEAGVRPGQTVAIVGAGGGLGSLAQQYAKAMGIRVVAVDGGDEKRAMCESLGTETVVDFTKSKDLVADVKAATPDGLGAHAV	
Sequenc	e VI LLAVSEKPFQQATEYVRSRGTIVAIGLPPDAYLKAPVINTVVRMITIKGSYVGNRQDGVEALDFFARGLIKAPFKTAPLKDLPKIYELM	
Γ	EQGRIAGRYVLEMPE	
$\leftarrow \rightarrow ($	→ ENCBIFeature Key: CDS	
1	50 NCBI Join Type: Join	
	note: Derived using Generous Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity	
	Transferred From: AN89/9.2 - 28682/7	
	Transferred Similarity: 09.42%	20(0277)
	Primary Match: 69.42%; 2,911,254 > 2,910,502 > 2,910,152 , 2842,527 - 2,884,2481; AUH1 EMENI AICOROI denydrogenase I (ADH I) CDS (AN8979.2 - 2868277) 9.2 -	- 2868277)
	TYTKGLKESGUP GULATVGAGGGGSLAQQTAKAMGLKVIAIDGGEEKKELUQUGGAESYDDFTKSDUPTKGLGPHAALL	
		_
chr_1_V2	Similarity: 40 %	%
	Find: Sest match	<u> </u>
	All matching spectraling >%	28
	All matching annotations	
		ද්ධා

12) Aromatic amino acid aminotransferase [BO96DRAFT_417179 - 37101885] from *A. niger* CBS 101883; L- 2623 bp



			Name: source Aspergillus niger CBS 101883		×
			Type: Source Length: 2601		
			Interval: <u>348,029 -> 345,429</u>		
			organism: Aspergillus niger CBS 101883		
<			mol_type: genomic DNA strain: CBS 101883		
Seque	nce View	Text View Lineage Info	culture_collection: CBS:101883		
$\leftarrow \rightarrow$	⊖ Extract	🕼 R.C. 🖄 Translate 🖄 Add/E	dit Annota type_material: culture from holotype of Aspergillus lacticoffea	atus	
1 :	250,000	500,000 750,000 1,000,000 ·	1.250,000 chromosome: Unknown NCBI Feature Key: source		
			note: Derived using Geneious Prime 2021.2.2 'Annotate from D	Database' based on nucleotide similarity	
			Transferred Similarity: 52.43%		
			Primary Match: 52.43%; <u>348,029 -> 345,429</u> ; source Aspergillus Primary Match Alignment: Open Alignment	s niger CBS 101883 (<u>BO96DRAFT 417179 - 3</u>	<u>7101885</u>)
			Source ATTGTGATAGCTGTTTTAGAGTTCAGCGAGG.	. CTATGAAAAGAAACACAATCGTTATCTAGCATC	AAAAGAG
chr_6_v2	3,000	340,000 342,000 344,000	346,00 Target GTGGCAGAGTGGATGGAAATGCCATAGAACACGCTCGAGA.	. CTCAAGCAATGAGAACTGCAGATGCTGAGAATG	CCACTGT
			gene; 52.43%	Similarity: 40 %	2⇒
				All matching appotations	*
			aromatic amino acid aminotransferase CDS; 62.06%		(2)
				Advanced	
		Name: aromatic amica and an	transforaça CDS		ж
		Type: CDS	uransierase CDS		
		Total Length: 1759 (over 3 interval	s)		
		Intervals: <u>347,514 -> 347,388</u> , <u>347,2</u>	<u>289 -> 346,715, 346,647 -> 345,591</u>		
		locus tag: BO96DRAFT 417179			
		codon_start: 1			
		transl_table: 1			
		protein id: XP 025448308.1	lotransierase		
		db_xref: <u>GI:1419042099</u>			
		db_xref: <u>GeneID:37101885</u>			
		Transferred Translation: MVYSTLS	SPPLDLSHHFSSVTKRREASETKSLYKYFFIPGIANLAGGLPNASYFPYDTLEATVAHP		
		QRFPATSDNDQIKPPSGSPSTERRIVP	KESPTTNLLKKIDLTTALQYGTAEGLPVMADFVRQFTRNHLHPNVPYAGGPGTLLTC	GATDGF	
		SKAIETFTNPWDPRRDWISQREGILCE	EFVYMNAIQTVKPRGLNIVPVAIDAQGMLAHGKGGLADVLENWDFKKGRLPHLMY	/TITIGQNP	
		TESKTIAPGCRI GWITAOPAIJERI TRI T	YWNLQYPSATAMEAGFRGSDAVDVIPRNYNAHGRSSGYDFLDSLVPSYLSVDTDG ETSTOOPSGEVOAMVAELIVGOOSEDGONATGASKNKSKKSEOAWOMDGWVR\	RVVRLD WLEGLRAGY	
<		EQRMTTMCTILEEGKYLIDSGSAWDDA	AQPMAEDETAWEVLDKMQMYEFSWPTGGMFVWVKVCIETHPLLEKYGPEKLIQAL	WLHLMQKPY	
Seque	nce View	LCLSGPGTMFAPTTELLDRAQTYYRLCF	AAMPAEDVLGITRRLVDGFRAFWQRKNLDGLDDEEIALSRLQAKGSGNLLGLGC		
	G Extract	NCBI Feature Key: CDS			
$\leftarrow \rightarrow$	CLAUACC	NCBI loin Type: join			
$\leftarrow \rightarrow$	250,000	NCBI Join Type: join note: Derived using Geneious Prim	e 2021.2.2 'Annotate from Database' based on nucleotide similarity		
 ← → 1 	250,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 Transforred Similarity: 62.06%	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885		
 ← → 1 	250,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_411 Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347,514</u> ->	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 <u>- 347.388, 347.289 -> 346.715, 346.647 -> 345.591;</u> aromatic amino acid ami	inotransferase CDS (<u>B096DRAFT 417179 - 3</u>	<u>7101885</u>)
 ← → 1 	250,000	NCBI Join Type: Join note: Derived using Genelous Prim Transferred From: BO96DRAFT_411 Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347.514</u> > Automatic Translation: MALPPPR	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 - <u>347.388, 347.289 -> 346.715, 346.647 -> 345.591;</u> aromatic amino acid ami DLSHIHFSYTKNRQASSYKGFYKYNIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPA	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ*	<u>7101885</u>)
 ← → 1 	250,000	NCBI Join Type: Join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 ⁻¹ Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347.514</u> -> Automatic Translation: MALPPPR E*WSCDQPHPGGFGGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVTYECHF	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 <u>• 347,388, 347,289 -> 346,715, 346,647 -> 345,591</u> ; aromatic amino acid ami DLSHHFSYVTKNRQASSVKGFYKYVAIPGIHNLAGGCPRGRRSPSPLPA+HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRISESSPSKCSICRGPRGNSVVRCNGW. PORTQGSQHCLRCHRQSGHAGIR+RWIGRCSGQLGLP+RSSSAFIVYYNDRSESH	inotransferase CDS (<u>BO96DRAFT 417179 - 3'</u> RQ★ AFQGARDLL RWQPLSG	<u>7101885</u>)
 ← → 1 	250,000	NCBI Join Type: Join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 ⁻ Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347,514</u> -> Automatic Translation: MALPPPRI E*WSCDQPHPGGFGGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVTYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV:	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • <u>347,388, 347,289 -> 346,715, 346,647 -> 345,591;</u> aromatic amino acid ami DLSHHFSYVTKNRQASSVKGFYKYVAIPGIHNLAGGCPRGRROSPSLPA*HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRISESSPSKCSICRGPRGNSVVRCNGW. PORTQTGSQHCLRCHRQSGHAGIR*RWIGRCSGQLGLP*RSSSAFIVYYNDRSES5H SIGFRNGGPFPWH*PRRTDSVLAQLQRGEIIGVRIPGLSCTVISLCRHGRSGRAPGi	inotransferase CDS (<u>BO96DRAFT 417179 - 3'</u> RQ≠ AFQGARDLL RWQPLSG HFLQDC	<u>7101885</u>)
< → 1	250,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 ⁻ Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347,514</u> -> Automatic Translation: MALPPPRI E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVTYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSR5SFGLDNGAARSR*ENHAYHRVLL GOVEYDENU SPC7CGHDVPU (SSP	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • <u>347,388</u> , <u>347,289 -> 346,715</u> , <u>346,647 -> 345,591</u> ; aromatic amino acid ami DLSHHFSYVTKNRQASSVKGFYKYVAIPGHNLAGGCPRGRROPSPSLPA*HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRISSESSPSKCSICRGPRGNSVVRCNGW. PDRQTQGSQHCLRCHRQSGHAGIR*#WIGRCSGQLGLP*RSSAFIVYYNDRSESH SIGFRNGGPFPWH*PRRTDSVLAQLQRGEIIGVRIPGLSCTVISLCRHGRSGRAPGI JPAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGAL*GAPRRLRTHAV	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HFLQDC JVVSYR	<u>7101885</u>)
← → 1 chr_6_v2	3,000	NCBI Join Type: Join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 Transferred Similarity: 62.06% Primary Match: 62.06%; <u>347.514</u> -2 Automatic Translation: MALPPPRI E*WSCDQPHPGGFGTGYGXAQGEWH2 CQLEPR*GLDSPAGRYSV*RVVIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDNGAARSR*ENHAYHRVLE GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGCCYGHIE	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346.715, 346.647 -> 345.591; aromatic amino acid ami DLSHHFSYVTKNRQASSVKGFYKYVAIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, PDRQTQGSQHCLRCHRQSGHAGIR*#WIGRCSGQLGLP*RSSSAFIVYYNDRSESH SIGFRNGGPFPWH*PRRTDSVLAQLQRGEIIGVRIPGLSCTVISLCRHGRSGRAPGI PPAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGAL*GAPRRLRATHAN QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL XVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R JDVFSYL	<u>7101885</u>)
< → 1 chr_6_v2	3,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: B096DRAFT_41' Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514 -> Automatic Translation: MALPPPR E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAVS SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • <u>347,388, 347,289 -> 346,715, 346,647 -> 345,591;</u> aromatic amino acid ami DLSHHFSYYTKNRQASSYKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, PDRQTQGSQHCLRCHRQSGHAGIR*RWIGRCSGQLGLP*RSSAFIVYYNDRSESH SIGFRNGGPFPWH*PRRTDSVLAQLQRGEIIGVRIPGLSCTVISLCRHGRSGRAPGi DPAAIRLPPVDRGAPHGTTGEGRVKRN*ERIYRLADGRLGAL*GAPRRLRATHAN QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL XVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R .DVFSYL Find: Best match	<u>7101885</u>)
← → 1 chr_6_v2	250,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPR E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIPCHF TQKGNLRHLSEIRHCHH*R*PLLESAV? SSRSSFGLDNGAARSR*ENHAYHRVLD GQVFPPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 - 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amin DISHHFSYVTKNRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, PDRQTQGSQHCLRCHRQSGHAGIR*RWIGRCSGQLGIP*RSSSAFIVYNDRSESH SIGFRNGGPPWH*PRRTDSVLAQLQRGGEIIGVRIPGCSCTVSLCRHGRSGRAPGI DPAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL XAVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL	Inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R 	7101885) ۲
← → 1 chr_6_v2	3,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41 Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPR E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAX SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885	Inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R _DVFSYL Find: O Best match ? All matching annotations	7101885)
← → 1 chr_6_v2	250,000 250,000 3,000	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: B096DRAFT_41 Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYTYECHE TQKGNLRHLSEIRHCHH*R*PLLESAX SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R 	7101885) 米 梁 梁
← → 1 chr_6_v2 ✓ ≥	3,000 Name A 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41' Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYTYECHE TQKGNLRHLSEIRHCHH*R*PLLESAX SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Rame: aromatic amino acid amin Type: CDS	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885	inotransferase CDS (<u>B096DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLYS*R DVFSYL Find: Best match ? All matching annotations Min Sequen Max Seque % GC	7101885) 冷 (欲 # Nu责
← → 1 chr_6_v2 ✓ 参	3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41' Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPRK E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYTYECHF TQKGNLRHLSEIRHCHH*R*PLLESAY: SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Name: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2 Length: 575	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amin DiSHHFSYVTKNRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA+HCUPA. SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW. PDROTQGSQHCLRCHRQSGHAGIR+RWIGRCSQQLGLP+RSSSAFIVYNDRSESH SIGFRNGGPFPWH+PRRTDSVLAQLQRGGEIIGVRIPGLSCTVISLCRHGRSGRAPGI PAAIRLRPVPDRGAPHGTTGEGRVKRN+ERIYNLADGRLGALA+GAPRRLRATHA QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL SAVGGRIPVVLAEDRSGRC+R+CGGYGCSPGAADMVTFACCFL Server 52.43% atomatic amino acid aminotransferase CDS; 62.06% Modified # Sequences otransferase CDS	inotransferase CDS (<u>B096DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLIX5*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) % # Nuffs
← → 1 chr_6_v2 ✓ ②	3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41' Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514-> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYTYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDNGAARSR*ENHAVHRVLL GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGg*LAVLPTVLCADAGGGCYGHIE Description Name: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2: 347.289 -> 346,715	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amin DISHHFSYTKINRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA+HCVPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW. 9DRQTQGSQHCLRCHRQSGHAGIR+RWIGRCSGQLGLP+RSSSAFIVYNDRSESH SIGFRNGGPFPWH+PRRTDSVLAQLQRGGEIIGVRIPGLSCTVISLCRHGRSGRAPGI 9PAAIRLRYPDRGAPHGTTGEGRVKRN+ERIYRLADGRLGALA+GAPRRLRATHAA QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL AVGGRPVVLAEDRSGRC+R*CGGYGCSPGAADMVTFACCFL source Aspergillus niger CBS 101883; 52.43% atomatic amino acid aminotransferase CDS; 62.06% Modified # Sequences lotransferase CDS	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLY5*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) % @ # Nu ^{FF} x
← → 1 chr_6_v2 ✓ ≥	3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41' Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514-> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDNGAARSR*ENHAVHRVLL GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Comme: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interval Interval 2: 147.289 -> 346.715 Interval 2: 847.549 -> 346.715 Interval 2: 347.289 -> 347.388, 347.347.384	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346.715, 346.647 -> 345.591; aromatic amino acid ami DiSHHFSYVTKINRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA+HCVPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW. 9DRQTQGSQHCLRCHRQSGHAGIR+RWIGRCSGQLGLP*RSSSAFIVYNDRSESH SIGFRNGGPFPWH+PRRTDSVLAQLQRGGEIIGVRIPGLSCTVISLCRHGRSGRAPGI 9PAAIRLRPVPDRGAPHGTTGEGRVKRN+ERIYRLADGRLGALA+GAPRRLRATHA QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL AVGGRIPVVLAEDRSGRC+R*CGGYGCSPGAADMVTFACCFL Segene: 52.43% atomatic amino acid aminotransferase CDS; 62.06% Modified # Sequences als) SGGGCTGGTG 289 -> 346.715, 346.647 -> 345.591	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLY5*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) ≫ ⊗ # Nutrs
← → 1 chr_6_v2 ✓ ≥	250,000 3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_41' Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514-> Automatic Translation: MALPPRH E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDNGAARSR*ENHAYHRVLL GQVFYPGHLLSPCCGHRHYRLLGSRR PVSG6*LAVLPTVLCADAGGGCYGHIE Description Rew Name: aromatic amino add amin Type: CDS Total Length: 1759 (over 3 Interva Interval 2 Length: 575 Interval 2 147,249 -> 346,715 Interval 2 347,289 -> 347,388, 347 Bases: ATGGCCCTTCGGCTCCTCG4 Interval: 2096PBC41 4171/20	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 347.388, 347.289 -> 346.715, 346.647 -> 345.591; aromatic amino acid ami DLSHHFSVTKNRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA+HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, PDRQTQGSQHCLRCHRQSGHAGIR+RWIGRCSGQLGLP*RSSSAFIVYNDRSESH SIGFRNGGPFPWH+PRRTDSVLAQLQRGGEIIGVRIPGLSCTVISLCRHGRSGRAPGI PDAAIRLRPVPDRGAPHGTTGEGRVKRN+ERIYRLADGRLGALA+GAPRRLRATHAA QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL AVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL gene; 52.43% atomatic amino acid aminotransferase CD5; 62.06% Modified # Sequences als) SGGCGTCGTG 1289 -> 346.715, 346.647 -> 345.591 C	inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLY5*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) 27 27 27 27 27 27 27 27 27 27 27 27 27
← → 1 chr_6_v2 ✓ ≥	3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Transferred From: BO96DRAFT_417 Transferred Similarity: 62.06% Primary Match: 62.06%; 347.514-> Automatic Translation: MALPPRH E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV: SSRSSFGLDNGAARSR*ENHAYHRVLC GQVFYPGHLLSPCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Name: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2 Langth: 575 Interval 2 1347.289 -> 346.715 Interval 2 1347.289 -> 346.715 Interval 2 Bases: GATGCCTCG4 Intervals: 347.514 -> 347.388, 347 Bases: ATGGCCTTCCGCCTCCTCG Iocus_tag: BO9BRAFT_417179 codon_start: 1	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid ami DLSHHFSVTKNRQASSVKGFYKYAIPGIHNLAGGCPRGRRGPSPSLPA+HCVIPAI SQSAEGNRPAHCSAIRHRGGISSPPVVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, DPRQTQGSQHCLRCHRQSGHAGIR+RWIGRCSQQLGL+*RSSSAFIVYNDRSESH SIGFRNGGPFPVH+*PRRTDSVLAQLQRGGEIIGVRIPGLSCTVISLCRHGRSGRAPGI DPAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA+GAPRRLRATHA QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL AVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL (gene: 52.43%) atomatic amino acid aminotransferase CDS; 62.06% Modified # Sequences atomatic amino acid aminotransferase CDS; 62.06% (gene: 52.43%) atomatic amino acid aminotransferase CDS; 62.06% (gene: 52.43%) atomatic amino acid aminotransferase CDS; 62.06% (gene: 52.43%) (gene: inotransferase CDS (<u>BO96DRAFT 417179 - 3</u> RQ* AFQGARDLL RWQPLSG HFLQDC HVLY5*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) 次 会 # Nu责	
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← → 1 chr_6_v2 ✓ ₽	3,000 Name ▲	NCBI Join Type: join note: Derived using Geneious Prim Irransferred From: BO96DRAFT_41 Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKGNLRHLSEIRHCHH*R*PLLESAV SRSSFGLDNGAARSR*ENHAYHRVLC GQVFPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Rame: aromatic amino acid amin Type: CDS Total Length: 1759 Interval 2: 347.289> 346,715 Interval 2: 347.289> 346,715 Interval 2: 347.289> 347.388, 347 Bases: ATGGCCTTCGCCTCCTGG locus_tag: B096DRAFT_417179 codon_start: 1 transl_table: 1 product: aromatic amino acid amin protein jd: XP_025448308.1 db_xref: Ginla10042099 db_xref: Ginla10042099 db_xref: Ginla10042099 db_xref: Ginla10042099 db_xref: SindBasplac1_417179 Transferred Translation: MVYSTI QRFPATSONDQIKPPSGSPSTERRIVI SKAIETGNWDDPBCMURCPDC710	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amino DISHHFSYVTKNRQASSVKGFYKYVAIPGIHNLAGGCPRGRRGPSPSLPA*HCVPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, PORQTQGSQHCLRCHRQSGHAGIR*RWIGRCSQCIGLP*RSSSAFIVYNDRSESH SIGFRNGGPPWH*PRRTDSVLAQLQRGGEIIGVRIPGCSCTVTSICRHGRSGRAPG PAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL SAVGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL Gene: 52,43% Source Aspergillus niger CBS 101883; 52,43% aromatic amino acid aminotransferase CDS (52,06%) Modified # Sequences als) GGGCGTCGTG inotransferase	Inotransferase CDS (B096DRAFT 417179 - 3' RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R 	7101885) 次 (动 # Nu责
← → 1 chr_6_v2 ✓ ≥	3,000 Name ▲ 8 Sequence	NCBI Join Type: join note: Derived using Geneious Prim Irransferred From: BO96DRAFT_41 Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVIPCHF QCKSNLRHLSEIRHCHH*R*PLLESAV SSRSSFGLDNGAARSR*ENHAYHRVLC GQVFPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description PVSGG*LAVLPTVLCADAGGGCYGHIE Name: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2: 347,289 -> 346,715 Interval 2: 347,289 -> 347,388, 347 Bases: ATGGCCTTCCG locus_tag: B096DRAFT_417179 Codon_start: 1 transl_table: 1 product: aromatic amino acid am protein_id: XP_025448308.1 db_xref: GiL1419042099 db_xref: GiL1419042099 db_xref: GiL1419042099 db_xref: RomeDiaT101885 db_xref: GiL1419042095 CREATEDDAGIKPPSGSPSTERRIVE SKALETFINFWDPRRDWISQREGILCI TGGTLSVERREFYALCROPTOIILEDDO	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346.715, 346.647 -> 345.591; aromatic amino acid amino DISHHFSYVTKNRQASSVKGFYKYVAIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW. PORTQGSQHCLRCHRQSGHAGIR*RWIGRCSQCIGLP*RSSSAFIVYNDRSESH SIGFRNGGPPWH*PRRTDSVLAQUQRGEGIIGVRIPGCSCTVSICLRGRSGRAPG PAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL SAVGGRIPVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL Segne: 52.43% Source Aspergillus niger CBS 101883; 52.43% aromatic amino acid aminotransferase CDS als) SGGGGTCGTG .289 -> 346.715, 346.647 -> 345.591 C Inotransferase	Inotransferase CDS (B096DRAFT 417179 - 3) RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R .DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC	7101885) 米 Nutīj
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347.514> Automatic Translation: MALPPRE E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIYECHF TQKSNLRHSLESERHCHH*R*PLLESAV SSRSSFGLDNGAARSR*ENHAYHRVLC GQVPYGHLLSPCCCGHRHYRLLGSRR PVSGG*LVLPTVLCADAGGGCYGHIE VSGG*LVLPTVLCADAGGGCYGHIE VSGG*LAVLPTVLCADAGGGCYGHIE Name: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2 Length: 575 Interval 2 Bases: GATGCCCTCGAC Total Length: 1759 (over 3 interva Interval 2 Length: 575 Interval 2 Bases: GATGCCCTCGAC Total Length: 1759 (over 3 interva Interval 2 Bases: GATGCCCTCGAC Interval 3 Bases: GATGCCCTCGAC Interval: 347.314> 347.388, 347 Bases: ATGGCCTTCGAC Interval: 10 product: aromatic amino acid am protein Jd: XP 025448308.1 db.xref: GI121419042099 db.xref: GI121419042099 db.xref: GIDB:Asplac1.417179 Transferred Translation: MVSTPH SKALETFINFWDPRROWNISGREGICL ICGB JDI Type: Join note: Derived using Genelous Prin Transferred Similarity: 62.06%; 347.514 Automatic Translation: MALPPPE E*WSCDOPHPGGFGTSYGAGGEWH QCLEPR*GLDSPAGRYSV*RVYPCEh TQKGNLRHLSERHCHH*R*PLLESAV	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 347.388, 347.289 > 346,715, 346,647 -> 345,591; aromatic amino acid ami DiSHHFSYVTKNRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA*HCUPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, 20RQTQGSQHCLRCHRQSGHAGIR*RWIGRCSQQLGLP*RSSSAFIVYNDRSESH SIGFRNGGPFVM+*PRRTDSVLAQLQRGGEIIGVRIPGCSCTVISLCRHGRSGRAPG 20PAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRRTLPRAVGALDTEAVPVPCRTRL 34VGGRIPVVLAEDRSGRC*R*CGYGCSPGAADMVTFACCFL 20PAAIRLRPVLAEDRSGRC*R*CGYGCSPGAADMVTFACCFL 20PAAIRLRPVLAEDRSGRC*R*CGYGCSPGAADMVTFACCFL 20PAGRIPVVLAEDRSGRC*R*CGYGCSPGAADMVTFACCFL 20PAGRIPVVLAEDRSGRC*R*CGYGCSPGAADMVTFACCFL 20PAGRIPVVLAEDRSGRC*R*CGYGCSPGAADMVTFACGFL 20PAGRIPVVLAEDRSGRC*R*CGYGCSPGAADMVTFACGFL 20PAGRIPVVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVCVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVCVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVCVLAEDRSGRC*A*CGYGCSPGAADMVTFACGFL 20PAGRIPVCVLAEDRSGRC*A*CGGYGCSPGAADMVTFACGFL 20PAGRIPVCVLAEDRSGRC*A*CGYGGYGADPAGRIPVCVCVCFL 20PAGRIPVCVLAEDRSGRC*A*CGYGYGAPAGRAADVVPCVLAEDRSGRC*CGYGAADVAEDV 20PAGRIPVCVLAEDRSGRC*GYGYAYADGYGGYTRNHLHPNVPVAGGPGTLTC 20PAGRIPVCVLAEDRSGRC*GSDAVDVPRNYNAHGRSSGYDFLDSLVPSYLSVDTDG 20PAGRIPVCVLAEDRSGRC*GSDAVDVPRNYNAHGRSSGYDFLDSLVPSYLSVDTDG 20PAGRIPVCVLAEDRSGRC*GSDAVDVPRNYNAHGRSSGYDFLDSLVPSYLSVDTDG 20PAGRIPVCVLAEDRSGRC*GYAYAYADGRNTGASKNSKKSKGQAVMOMGWWR 20PAGPAGBVQAMVELUNGQSEGGNATGASKNSKKSKGQAVMOMGGWR 20PAGRIPVCVLACGYGARGPGSDAVDVPRNYNAHGRSSGYDFLDSLVPSYLSVDTDG 20PAGRIPVCVLACGARFHQGGSDAVDVPRNYNKNSKKSKGQAVMOMGWR 20PAGRIPVCVLACGARFHQGGSDAVGASKNSKKSKGQAVMSGWMDGWR 20PAGRIPVCVKNRGAASSKCSFKYYAPGIGHNLAGGGCFGRGRGPSEDFACHCVTATA 20SGRNRGGGPFWH*TRRTDSVLAQLQRGGEIIGVRIPCLSCTVISLCHGRSGRSPLACHCVTATA 20SGRNRGGFFWH*TRRTDSVLAQLQRGGEIIGVRIPCLSCTVISLCHGRSGRAFDSEDFACHCVTATA 20SGRNRGGFFWH*TFRRTDSVLAQLQRGGEIIGVRIPCLSCTVISLCHGRSGRAFGSDASFLPA+HCVTATA	Inotransferase CDS (BO96DRAFT 417179 - 3' RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC CGATDGF YTTTIGQNP IRVVRLD WLFGLRAGY LWLHLMQKPY Minotransferase CDS (BO96DRAFT 417179 - 3 RQ* /AFQGARDLL HRWQPLSG HFLQDC	7101885) 米 Nu变
← → 1 chr_6_v2 ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ●<	a,000 Name ▲ 8 Sequence 8 Sequence 250,000 Control Control	NCBI Join Type: join note: Derived using Geneious Prim Iransferred From: BO96DRAFT_41' Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPRH E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIFCHF TQKSNLRHLSEIRHCHH*R*PLLESAV SSRSSFGLDNGAARSR*ENHAYHRVLC GQVFPGHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE STotal Length: 1759 (over 3 interva Interval 2 Length: 575 Interval 2 Laength: 179 Gudon, start: 1 transf commatic amino acid am protein_id: XP_025448308.1 db_xref: Girla1094209 db_xref: Girla1094209 db_xref: GIDB:Asplac1_417179 Transferred Translation: MVXTPI RCRLSpredRiverApAIERLTRL EQRMTTMCTILEGKYLIDSGAMDUSGREGUCT IGGLSVERREIVALCROPDIILEDDI TFSKTLAPGCRLSWTAQPAIERLTRL EQRMTTMCTILEGKYLIDSGAMDU LCLSGPGTMFAPTTELLDRAQTYRLC NCBI Jeature Key: COS	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 - 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amino DISHHFSYVTKNRQASSVKGFYKYYAIPGIHNLAGGCPRGRRGPSPSLPA*HCVIPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, 20RQTQGSQHCLRCHRQSGHAGIR*RWIGRCSQQLGLP*RSSSAFIVYNDRSESH SIGFRNGGPPWH*PRRTDSVLAQLQRGGEIIGVRIPGCSCTVISLCRHGRSGRAPG 20PAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL 34VGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL Commatic amino acid aminotransferase CDS; 62.06% Modified # Sequences also SGGCGTCGTG 289 - 346,715, 346,647 -> 345,591 C inotransferase SPPLDLSHHFSSYTKRREASETKSLYKYFFIPGIANLAGGLPNASYFPYDTLEATVAHP PXKESPTTNLLKKIDLTTALQYGTAEGLPVMADFVRQFTRNHLHPNVPYAGGPGTLTT EFFYVMNAUGYVKPRGLNPVVIDAQGMLAHGKGGLAVLEAVUENSKKREQAWQMDGWVR SQGMADETAWEVLDKMQMYEFSWPTGGMFVMXVCCIETHPLLEKYSPKUDTG TETSTQQPSGFVQAMVAELVGQSEDGQNATGASKNKSKKSEQAWQMDGWVR SQGMADEDTAWEVLDKMQMYEFSWPTGGMFVMXVCCIETHPLLEKYSPEKILGGC me 2021.2.2 'Annotate from Database' based on nucleotide similarity 17179 - 37101885	Inotransferase CDS (BO96DRAFT 417179 - 3' RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC CGATDGF YITTIGQNP RVVRLD WLEGLRAGY WVLEGLRAGY WVLEGLRAGY WVLEGLRAGY WVLEGLRAGY HRQP AFQGARDLL HRWQPLSG HFLQDC VHVLYS*R LDVFSYL	7101885) 米 Nu责
← → 1 chr_6_v2 ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ● ✓ ●<	ence View → Extrar 250,000 ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■	NCBI Join Type: join note: Derived using Geneious Prim Iransferred From: BO96DRAFT_41 Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPR E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIPCHF TYKSDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIPCHF TYKSDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVYIPCHF TYKSDQFLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Description Reme: aromatic amine acid amin Type: CDS Total Length: 1759 (over 3 interva Interval 2 Length: 575 Interval 2 Langth: 575 Interval 3 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 3 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 3 Langth: 575 Interval 3 Langth: 575 Interval 3 Langth: 575 Interval 7 Langth: 575 Interval 7 Langth: 575 Interval 2 Langth: 575 Interval 7 Langth: 575 Interval 7 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth: 575 Interval 2 Langth:	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 • 347.388, 347.289 -> 346,715, 346,647 -> 345,591; aromatic amino acid amino DISHHFSYVTKNRQASSVKGFYKYAIPGIHNLAGGCPRGRRGPSPSLPA+HCVPA SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, 20RQTQGSQHCLRCHRQSGHAGIR*RWIGRCSQQLGLP*RSSSAFIVYNDRSESH SIGFRNGGPPWH+PRRTDSVLAQLQRGGEIIGVRIPGCSCTVISLCRHGRSGRAPG 20PAAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV QSAHVRLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL 34VGGRIPVVLAEDRSGRC*R*CGGYGCSPGAADMVTFACCFL	Inotransferase CDS (BO96DRAFT 417179 - 3' RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R DVFSYL Find: Best match All matching annotations Min Sequen Max Seque % GC CGATDGF YITTIGQNP RVVRLD WLEGLRAGY WLFGLRAGY WLFGLRAGY WLFGLRAGY WLFGLRAGY WLFGLRAGY LDVFSYL AFQGARDLL HRWQPLSG HFLQDC YHVLYS*R LDVFSYL	7101885) 米 Nu责
← → 1 chr_6_v2 ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥ ✓ ♥<	ence View 	NCBI Join Type: join note: Derived using Geneious Prim Iransferred From: BO96DRAFT_41 Transferred Similarity: 62.06%; Primary Match: 62.06%; 347.514> Automatic Translation: MALPPPR E*WSCDQPHPGGFGTGYGAQGEWHS QCLEPR*GLDSPAGRYSV*RVIYECHF QCLEPR*GLDSPAGRYSV*RVIYECHF QCKDRLHSLESIRHCHH*R*PLLESAV SSRSSFGLDNGAARSR*ENHAYHRVLC GQVFPCHLLSPCCCGHRHYRLLGSRR PVSGG*LAVLPTVLCADAGGGCYGHIE Bame: aromatic amino acid amin Type: CDS Total Length: 1759 (over 3 interval 1 tarval 2: 347.289> 346,715 Interval 2: 347.289> 346,715 Interval 2: 347.289> 346,715 Interval 2: 347.289> 347,388, 347 Bases: ATGGCCTTCCGC Iocus tag: BO96DRAFT_417179 codon, stre: 1 product: aromatic amino acid am protein id: XP_025448308.1 db,xref: Gil1a10042099 db,xref: Gil1a10042099 db,xref: GIDB:Asplac1_417179 Transferred Translation: MVSTL QRFPATSDNDQIKPPSGSPSTERRIV SKAIETFINPWDPRRDWISGREGLCL TGGLSVERRREIYALCRQFDIIIEDDI TFSKTAPGCRLGWTAQPAILERLTRL EQRMITMCTLIEGGKVTLOSGSAVDCU LCLSSPGTMFAPTTELLDRAQTYRLC NCBI Jein Type: Join note: Derived using Genelous Prim Transferred From: BO96DRAFT_4 Transferred Similarity: 62.06%; Primary Match: 62.06%; 347,514 Automatic Translation: MMSTFI	e 2021.2.2 'Annotate from Database' based on nucleotide similarity 7179 - 37101885 347.388, 347.289 -> 346.715, 346.647 -> 345.591; aromatic amino acid ami DiSHHFSYVTKNRQASSVKGFYKYAIPGIHNLAGGCPRGRRGPSPSLPA*HCUPA- SQSAEGNRPAHCSAIRHRGGISSPPVSSRIRSESSPSKCSICRGPRGNSVVRCNGW, 20RTQGSQHCLRCHRQSGHAGIR*RWIGRCSQCIGLP*RSSSAFIVYNDRSESH SGGRAGGPWH*PRRTDSVLAQUQRGGEIIGVRIPGCSCTVSICLRGRSGRAPG 20PAIRLRPVPDRGAPHGTTGEGRVKRN*ERIYRLADGRLGALA*GAPRRLRATHAV 20SAHVLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL 20SAHVLLMAPRRHVCLGKDTVGHTSAIREIPPRETLPRAVGALDTEAVPVPCRTRL 20SATUADRSGC*R*CGGYGCSPGADMVTFACCFI 2000000000000000000000000000000000000	Inotransferase CDS (BO96DRAFT 417179 - 3' RQ* AFQGARDLL RWQPLSG HFLQDC /HVLYS*R DVFSYL Find: Best match ? All matching annotations Min Sequen Max Seque % GC CGATOGF Min Sequen Max Seque % GC CGATOGF YHTTIGQNP RVVRLD WULGLRAGY WVLEGLRAGY WVLHLMQKPY hinotransferase CDS (BO96DRAFT 417179 - 3 RQ* VFQARDLL 4RWQPLSG HFLQDC VHVLVS*R LDVFSYL Find: Best match ?	7101885) % % ** Nu Ţ

\checkmark		Name 🔺	Description	Modified	# Sequences Min Sequen	Max Seque % GC	# Nut
		8 Sequence	Name: aromatic amino acid aminotransferase CDS				x
	-	o sequence	Type: CDS				
			Total Length: 1759 (over 3 intervals)				
			Interval 1 Length: 127				
			Interval 1: 347,514 -> 347,388				
			Interval 1 Bases: ATGGCCCTTCCGCCTCCTCGC				
			Intervals: 347,514 -> 347,388, 347,289 -> 346,715, 346,647 -> 345,591				
			Bases: ATGGCCCTTCCGCCTCCTCGC				
			locus_tag: BO96DRAFT_417179				
			codon_start: 1				
			transl_table: 1				
			product: aromatic amino acid aminotransferase				
			protein_id: XP_025448308.1				
			db_xref: GI:1419042099				
			db_xref: GeneID:37101885				
			db_xref: JGIDB:Asplac1_417179				
			Transferred Translation: MVYSTLSPPLDLSHHFSSVTKRREASETKSLYKYFFIF	GIANLAGGLPNASYF	PYDTLEATVAHP		
			QRFPATSDNDQIKPPSGSPSTERRIVPKESPTTNLLKKIDLTTALQYGTAEGLPVMA	DFVRQFTRNHLHPNV	PYAGGPGTLLTCGATDGF		
			SKAIETFTNPWDPRRDWISQREGILCEEFVYMNAIQTVKPRGLNIVPVAIDAQGMI	AHGKGGLADVLENW	DFKKGRLPHLMYTITIGQNP		
			TGGTLSVERRREIYALCRQFDIIIIEDDPYWNLQYPSATAMEAGFRGSDAVDVIPRN	IYNAHGRSSGYDFLDSI	LVPSYLSVDTDGRVVRLD		
			TFSKTIAPGCRLGWITAQPAIIERLTRLTETSTQQPSGFVQAMVAELIVGQQSEDGC	NATGASKNKSKKSEQ	AWQMDGWVRWLEGLRAGY		
	<		EORMTTMCTILEEGKYLIDSGSAWDDAOPMAEDETAWEVLDKMOMYEFSWPTG	GMFVWVKVCIETHPLL	LEKYGPEKLIOALWLHLMOKPY		
			LCLSGPGTMFAPTTELLDRAQTYYRLCFAAMPAEDVLGITRRLVDGFRAFWQRKNL	DGLDDEEIALSRLQAK	GSGNLLGLGC		
5	eque	nce View	NCBI Feature Key: CDS				
Ξ.		G. Evitive et	NCBI Join Type: join				
	\neg		note: Derived using Geneious Prime 2021.2.2 'Annotate from Database'	based on nucleotide	similarity		
1		250,000	Transferred From: BO96DRAFT_417179 - 37101885		-		
			Transferred Similarity: 62.06%				
			Primary Match: 62.06%; <u>347,514 -> 347,388</u> , <u>347,289 -> 346,715</u> , <u>346,647</u>	-> 345,591; aromatic	amino acid aminotransferas	e CDS (<u>BO96DRAFT_417179 -</u>	<u>37101885</u>)
			Automatic Translation: MALPPPRDLSHHFSYVTKNRQASSVKGFYKYYAIPGI	HNLAGGCPRGRRGPS	PSLPA*HCVIPARQ*		
			E*WSCDQPHPGGFGTGYGAQGEWHSQSAEGNRPAHCSAIRHRGGISSPPVVSSI	RIRSESSPSKCSICRGPF	RGNSVVRCNGWAFQGARDLL		
			QCLEPR*GLDSPAGRYSV*RVYIYECHPDRQTQGSQHCLRCHRQSGHAGIR*RWI	GRCSGQLGLP*RSSSA	FIVYYNDRSESHRWQPLSG		
			TQKGNLRHLSEIRHCHH*R*PLLESAVSIGFRNGGPFPWH*PRRTDSVLAQLQRG	GEIIGVRIPGLSCTVISL	CRHGRSGRAPGHFLQDC		
			SSRSSFGLDNGAARSR*ENHAYHRVLDPAAIRLRPVPDRGAPHGTTGEGRVKRN*	ERIYRLADGRLGALA*	GAPRRLRATHAVHVLYS*R		
		3.000	GQVFYPGHLLSPCCCGHRHYRLLGSRRQSAHVRLLMAPRRHVCLGKDTVGHTSAI	REIPPRETLPRAVGALD	DTEAVPVPCRTRLDVFSYL		
ch	r 6 v2	~~	PVSGG*LAVLPTVLCADAGGGCYGHIEAVGGRIPVVLAEDRSGRC*R*CGGYGCSI	PGAADMVTFACCFL			
					Similarity.	•	10 524
			gene; 52.43 <mark>%)</mark>		Find:	est match	4
					Tind. 😈 D		~
			source Aspergillus	niger CBS 101883; 52	2.43% O A	ll matching annotations	
			aromatic amino acid aminotr	ansferase CDS; 62.06	5%	han be Advanced	<u>{ô</u> }
			,		A	Advanced	

13) tRNA Isopentenyltransferase [ANOM_005322 - 26807126] from *Aspergillus nomiae* NRRL 13137; L- 1458 bp



	Name: source Aspergillus nomiae NRRL 13137		×
	Type: Source		
	Length: 1505		
	Interval: <u>502,129 -> 503,633</u> Bases: GCACTATTCAATTTCCATTTG		
	organism: Aspergillus nomiae NRRL 13137		
	mol_type: genomic DNA		
	strain: NRRL 13137		
	isolation_source: plant tissue		
	host: wheat		
	dh vref: taxon:1509407		
	chromosome: Unknown		
Sequence View Text View Lineage Info	country: USA: Peoria, Illinois		
← → ⊖ Extract @ R C & Translate (**) Add/Edit Ann	lat_lon: 40.6936 N 89.589 W		
	collection_date: 1,987		
500,000 1,000,000 1,500,000	NCBI Feature Key: source	base' based on pucleotide similarity	
	Transferred From: ANOM 005322 - 26807126	base based on nucleotide similarity	
	Transferred Similarity: 62.29%		
	Primary Match: 62.29%; 502,129 -> 503,633; source Aspergillus nor	miae NRRL 13137 (<u>ANOM 005322 - 2680712</u>	6)
	Primary Match Alignment: Open Alignment		
	Source CTAGTGATTCACTGCCTGTGTCACGAGAGATCCTTCCGTCA	GTACCCGTGGCACCGACTATGAGGGGC	TCCAT
	Target GCACTATTCAATTTCCATTTGCGCTAGACTGGGTATTCTTCAG	GTACCCGTAGCACCAACGACAGCTATGAGTGGT	TCCAT
501,000 502,000	503,000 504,000 505,000		\Box
chr_3_v1g	ene; 52.29%	Similarity:	r?5
source Asp	ergillus nomiae 🔪	Find: O Best match	
*tRNA isop	entenyltransfe	All matching appotations	%
trna	isopentenyltr	· · ·	-^-
Z 😺 8 Sequences MP C322 -	17 Sep 2021 8:21 am 8 1,92	27,652 4,110,925 49.1% 2	23,811^
Interne			
Interva Interva Bases: Iocus.tr codon.s transl.j product product <td><pre>il 2 Bases: CTTGCAGTGGACTTGGCGTCC ils: 503,633 -> 503,583, 503,467 -> 502,129 ATGGAACCACTCATAGCTGTC ag: ANOM_005322 start: 1 table: 1 t: tRNA isopentenyltransferase</pre></td> <td>YRGLPIITNQIPMDERNNIPHHLISCI ARSDGETEESSTKWPILDAPTDVVLRKLR QLRFPTMVFWIHSEREALIARLDKRVDVMIEQG CIESIKIATRQYSASQVKWIRNKLWNTLAE QKGPGFAAKCFTCDVCRKTMVNEEQWNIHL ed on nucleotide similarity attenyltransferase CDS (ANOM 005322 - 268 /HGLPIITNQIPVEERNGIPHHMIGCIGL DSESSSPEKWPILNAPTDVLLQKLREVDPA</td> <td>0<u>07126</u>)</td>	<pre>il 2 Bases: CTTGCAGTGGACTTGGCGTCC ils: 503,633 -> 503,583, 503,467 -> 502,129 ATGGAACCACTCATAGCTGTC ag: ANOM_005322 start: 1 table: 1 t: tRNA isopentenyltransferase</pre>	YRGLPIITNQIPMDERNNIPHHLISCI ARSDGETEESSTKWPILDAPTDVVLRKLR QLRFPTMVFWIHSEREALIARLDKRVDVMIEQG CIESIKIATRQYSASQVKWIRNKLWNTLAE QKGPGFAAKCFTCDVCRKTMVNEEQWNIHL ed on nucleotide similarity attenyltransferase CDS (ANOM 005322 - 268 /HGLPIITNQIPVEERNGIPHHMIGCIGL DSESSSPEKWPILNAPTDVLLQKLREVDPA	0 <u>07126</u>)
Linterva Bases: Iocus_tri codon_j transl_j produci protein db_xref Transfe ELDEEP EVDPVN LMSEAC TGTAHF Sequence View Text View Lineage Info NGBI F ← → ⊖ Extract @ R.C. @ Translate ➡ Add/(NCBI Jo note: D 500000 1.000,000 1.50 Transfe Transfe Primary Automa EKEPVP MASRW ERMLUY	<pre>il 2 Bases: CTTGCAGTGGACTTGGCGTCC ils: 503,633 -> 503,583, 503,467 -> 502,129 ATGGAACCACTCATAGCTGTC ag: ANOM_005322 start: 1 table: 1 t: tRNA isopentenyltransferase</pre>	YRGLPIITNQIPMDERNNIPHHLISCI ARSDGETEESSTKWPILDAPTDVVLRKLR QLRFPTMVFWIHSEREALIARLDKRVDVMIEQG CIESIKIATRQYSASQVKWIRNKLWNTLAE QKGPGFAAKCFTCDVCRKTMVNEEQWNIHL ed on nucleotide similarity thenyltransferase CDS (ANOM 005322 - 268 HGLPIITNQIPVEERNGIPHHMIGCIGL DSESSSPEKWPILNAPTDVLLQKLREVDPA LIFWVHADKEILNSRLDARVDTMIEQGLMSEA CTATRQYAVSQIKWIRNKLWKALAETRMTS TSGLPKCVTCDMCRKTMMNEEQWTVHVKGQC Similarity:	0 <u>77126</u>) 5H
Interva Bases: Iocus_tr codon_s transl_t product protein db_xref Transfe ELDEEP EVDPVN LMSEAC TGTAHF NGBIF ← → → Extract @ R.C. Translate → Add/(NCBI Jo note: D 50000 1.000,000 1.50 Transfe Frimary EKEPVN MASRW ERMLU Source Asp	<pre>il 2 Bases: CTTGCAGTGGACTTGGCGTCC ls: 503,633 -> 503,583, 503,467 -> 502,129 ATGGAACCACTCATAGCTGTC ag: ANOM_005322 start: 1 table: 1 t: tRNA isopentenyltransferase tid: XP_015406736.1 t: GI-92086412 t: GeneID:26807126 erred Translation: MEPLIAIVGATGTGKSKLAVDLATRFNGEIINGDAMQMI WRIGLFKSECLRIIKDIHSRGKLPILVGGTHYYTQAVLFKDQLVGEGSDEIQGSG AADRWHPNDTRKIRRSLEIYFQTGRPASEVYADQKRLKQALAINGDPSAGEGI RMSDYIRERRTQGCSIDPTRGVWVAIGFKELAPYFEALHKRSLSGDELESLKES RLYLDSTNVEDWGSCITEPSELLTQALLKDESAPDPKSLSELAKTILGAKEAQSC KRVLKLWPKERNGRNLYRLGKTIREGSLVTQAVNH eature Key: CDS bin Type: join errived using Genelous Prime 2021.2.2 'Annotate from Database' base erred From: ANOM_005322 - 26807126 erred Similarity: 65.05% y Match: 65.05%; 503,633 -> 503,583, 503,467 -> 502,129; tRNA isopen atic Translation: MEPLIAVVGATGTGKSKUAVDLASRFNGEIINGDAMQMY WGIFKKECLRIKIDHSRGKLPLVGGTHYYTQTVLFKDQLVDREEAGSGSELDU (HPNETRKIRRSLEIYFQTGRPASEIYAEQRCQRQIASMDENSAGGRGGLRYQTI 10EKQSQGLTVDQTKGVWVSIGFKELGPYFSALRDGSSTGELENLRRSCIESIK SSNVDHWDTCVKQASERLVHSFLINPECPGPKSLSELARVTLEAKEAQAQKPT VAKRAEREFLRKRESLKNTQSSANGN*IV ene; 62.29% ergillus nomiae</pre>	YRGLPIITNQIPMDERNNIPHHLISCI ARSDGETEESSTKWPILDAPTDVVLRKLR QLRFPTMVFWIHSEREALIARLDKRVDVMIEQG SCIESIKIATRQYSASQVKWIRNKLWNTLAE QKGPGFAAKCFTCDVCRKTMVNEEQWNIHL ed on nucleotide similarity Attenyltransferase CDS (ANOM 005322 - 268 YHGLPIITNQIPVEERNGIPHHMIGCIGL SESSSPEKWPILNAPTDVLLQKLREVDPA LIFWVHADKEILNSRLDARVDTMIEQGLMSEA CTATRQYAVSQIKWIRNKLWKALAETRMTS FSGLPKCVTCDMCRKTMMNEEQWTVHVKGQG Similarity:40 % Find: § Best match	оотіго) жн
Linterva Linterva Bases: locus_tr codon_j transl_ transl_ transl_ produci protein db_xref Transfe EUDEEP EVDPVA LMSEAC TGTAHF Sequence View Text View Lineage Info NCBI Jo Solooo 1.000.000 1.50 Transfe Primary Automi EKEPWi MASRW ERMLLY Soluce Asp MCN I F Soluce Asp	<pre>il 2 Bases: CTTGCAGTGGACTTGGCGTCC ils: 503,633 -> 503,583, 503,467 -> 502,129 ATGGAACCACTCATAGCTGTC ag: ANOM_005322 start: 1 table: 1 t: tRNA isopentenyltransferase tid: XP_015406736.1 : GI-92086412 : GeneID:26807126 erred Translation: MEPLIAIVGATGTGKSKLAVDLATRFNGEIINGDAMQMI WRIGLFKSECLRIIKDIHSRGKLPILVGGTHYYTQAVLFKDQLVGEGSDELQGSG AADRWHPNDTRKIRRSLEIYFQTGRPASEVYADQKRLKQAALANGDFSAGEG KMSDYIRERRTQGCSIDPTRGVWXIGFKELAPYFEALHKRSLSGDELESLKES RLYLLDSTNVEDWGSCITEPSELLTQALLKDESAPDPKSLSELAKTILGAKEAQSC KRVLKUWPKERNGRNLYRLGKTIREGSLVTQAVNH eature Key: CDS in Type: join errived using Genelous Prime 2021.2.2 'Annotate from Database' base erred From: ANOM_005322 - 26807126 erred Similarity: 65.05% y Match: 65.05%; 503,633 -> 503,583, 503,467 -> 502,129; tRNA isopen atic Translation: MEPLIAVVGATGTGKSKLAVDLASRENGEIINGDAMQMI WSIGFKKECLRLIKDIHSRGKLPILVGGTHYYTQTVLFKDQLVDREEAGSGSELDD HPNETRKIRRSLEIYFQTGRPASEIYAEQRCQRQIASMDENSAGGRGGLRYQTI 10EKQSQGLTVDQTKGWNVSIGFKELGPYFSALRDGSSTGELENLRRSCIESIK SSNVDHWDTCVKQASERLVHSFLINPEPCPGPKSLSELARVTLEAKEAQAQKFT ergeillus nomiae entenyltransfe</pre>	YRGLPIITNQIPMDERNNIPHHLISCI ARSDGETEESSTKWPILDAPTDVVLRKLR QLRFPTMVFWIHSEREALIARLDKRVDVMIEQG CIESIKIATRQYSASQVKWIRNKLWNTLAE QKGPGFAAKCFTCDVCRKTMVNEEQWNIHL ed on nucleotide similarity Attenyltransferase CDS (ANOM 005322 - 268 VHGLPIITNQIPVEERNGIPHHMIGCIGL DSESSSPEKWPILNAPTDVLLQKLREVDPA LIFWVHADKEILNSRLDARVDTMIEQGLMSEA TATRQYAVSQIKWIRNKLWKALAETRMTS FSGLPKCVTCDMCRKTMMNEEQWTVHVKGQG Similarity:40 % Find: @ Best match ? All matching annotations	о отта отта отта отта отта отта отта от

✓	Name 🔺	Description		Modified	# Sequences	Min Sequen	Max Seque	% GC	# Nu⊞
✓		-	1	17 Sep 2021 8:21 am	8	1,927,652	4,110,925	49.1%	23,811^
S S	 ✓ Usequences for C222 ✓ Text View Lin → ⊕ Extract @ R.C. ② Tran 	eage Info slate ⇔ Add/	Name: tRNA isopentenyltransferase C Type: CDS Total Length: 1390 (over 2 intervals) Interval 1 Length: 51 Interval 1: 503,633 -> 503,583 Interval 1 Bases: ATGGAACCACTCATA Intervals: 503,633 -> 503,583 Gases: ATGGAACCACTCATAGCTGTC Iocus_tag: ANOM_005322 codon_start: 1 transl_table: 1 product: tRNA isopentenyltransferase protein_id: XP_015406736.1 db_xref: GeneID:26807126 Transferred Translation: MEPLIAIVG/ ELDEEPWRIGLFKSECLRIKDIHSRGKLPIL EVDPVMADRWHPNDTRKIRRSLEIYFQTG LUSEAQRMSDYIRERRTQGCSIDPTRGVW TGTAHRLYLLDSTNVEDWGSCITEPSELIT NGHSHKRVLKLWPKERNGRNLYRLGKTIR NCBI Feature Key: CDS NCBI Join Type: join Date: Derived using Geneious Prime 2	AGCTGTC -> 502,129 ATGTGKSKLAVDLATRI .VGGTHYYTQAVLFKDG SRPASEVYADQKRLKQ, WAIGFKELAPYFEALH QALLKDESAPDPKSLS SEGSLVTQAVNH 021,2,2,1 Appostate fro	- FNGEIINGDAM 2UVGEGSDEIQ AALANGDPSAC RSLSGDELESL ELAKTILGAKEA	QMYRGLPIITN SSGARSDGTEI SEGQLRFPTMVI KESCIESIKIATR QSQKGPGFAAI	QIPMDERNNIPI SSTKWPILDAP WIHSEREALIAF QYSASQVKWIR (CFTCDVCRKTM	HHLISCI TDVVLRKLR RLDKRVDVMIEC NKLWNTLAE IVNEEQWNIHL	23,01 ×
	500 <mark>000 1,000,00</mark>	1,50	Transferred From: ANOM_005322 - 26	5807126			o ciae o initiarity		
			Transferred Similarity: 65.05% Primary Match: 65.05%; 503,633 -> 50 Automatic Translation: MEPLIAVVGA EKEPWRVGIFKKECLRIIKDIHSRGKLPILV MASRWHPNETRKIRRSLEIYFQTGRPASEI ERMLLYIQEKQSQGLTVDQTKGWVSIGF RLYLLDSSNVDHWDTCVKQASERLVHSFL KRALKAAAKRAEREEFLRKRESLKNTQSSA	3,583, 503,467 -> 502 TGTGKSKLAVDLASRF GGTHYYTQTVLFKDQL IYAEQRCQRQIASMDE :KELGPYFSALRDGSSTG HNEPCPGPKSLSELAR INGR*IV	129; tRNA iso NGEIINGDAMO VDREEAGSGSE NSAGGRGGLR [\] GEELENLRRSCI VTLEAKEAQAQ	pentenyltransf QMYHGLPIITNC EDDSESSSPEKV YQTLIFWVHADI ESIKTATRQYAV	erase CDS (<u>ANG</u> QIPVEERNGIPHI VPILNAPTDVLL KEILNSRLDARVI SQIKWIRNKLW CDMCRKTMMN	DM 005322 - 20 HMIGCIGL QKLREVDPA DTMIEQGLMSE KALAETRMTS EEQWTVHVKG(<u>\$807126</u>) A QGH
ch	r_3_v1	502,000	gene: 62.29% urce Aspergillus nomiae RNA isopentenyltransfe tRNA isopentenyltr	u 303,00 1	v	Similarity: Find: O Be	est match I matching ann	- 40 % ? otations	

14) Putative C-8 sterol isomerase (Erg-1) [P174DRAFT_442147 - 36534834] from *Aspergillus novofumigatus* IBT 16806; L- 1099 bp



		582,000	582,500	583,000	583,500	D	584,000	
chr_5	5_V2			source Aspergill CDS; 73.79% unnamed pi	ne; 69.66% us oryzae RIB4 rote	0; 69.66%		
< Sequence → (c) 250	e View Text → Extract @ 1 .000 500,000	View Lineage Info R.C. ② Translate ⇔ Ad 750,000 1.000,000	Name: source A: Type: Source Length: 1002 Interval: <u>582,833</u> Bases: ATGTCAG organism: Asper mol_type: genor strain: RIB40 db_xref: taxon.5' chromosome: 8 note: Derived us Transferred Froo	spergillus oryzae RIB40 2 -> 583,840 CAATCCAGGACCAG gillus oryzae RIB40 mic DNA 10516 ay: source ing Geneious Prime 2021.2.2 m: A0090103000287 - 599898 Jionim Co Change - 599898	'Annotate from Datal	base' based on nucl	eotide similarity	×
hr_5_v2 -	582,000	582,500	Iransferred Sim Primary Match: Primary Match Source ATGGC Target ATGTC 583,000 51 (sene; 69.66% source Aspergillus oryzae CDS; 73.79% unnamed prote	IIIarity: 59,65% 69,66%; 582,839 -> 583,840; s Aligimment: Open Alignment TGGAATCCAGGACCGACTAGACC AGCAATCCAGGACCGGCTAACC 83,500 584,000 RIB40; 69,66%	Source Aspergillus ory	rzae RIB40 (A00901) rt Trt GGCGCTCCAGJ TTCT AAAGCTTAGG Source: 25 Similarity: Find: Best match All match	13000287 - 5998989) ISOCAGGCTAATGTATTTA ISOCAAGCGAAGGTCTTCC 40 % th ? ing annotations Advanced	AC G TGA AC C TGA ↓ ↓ ↓ ↓ ↓ ↓
< Sequence ← → () 25	e View Tex → Extract & α,οοο 5οφ,οος	t View Lineage Inf R.C. 🛞 Translate 🖒 Ad 750,000 1.000,000	Name: CDS Type: CDS Total Length: 885 Interval 1 Length: Interval 1: 582,839 Interval 1 Bases: A Interval 582,839 Bases: ATGTCAGC4 locus_tag: A00901 note: unnamed pro codon_star: 1 transl_table: 1 protein_id: XP_001 db_xref: GL:169784 db_xref: GL:169784 db_xref: GL:169784 db_xref: GL:169784 NCBI Join Type: Joi note: Derived using Transferred From: Transferred From:	(over 3 intervals) 557 557 ATGTCAGCAATCCAGGACCAG. -> 583,395, 583,457> 583,61 VATCCAGGACCAG 03000287 otein product; Zn-dependent 826867.1 812 198989 lation: MAGIQTQLDQGAVPN .WETGSGKDYPTVWGPAIADIF. TGADVGYVLEHYLKLSLNWKTFI STQRLKHLQRITKGQVIPGHDK in g Geneious Prime 2021.2.2 'A A0090103000287 - 5998989 arity: 73.79%	 6, <u>583,673 -> 583,840</u> hydrolases, including MISNVCPPGTKFQVLEV ARVKYEPRHELRAAVEA NDQTLDFCQGITLHHLF ETFLALQSQANVFT .nnotate from Databa:	glyoxylases /GWLECDKGFVIRGG \TGHKLDDIKKIIIGHL PGHTDGLIGMQINM se' based on nucleo	NTSTKSTETGSFVNERCEI HLDHAGGLDEFLHRTDV LNTGTFFFISDHCHVIENV btide similarity	M EVW VRDGIP
:hr_5_v2	582,000	582,500	Transferred Simila Primary Match: 73 Automatic Transla VCILIEHPHEGLILWE EKELLSAFWSVATGA WLARDHPAWFRSTC \$33,000 £ ene; 69,669 source Aspergillus oryzae CDS; 73,79% Unnamed prote	arity: 73.79% 1.79%; 582,839 -> 583,395, 583 ation: MSAIQDQLNRGAVPNA TGSGKDYPRTWGPVVSDIFSRV DTGVYLEHYLKLSLNWKTFRDE RILHERITRGQVIPGHDKETF 0007000 0007000 8 RIB40: 69.66%	3,457 -> 583,616, 583, VSNVCPPGTKFHVLEVC (RYEPEHELRAAVEATGI TLDFCQGITLHHLPGHT LKLKQQAKVFT* >004,500	673 -> 583,840; CDS GWLECDEGFVVRGGM NRIEDIKKIIIGHLHLD IDGLIGMQINLLNTG Similarity: Find: Best mat All match Apply	(AO090103000287 - 599 JTSLKSTENEKFVNKRREN HAGGLDEFLDRKDVEIW TFFFISDHCHVIENWRDG (h ?) ing annotations Advanced	8989) APM VH IPQG 後 後 家

15) Hypothetical protein [AO090103000287 - 5998989] from *A. oryzae* RIB40; L- 987bp; Accession no: 5998989

	Name: CDS Type: CDS Total Length: 885 (over 3 intervals) Interval 2 Length: 160 Interval 2 583,457 -> 583,616 Interval 2 Bases: GAACTGGAAGACCTTTAGGGA	×
	Bases: ATGTCAGCAATCCAGGACCAG locus_tag: A0090103000287 note: unnamed protein product; Zn-dependent hydrolases, including	ı giyoxylases
	codon_start: 1 transl_table: 1 protein_id: XP_001826867.1 db_xref: <u>GI:169784812</u>	
Sequence View Text View Lineage Info	db_xref: <u>GeneID:5998989</u> Transferred Translation: MAGIQTQLDQGAVPNAISNVCPPGTKFQVLEV	/GWLECDKGFVIRGGNTSTKSTETGSFVNERCEM
\leftarrow \rightarrow \ominus Extract $@$ R.C. $@$ Translate $ riangle$ Add/Edit Ar	VHEKELTNAFWSVATGADVGVYLEHYLKLSLNWKTFNDQTLDFCQGITLHHL	PGHTDGLIGMQINMLNTGTFFISDHCHVIENWRDGIP
1 250,000 500,000 750,000 1.000,000 1.250,00	QGWLARDHPSWFRSTQRLKHLQRITKGQVIPGHDKETFLALQSQANVFT NCBI loin Type: join	
	note: Derived using Geneious Prime 2021.2.2 'Annotate from Databa	se' based on nucleotide similarity
	Transferred From: AO090103000287 - 5998989 Transferred Similarity: 73.79%	
	Primary Match: 73.79%; 582,839 -> 583,395, 583,457 -> 583,616, 583, Automatic Translation: MSAIQDQLNRGAVPNAVSNVCPPGTKFHVLEV YCILIEHPHEGLILWETGSGKDVPRTWGPVVSDIFSRVRYEPEHELRAAVEATG EKELLSAFWSVATGADTGVYLEHYLKLSLNWKTFRDETLDFCQGITLHHLPGH WI ARDIPAWFRSTORI KHI FRITRGOVIPGHDKFTF KI KI KOQAKVFT*	673 -> 583,840; CDS (AO090103000287 - 5998989) GWLECDEGFVVRGGNTSLKSTENEKFVNKRREMPM NRIEDIKKIIIGHLHLDHAGGLDEFLDRKDVEIWVH TDGLIGMQINLLNTGTFFFISDHCHVIENWRDGIPQG
chr 5 v2	gene: 69.66%	Similarity:
source	e Aspergillus oryzae R B40; 69.66%	Find: • Best match
	DS; 73.79%	All matching annotations
Sequence View Text View Lineage Info ← → ⊖ Extract @ R.C. ⑧ Translate ⇒ Add/Edit 1 250,000 500,000 750,000 1.000,000 1.259.	Type: CDS Total Length: 885 (over 3 intervals) Interval 3 Length: 168 Interval 3 : S83,673 ~> 583,840 Interval 3 : S83,673 ~> 583,840 Interval 3 : S83,673 ~> 583,840 Interval 5 : S82,839 ~> 583,3457 ~> 583,616, 583,673 ~> 583,847 Bases: ATGTCAGCAATCCAGGACCAG locus, tag: AO090103000287 note: unnamed protein product; Zn-dependent hydrolases, includit codon_start: 1 trans[_table: 1 protein_id: XP_001826867.1 db_xref: GreneID:5998989 Transferred Translation: MAGIQTQLDQGAVPNAISNVCPPGTKFQVL PMYCLIDHPHEGLILWETGSGKDYPTVWGPAIADIFARVKYEPRHELRAAW V HEKELTNAFWSVATGADVGVYLEHYLKLSLNWKTFNDQTLDFCQGTILHF QGWLARDHPSWFRSTQRLKHLQRITKGQVIPGHDKETFLALQSQANVFT NCBI Join Type: Join note: Derived using Geneious Prime 2021.2.2 'Annotate from Datal Transferred From: AO090103000287 - 599899	IQ 1g glyoxylases EVGWLECDKGFVIRGGNTSTKSTETGSFVNERCEM EATGHKLDDIKKIIGHLHLDHAGGLDEFLHRTDVEVW iLPGHTDGLIGMQINMLNTGTFFFISDHCHVIENWRDGIP base' based on nucleotide similarity
	Transferred Similarity: 73.79%	
582.000 582.500 5	Primary Match: 73.79%; <u>582,839 -> 583,395</u> , <u>583,457 -> 583,616</u> , <u>58</u> Automatic Translation: MSAIQDQLNRGAVPNAVSNVCPPGTKFHVLE YCILIEHPHEGLILWETGSGKDYPRTWGPVVSDIFSRVRYEPEHELRAAVEAT EKELLSAFWSVATGADTGVYLEHYLKLSLNWKTFRDETLDFCQGITLHHLPG WLARDHPAWFRSTQRLKHLERITRGQVIPGHDKETFLKLKQQAKVFT*	3.673 -> 583.840; CDS (AC090103000287 - 5998989) VGWLECDEGFV/RGGNTSLKSTENEKFV/NKRREMPM GNRIEDIKKIIIGHLHLDHAGGLDEFLDRKDVEIWVH HTDGLIGMQINLLNTGFFFISDHCHVIENWRDGIPQG
chr_5_v2	gene; 69.66%	Similarity: 40 %
sou	CDS; 73.79%	Find: • Best match ?
	unnamed prote	All matching annotations
		Apply Advanced

16) ADH2: alcohol dehydrogenase [ADH2 - 3640751] from *C. albicans* SC5314; L-1047 bp

1	500,000) 1,0	000,000	1,500,000	2,000,00	0 2,5	00,000	,000,000	3,500,000) 4,1	110,925
chr 1 V2)4,000	2,905,000	2,906,000	2,907,000	2,908,000	2,909,000	2,910,000	2,911,000	2,912,000	2,913,000	2,9'
					(
							30				

	Name: source Candida albicans SC5314
1 500,000 1,000,000 1,500,000	^{2.00} Type: Source
	Length: 1047
	Interval: 2 910.208 -> 2 911.254
	Bases: GGTCAACAGTCTTGAATGGGG
	organism: Candida albicans SC5314
	mol type: genomic DNA
	strain SCS314
	het: Hono spiens
	culture collection: ATCC:MVA-2976
00 2,910,250 2,910,500 2,910,750 2,911	db weft taxon 27561
chr_1_V2 * ADH2 CDS; 46.21%	
ADH2 gene; 46.21%	
ADH2 mRNA: 46.21%	napiotype: A
source Candida albicans SC5314: 4	country: USA: New York
Source candida albicans Sessira, 4	collected_by: Margarita Silva-Huther
	NCBI Feature Key: source
	note: Derived using Genelous Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity
	Transferred From: ADH2 - 3640751
	Transferred Similarity: 46.21%
	Primary Match: 46.21%; 2,910,208 -> 2,911,254; source Candida albicans SC5314 (ADH2 - 3640751)
	Primary Match Alignment: Open Alignment
<	Source TTATTTGTCGTTGTCCAAGACATATCTACCCAAGATTTTATTTCGAAAATAACAGCTTTTTGAGTAGTTGGGACAGACAT
Mouse over base 2,911,114 (A), residue 47 (D/Asp/Aspartic acid	Target GGTCAACAGTCTTGAATGGGGCCTTGATGAGACCGCGTCTCCTCGACGAGCCTGAGCCCATTGAAGCTTTGGAAGAGACAT
	Name: ADH2 CDS *
	Langth: 1047
	Inferval 2 011 254 -> 2 010 208
	ane Alter and a second and a second a
<	note: Alcohol debudrogenase: soluble in hyphae: expression regulated by white-onague switching: regulated by Ssn6:
	indued by Mn11 in weak acid, stress: protein enriched in stationary place wast cultures: Spider biofilm indued
Sequence View Text View Lineage Info	codon start: 1
← → ⊖ Extract @ R C Translate Add/Ed	transl table: 12
	product: alcohol dehvdrogenase
1 500,000 1,000,000 1,500,000	protein id: XP 717649.1
	db xref: GI:68476713
	db xref: CGD:CAL0000200753
	db xref: GeneID:3640751
	Transferred Translation: MSVPTTQKAVIFETNGGKLEYKDIPVPKPKANELLINVKYSGVCHTDLHAWKGDWPLATKLPLVG
	GHEGAGVVVALGENVKGWKVGDYAGVKWLNGSCLNCEYCOSGAEPNCAEADLSGYTHDGSFOOYATADAVOAARIPAGTDLANVAPILCA
	GVTVYKALKTAELEAGQWVAISGAAGGLGSLAVQYAKAMGYRVLAIDGGEDKGEFVKSLGAETFIDFTKEKDVVEAVKKATNGGPHGVIN
	VSVSERAIGOSTEYVRTLGKVVLVGLPAGAKISTPVFDAVIKTIOIKGSYVGNRKDTAEAVDFFTRGLIKCPIKIVGLSELPEVYKLMEE
	GKILGRYVLDNDK
00 2,910,250 2,910,500 2,910,750 2,911,	NCBI Feature Kev: CDS
chr_1_V2 * ADH2 CDS; 16.21%	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity
ADH2 gene; 45.21%	Transferred From: ADH2 - 3640751
ADH2 mRNA; 46.21%	Transferred Similarity: 46.21%
source Candida albicans SC5314; 46	Primary Match: 46.21%; 2,911,254 -> 2,910,208; ADH2 CDS (ADH2 - 3640751)
	Primary Match Alignment: Open Alignment
	Source ATGTCTGTCCCAACTACTCAAAAAGCTGTTATTTTCGAAATAAAATCTTGGGTAGATATGTCTTGGACAACGACAACGACAAATAA
	Target ATGTCTCTTCCAAAGCTTCAATGGGCTCAGGTCGTCGAGGAGACGCGGGTCTCATCAAGGCCCCATTCAAGACTGTTGACC
	Automatic Translation: MSLPKLQWAQVVEEAGGPVIYKQIPVPTPGPDEVLVNIKYTGVCHTDSHARKGDWPLPVKMPLVGGH
	EGAGVVVAKGQLVNNVNIGDHAGVKWSNGSCLSCEFCRTSDESLCPDASLSGYTVDGTFQQFCIAKAAHVSILPKDVPSDAVAPVSCAGI
	TVYKGSKESGIQPGQTLAIVGAGGGLGSLAQQYAKAMGSRVIAIDGGEEKKESCQQLGAEVTISSPFLALNARNLLTFLFSLRLSPTSTS
4	PSLRTSLRTSLRDPPPTVSVLTSLSCWSSPKFHSNRPPPTSAPVVSSSLLVCSQVPSLRLSSSAPLSA*SRSGVVMSVTDRMESKPSISSD
Mouse over hase 2 910 838 (G) residue 139 (T/Te/Tsoleucine)	

17) ybjG: undecaprenyl pyrophosphate phosphatase [ybjG - 6301165 (discontinued)] from *Erwinia tasmaniensis* Et1/99; L- 585 bp





18) Probable C-8 sterol isomerase erg-1 [FFUJ_02631 - 35396113] from *Fusarium fujikuroi* IMI 58289; L-760 bp



	Name: probable C-8 sterol isomerase erg-1 CDS
	Type: CDS
	Total Length: 706 (over 2 intervals)
	Interval 1 Length: 368
	Interval 1: 223,525 -> 223,892
٢	Interval 1 Bases: TTCGTAAATAAATTCATCATG
Converse View Text View Lineage	, Intervals: <u>223,525 -> 223,892,</u> <u>223,930 -> 224,267</u>
Sequence view Text view Lineage II	Bases: TTCGTAAATAAATTCATCATG
← → ⊖ Extract 🕼 R.C. 🛞 Translate 🖄 A	(locus_tag: FFUJ_02631
1 200,000 700,000 1,000,000	- codon_start: 1
1 2:0,000 500,000 7:50,000 1,000,000	transl_table: 1
	product: probable C-8 sterol isomerase erg-1
	protein_id: XP_023427749.1
	db_xref: <u>GI:1328525023</u>
	db_xref: EnsemblGenomes-Gn:FFUJ_02631
	db_xref: EnsemblGenomes-Tr:CCT65668
	db_xref: GeneID:35396113
	db_xref: GOA:S0DYX8
0 223,200 223,400 223,600 22	db_xref: InterPro:IPR006716
chr 6 v2	db_xref: UniProtKB/TrEMBL:S0DYX8
source Eusariu	Transferred Translation: MAKSKSKSKSSATAASQGSGLNKLLLVLGLLTALLSSVVYFVEQNLNQFYIFDLDHLDDLSKRAI
exop 1: 54 69	AKHGEDTRSVVQYIVTELNEKVPEHINLKEEWVFNNAGGAMGAMYIIHASVTEYLIIFGTAIGTEGHTGRHTADDYFHILSGTQLAYVPG
probable C-8 st	EYKAEVYPAGSIHHLRRGDVKQYKMPEGCFALEYARGWIPPMLFFGFADGLSSTLDFPTLWDTTRITGREMINNLLKGKL
probable C-8 s	NCBI Feature Key: CDS
probable	NCBI Join Type: join
	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity
	Transferred From: FFUJ_02631 - 35396113
	Transferred Similarity: 63.29%
	Primary Match: 63.29%; 223,525 -> 223,892, 223,930 -> 224,267; probable C-8 sterol isomerase erg-1 CDS (FFU] 02631 - 35396113
	Automatic Translation: FVNKFIMGSKPRSGAKNCSCGIHKLGLVAIVLALFTALYSYLNARLDQFYIFEPEYLHDLSQRAIST
<	HGNDTKAVVSFIVDELDQRETGAYVNKDQEWVFNNAGGAMGAMYVIHASXXXXL*YCHWN*RPHWTPHCGRLLQHPPGNPTRLRPRGIR
Cursor before base 223,090. Mouse over base 223,748 (1) AGGVPTRQRPSPPPRRGQTVQDGVVVFRAGIRPGLDPSHAFLRVR*YLYEHS*FPDIVGHHADHRQRDDFKPVPIEAV

	Name: probable C-8 sterol isomerase erg-1 CDS	×					
	Type: CDS						
	Fotal Length: 706 (over 2 intervals)						
	nterval 2 Length: 338						
	Interval 2: 223,930 -> 224,267						
	Interval 2 Bases: GTACTGCCATTGGAACTGAAG						
	Intervals: 223,525 -> 223,892, 223,930 -> 224,267						
	Bases: TTCGTAAATAAATTCATCATG						
	locus_tag: FFUJ_02631						
	codon_start: 1						
	transl_table: 1						
	product: probable C-8 sterol isomerase erg-1						
	protein_id: XP_023427749.1						
	db_xref: GI:1328525023						
	db_xref: EnsemblGenomes-Gn:FFUJ_02631						
	db_xref: EnsemblGenomes-Tr:CCT65668						
	db_xref: GeneID:35396113						
<	db_xref: GOA:S0DYX8						
Seguence View Text View Lineage	db_xref: InterPro:IPR006716						
Sequence view liext view Lineage In	db_xref: UniProtKB/TrEMBL:S0DYX8						
← → ⊖ Extract 🕼 R.C. 🛞 Translate 🖄 A	Transferred Translation: MAKSKSKSKSSATAASQGSGLNKLLLVLGLLTALLSSVVY	FVEQNLNQFYIFDLDHLDDLSKRAI					
1 30,000 500,000 750,000 1,000,000	AKHGEDTRSVVQYIVTELNEKVPEHINLKEEWVFNNAGGAMGAMYIIHASVTEYLIIFGT	AIGTEGHTGRHTADDYFHILSGTQLAYVPG					
1 220,000 500,000 750,000 1,000,000	EYKAEVYPAGSIHHLRRGDVKQYKMPEGCFALEYARGWIPPMLFFGFADGLSSTLDFPTL	WDTTRITGREMINNLLKGKL					
	NCBI Feature Key: CDS						
	NCBI Join Type: join						
	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' base	d on nucleotide similarity					
	Transferred From: FFUJ_02631 - 35396113						
	Transferred Similarity: 63.29%						
	Primary Match: 63.29%; <u>223,525 -> 223,892</u> , <u>223,930 -> 224,267</u> ; probable C-8	sterol isomerase erg-1 CDS (FFUJ 02631 - 35396113)					
	Automatic Translation: FVNKFIMGSKPRSGAKNCSCGIHKLGLVAIVLALFTALYSYL	NARLDQFYIFEPEYLHDLSQRAIST					
0 223,200 223,400 223,600 22	HGNDTKAVVSFIVDELDQRETGAYVNKDQEWVFNNAGGAMGAMYVIHASXXXXL*1	CHWN*RPHWTPHCGRLLQHPPGNPTRLRPRGIR					
chr 6 v2	AGGVPTRQRPSPPPRRGQTVQDGVVVFRAGIRPGLDPSHAFLRVR*YLYEHS*FPDIVG	HHADHRQRDDFKPVPIEAV					
source Fusariu	m fujikuroj IMI 58289: 60.55%						
exon 1: 54.69	1% exon 2: 72.78%	Find: O Best match					
probable C-8 st	ero*	All matching annotations					
	-8						
probable of		Apply Advanced					



19) Alcohol dehydrogenase [FOYG_00855 - 42008387] from *F. oxysporum* NRRL 32931; L-2,722 bp

20) Related to ADH5-alcohol dehydrogenase V [FPRO_06554 - 42051433] from *Fusarium proliferatum* ET1; L-1,183 bp



				Name and the FDDO and	- 1-1000 F				×		
				Name: contig: FPRO scan	rold003 source Fusariur	n proliferatum ETT					
				Type: Source							
				Length: 1218	igth: 1218						
				Interval: <u>50,545 -> 49,328</u>	3						
				Bases: ATGTCTACCATCAG	STCTTCCC						
				organism: Fusarium prol	iferatum ET1						
				mol_type: genomic DNA							
Sequen	ce View Tex	t View	Lineage Info	strain: ET1							
· ·				db_xref: taxon:1227346							
$\leftarrow \rightarrow$	\ominus Extract 🛛 😰	R.C. 🔇	🖇 Translate 🛛 🖄 Ad	chromosome: Unknown							
1 25	0.000 500.00	0 7	50.000 1.000.000	note: contig: FPRO_scaffo	ld003						
		• •	.,	- NCBI Feature Key: sourc	e						
				note: Derived using Gene	ious Prime 2021.2.2 'Ar	nnotate from Database	' based on nucleotide similarity				
				Transferred From: FPRO	_06554 - 42051433						
				Transferred Similarity: 6	52.41%						
				Primary Match: 62.41%;	50,545 -> 49,328; contig	g: FPRO scaffold003 sou	rce Fusarium proliferatum ET1 (FPRO_06554 - 420)51433)		
				Primary Match Alignme	nt: Open Alignment						
				Source ATGTCAA	TCCCCCTCTCAACAA	CGAGCAGCAGTCAAG	TCGCTGGACGATGCGTTCTCGAGGT	TGC T G CT TAG			
				Target ATGTCTACCATC	AGTCTTCCCCAACAACAA	CG G GC C G TC GTCAAC	TTGCTGGTCGTGTCGTTTTGAAGGT	TGC C G AA TAA			
									\rightarrow		
	4	9,500	50,000	50,500	51.000	51,500	Similarity:	40 %	-		
chr_5_v2			gene; 62.419						[?>		
							Find: 💽 Best match	(?)			
	_		contig: FP	FPRO scaffold003 source Fusarium proliferatum ETI; 62.41%							
				1				ino caciono			

21) ADH1: alcohol dehydrogenase ADH1 [ADH1 - 854068] from *S. cerevisiae* S288C; L-1,047 bp



			Name: ADH1 CDS	C
\checkmark		Name Description	Type: CDS	
\checkmark	🗦 🛛	8 Sequences MP C322 -	Length: 1047	-
			Interval: 2,911,254 -> 2,910,208	≠ Nu
			Bases: ATGTCTCTTCCAAAGCTTCAA	3,81
			gene: ADH1	
			locus_tag: YOL086C	
			gene_synonym: ADC1	
			EC_number: 1.1.1.1	
			experiment: EXISTENCE:direct assay:GO:0004022 alcohol dehydrogenase (NAD+) activity [PMID:6985717]	1
			experiment: EXISTENCE:direct assay:GO:0005737 cytoplasm [PMID:2937632]	
			experiment: EXISTENCE:direct assay:GO:0005886 plasma membrane [PMID:16622836]	
			experiment: EXISTENCE:direct assay:GO:0006116 NADH oxidation [PMID:3546317]	
			experiment: EXISTENCE:direct assay:GO:0019170 methylglyoxal reductase (NADH-dependent) activity [PMID:12722185]	
			experiment: EXISTENCE:direct assay:GO:0045069 regulation of viral genome replication [PMID:31648290]	
			experiment: EXISTENCE:direct assay:GO:0046809 replication compartment [PMID:31648290]	
			experiment: EXISTENCE:direct assay:GO:1904408 melatonin binding [PMID:31708896]	
			experiment: EXISTENCE:genetic interaction:GO:0000947 amino acid catabolic process to alcohol via Ehrlich pathway	
			[PMID:12499363]	
			experiment: EXISTENCE:mutant phenotype:GO:0006116 NADH oxidation [PMID:15082781 PMID:23744286]	
			experiment: EXISTENCE:mutant phenotype:GO:0043458 ethanol biosynthetic process involved in glucose fermentation to	
			ethanol [PMID:15082781]	
			note: Alcohol dehydrogenase; fermentative isozyme active as homo- or heterotetramers; required for the reduction of	
	<		acetaldehyde to ethanol, the last step in the glycolytic pathway; ADH1 has a paralog, ADH5, that arose from the whole	
5	equen	ce View Text View Lineage Info	genome duplication	
			codon_start: 1	
÷	\rightarrow	\ominus Extract 🛱 R.C. 🛞 Translate 🖆 Add/E	d transi_table: 1	
1		500,000 1,000,000 1,500,000	- product: alconol denydrogenase ADH1	3387
[_protein_i0: NP_014555.1	838
			aD_xref: <u>G1:6324486</u>	838
			GID WALKSAN KANN GWARNED HAUNWENGSCHWEIGEN CELGHES HEN GEGVEN HAUN WANN GWARNEG HAUNWENGSCHWEIGEN CELGHES HEN G	
			COT/GDV/M/DTKK	
		2,910,000 2,910,200 2,910,400	NRI Feature Key (DS	
ch	r_1_v2	*	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity	
		22	Transferred From: ADH1 - 854068	4
		222	Transferred Similarity: 48.13%	[?*
		source Sacch	Primary Match: 48.13%: 2.911.254 -> 2.910.208: ADH1 CDS (ADH1 - 854068)	
			Primary Match Alignment: Open Alignment	\times
				1

22) ADH2: alcohol dehydrogenase ADH2 [ADH2 - 855349] from *S. cerevisiae* S288C; L-1,047 bp



Name: ADH2 CDS	ж
Type: CDS	
Length: 1047	
Interval: <u>2,911,254 -> 2,910,208</u>	
Bases: ATGTCTCTTCCAAAGCTTCAA	
gene: ADH2	
locus_tag: YMR303C	
gene_synonym: ADR2	
EC_number: 1.1.1.1	
experiment: EXISTENCE:direct assay:GO:0004022 alcohol dehydrogenase (NAD+) activity [PMID:3546317]	
experiment: EXISTENCE:direct assay:GO:0005737 cytoplasm [PMID:11914276 PMID:14562095]	
experiment: EXISTENCE:direct assay:GO:0006067 ethanol metabolic process [PMID:3546317]	
experiment: EXISTENCE:direct assay:GO:0006116 NADH oxidation [PMID:3546317]	
experiment: EXISTENCE:genetic interaction:GO:0000947 amino acid catabolic process to alcohol via Ehrlich path	way
[PMID:12499363]	
note: Glucose-repressible alcohol dehydrogenase II; catalyzes the conversion of ethanol to acetaldehyde; involve	d in the
production of certain carboxylate esters; regulated by ADR1	
codon_start: 1	
transl_table: 1	
product: alcohol dehydrogenase ADH2	
protein_id: NP_014032.1	
db_xref: <u>GI:6323961</u>	
db_xref: GeneID:855349	
db_xref: SGD:S000004918	
Transferred Translation: MSIPETQKAIIFYESNGKLEHKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGDWPLPTKLPLVG	
GHEGAGVVVGMGENVKGWKIGDYAGIKWLNGSCMACEYCELGNESNCPHADLSGYTHDGSFQEYATADAVQAAHIPQGTDLA	EVAPILCA
GITVYKALKSANLRAGHWAAISGAAGGLGSLAVQYAKAMGYRVLGIDGGPGKEELFTSLGGEVFIDFTKEKDIVSAVVKATNGGAH	GIIN
VSVSEAAIEASTRYCRANGTVVLVGLPAGAKCSSDVFNHVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGLSSLPEIYEKME	K
GQIAGRYVVDTSK	
NCBI Feature Key: CDS	
note: Derived using Genelous Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity	
Transferred From: ADH2 - 855349	
di Prince di Similarity: 47.79%	
Primary Match: 47.79%; 2,917,254 > 2,910,208; ADH2 CDS (ADH2 - 855349)	
Firmary Match Algoment. Open Algoment	
Source Arteroraticcabaacticabaacacaticcertaticticated	A
Internation Translation: MSI DKI OWAOVA/EEAGGDV/VKOID/DTDGDDEV//V/IKVTG/CHTDLHAPKCDW/DID//KMDI//C/	.с :ц
	IS I
	_

23) PHO8: alkaline phosphatase PHO8 [PHO8 - 852092] from *S. cerevisiae* S288C; L-1,701 bp



	Name: PHO8 CDS
	Type: CDS
	Length: 1680
	Interval: 1.948.332 -> 1.946.653
	Bases: CTAACCGGCGAACGAGTCAAG
	aene: PHO8
	locus tag: YDR481C
	EC number: 3.1.3.1
	EC number: 3.1.3.54
	EC number: 3176
	experiment: EXISTENCE:direct assay:GO:0000329 fungal-type vacuole membrane [PMID:2676517] PMID:26928762]
	experiment: EXISTENCE: direct assay: GO:0004035 alkaline phosphatase activity [PMID:8499492]
	experiment: FXISTENCE: direct assay:G0:0006470 protein dephosphorylation [PMID:8499492]
	experiment: EXISTENCE: mutant observoe: GO:0046496 nicotinamide nucleotide metabolic process [PMID:21349851]
	note: Repressible vacuolar alkaline phosphatase: controls polyphosphate content: regulated by levels of Pi and by Pho4p
	Phone Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose Phose
	nicotinamide rihoside from NMN
	rodon start 1
	transl table: 1
<	product: alkaline phosphatase PHO8
	protein id: NP 010769 3
Sequence View Text View Lineage In	db xref: GI:398366635
$\leftarrow \rightarrow \ominus$ Extract $\bigotimes B \subset \bigotimes$ Translate $\bowtie A$	db xref: GeneID:852092
	db xref: SGD:S00002889
1 250,000 500,000 750,000 1.000,000	Transferred Translation: MMTHTLPSEOTRLVPGSDSSSRPKKRRISKRSKIIVSTVVCIGLLLVLVOLAFPSSFALRSASHK
	KKNVIEEVIDGMGPASI SMARSENOHVNDI PIDDII TI DEHEIGSSRTRSSDSI VTDSAAGATAFACAL KSYNGAIGVDPHHRPCGTVLE
	AAKLAGYLTGLVVTTRITDATPASESSHVDYRWOEDLIATHOLGEYPLGRVVDLLMGGGRSHFYPOGEKASPYGHHGARKDGRDLIDEAO
	SNGWOYVGDRKNFDSLLKSHGENVTLPFLGLFADNDIPFEIDRDEKEYPSLKEOVKVALGALEKASNEDKDSNGFFLMVEGSRIDHAGHO
	NDPASOVREVLAFDEAFOYVLEFAENSDTETVLVSTSDHETGGLVTSROVTASYPOYVWYPOVLANATHSGEFLKRKLVDFVHEHKGASS
	KIENEIKHEILEKDI GIYDYTDSDI ETI IHI DDNANAIODKI NDMVSERAOIGWTTHGHSAVDVNIYAYANKKATWSYVI NNI OGNHENT
	EVGOELENELELNINEVTDLIRDTKHTSDEDATEIASEVOHYDEYYHELTN
	NCBI Feature Key: CDS
1,946,000 1,947,000	note: Derived using Geneious Prime 2021.2.2 'Annotate from Database' based on nucleotide similarity
chr_4_v1	Transferred From: PHO8 - 852092
	Transferred Similarity: 47.17%
* PHO8 (Primary Match: 47 17%: 1 948 332 -> 1 946 653: PHO8 CDS (PHO8 - 852092)
PHO8 ge	Primary Match Alignment: Open Alignment
PHO8 m	SOUTCE ATGACTCACACATTACCAAGCGAACAGACAGGCTCTTGACAACATTATGACGAATACTACCATGAGTTGACCAACTGA
source Saccha	Target_CTAACCGGCGAACGAGTCAAGGGGGAGCAACTGCAAGACAACTCCAGTCGTGGCGTATGGTCGGTGGCCTGGAGAAT
	Automatic Translation: I IGERVKREQI ODNGRRAEWKEVGI FAWASIATIAVIVIAVVYOHEIAKIHRANPPWSPKEKPTGKR
	NUTEMVSDGMGPTSLSMTRSFROFTDGLPIDDILVLDKHHIGTSRTRSSSSLVTDSAAGATAFSCGDKSYNGAISVLPDHSPCGTVLFAA
	ALAGYKTGLVVTTRITDATPACFASHVNMROYEDKIAAOEIGEHPLGRVVDLILGGGRCHFLPNSTGGSCRGDDRDLIAAAHGNGFSVIN
	DRAGEDALKOGTAAKLPLLGLFADODIPYFIDRRSONDVYPSLEEMARTALTALSEATKDSEOGEFLMIEGSRIDHAGHGNDPAAOVHEV

Section IV: Preliminary NCBI Homology Searches

The bioinformatic analysis was conducted to assess the similarity between the *M. purpureus* YY1 genome and those of *A. terreus* NIH2624 (NCBI Reference: NZ_AAJN00000000.1) and *P. sclerotiorum* strain 113 (NCBI Reference: KV784220) using NCBI-BLAST-N with the Multiple Sequence Alignment tool. The preliminary searches were performed using the reference *M. purpureus* YY1 genome (Table 53) available in the NCBI database, as the *M. purpureus* C322 genome was not yet assembled. Homology searches for QSM genes included comparisons with the *A. terreus* and *P. sclerotiorum* genomes, as both organisms have been previously used in situ for quorum sensing experiments, where QS has been established (Table 54). Although complete biosynthetic pathways are not available, some genes responsible for encoding butyrolactones are present in the NCBI databases have been explored in this section.

Chromosome	NCBI Accession No.
1	CM009898.1
2	CM009899.1
3	CM009900.1
4	CM009901.1
5	CM009902.1
6	CM009903.1
7	CM009904.1
8	CM009905.1

Table 53: Accession numbers of each chromosome of M. purpureus YY-1

Table 54 Identified gamma-butyrolactone genes from NCBI database

Gamma- Butyrolactone Gene	Gene Accession no.	Gene Length (bp)	M. purpureus YY1 Accession No- QDGY0000000 0.1	<i>P. sclerotiorum</i> Strain 113 Accession no: KV784220.	A. terreus NIH2624 Accession no: NZ- AAJN000000 00.1
SSOG_RS39990 gamma- butyrolactone biosynthesis protein	NZ_GG6577 54.1	1025	Chr1- 32/33 bp 97% similarity, Chr3- 85/85 bp 100% similarity and Chr4- 234/307 bp 76% similarity	No Similarity	378/510 bp 74%, 122/144 bp 78%, 54/64 bp 84%, 33/33 100%
NH08_RS0115770 putative gamma- butyrolactone biosynthesis enzyme [<i>Streptomyces</i> <i>rimosus subsp.</i> <i>rimosus</i>]	NZ_JNYR0 1000009.1	1130	No Similarity	No Similarity	105/131bp 80% similarity
A3L23_RS24285 gamma- butyrolactone biosynthesis protein of the AfsA family [<i>Rhodococcus</i> <i>fascians D188</i>]	NZ_CP0152 36.1	971	No Similarity	No Similarity	No Similarity
pFi_060 putative gamma- butyrolactone biosynthesis protein of the AfsA family [<i>Rhodococcus</i> <i>fascians D188</i>]	NC_021080. 1	971	No Similarity	No Similarity	No Similarity

AQJ27_RS33045 gamma- butyrolactone biosynthesis enzyme [<i>Streptomyces</i> <i>olivochromogenes</i>]	NZ_KQ948 465.1	1118	No Similarity	No Similarity	698/923bp 76% similarity, 156/192 bp 81% similarity, 57/84 bp 84% similarity, 32/33 bp 97% similarity
SU9_RS04625 gamma- butyrolactone biosynthesis enzyme [<i>Streptomyces</i> <i>auratus AGR0001</i>]	NZ_JH72538 7.1	959	No Similarity	275/378bp 78% similarity, 68/81bp 84% similarity	31/32bp 97% similarity
NMI01S_RS24115 gamma- butyrolactone biosynthesis protein [<i>Nocardia mikamii</i> <i>NBRC 108933</i>]	NZ_BDCM 01000014.1	977	No Similarity	No Similarity	No Similarity

Among the identified gene sequences for gamma butyrolactone biosynthesis, the gene SSOG_RS39990 from *Streptomyces himastatinicus* ATCC 53653 showed some similarity with the *M. purpureus* YY1 genome, suggesting a potential for gamma butyrolactone biosynthesis (Table 54).

The BLAST alignments for the gamma butyrolactone biosynthesis genes with M. purpureus

YY1, P. sclerotiorum strain 113, and A. terreus NIH 2624 are provided below.

1. SSOG_RS39990 gamma-butyrolactone biosynthesis protein [Streptomyces

himastatinicus ATCC 53653] Accession No: NZ_GG657754.1

Gene symbol	SSOG_RS39990					
Gene description	gamma-butyrolactone biosynthesis protein					
Locus tag	SSOG_RS39990					
Gene type	protein coding					
Organism	Streptomyces himastatinicus ATCC 53653					
	(strain: ATCC 53653, culture-collection:					
	ATCC:53653, old-name: Streptomyces					
	hygroscopicus ATCC 53653)					
Lineage	Bacteria; Actinobacteria; Streptomycetales;					
	Streptomycetaceae; Streptomyces					
Old locus tag	SSOG_07932					

A) Pairwise alignment with M. purpureus YY1 genome

• Chromosome 1:

lob title: CM009898:Monascus purpureus strain YY-1 chromosome...

 RID
 AM28UA22114 (Expires on 04-08 22:28 pm)

 Query ID
 CM009898.1
 Subject ID
 NZ GG657754.1

 Description
 Monascus purpureus strain YY-1 chromosome I
 Description
 None

 Molecule type
 dna
 Molecule type
 dna

 Query Length
 109886
 Subject Length
 10901646

 Program
 BLASTN 2.9.0+ ▷ Citation

Sequences producing significant alignments:

Sel	ect: <u>All None</u> Selected:0						
11	Alignments Download GenBank Graphics						0
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	Streptomyces himastatinicus ATCC 53653 supercont1.1, whole genome shotgun sequence	56.5	56.5	0%	4e-04	96.97%	NZ GG657754.1

Downl	oad 👻 <u>Gen</u>	Bank Graphic	<u>s</u>		
Strepto ^{Sequenc}	myces hima ⊫ ID: <u>NZ_GG</u>	astatinicus A 657754.1 Ler	FCC 53653 superc ngth: 10901646 Num	ont1.1, whole ge ber of Matches: 1	enome shotgun sequence
Range 1	: 9937969 to	9938001 GenB	ank Graphics		🔻 Next Match 🔺 Previous Match
Score		Expect	Identities	Gaps	Strand
56.5 bit	ts(30)	4e-04	32/33(97%)	0/33(0%)	Plus/Plus
Query	3973064	CCCCAGTGG	ATGAAGATGCCGAAC [.]	TTGGCATCG 39	73096
	9937969	CCCCAGTGG	ATGAAGATGCCGAAC	ttööcotco 99	38001
Sbjct					

• Chromosome 3

Sequences producing significant alignments:

Select: All None Selected:0

Â	Alignments 🖥 Download 👻 GenBank Graphics						0
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	Streptomyces himastatinicus ATCC 53653 supercont1.1, whole genome sholgun sequence	54.7	160	0%	0.001	100.00%	NZ GG657754.1

nments			

■Download - <u>GenBank</u> <u>Graphics</u> so	ort by: E value	Y Next 🛦 Previous 🛓 Descriptions
Streptomyces himastatinicus ATCC	53653 supercont1.1, whole ge	nome shotgun sequence

Sequence ID: <u>NZ_GG657754.1</u> Length: 10901646 Number of Matches: 3

Range 1	: 812841 to 8	12869 GenBar	•	🔻 Next Match 🔺 Previous Match			
Score		Expect	Identities	Gaps	Strand		
54.7 bit	:s(29)	0.001	29/29(100%)	0/29(0%)	Plus/Plus		
Query	3333872	GGGCAGCGG		A 3333900			
Sbjct	812841	GGGCAGCGG	CAGCGGCAGCGGCAGCGG	A 812869			

Range 2	812848 to 8	312875 GenBank	Graphics	Vext Match	🛦 Previous Match 🧃	First Match
Score		Expect	Identities	Gaps	Strand	
52.8 bil	s(28)	0.005	28/28(100%)	0/28(0%)	Plus/Plus	
Query	3333873	GGCAGCGGCA	GCGGCAGCGGCAGCGGCA	3333900		
Sbjct	812848	GGCAGCGGCA	GCGGCAGCGGCAGCGGCA	812875		

Range 3	: 812854 to 8	312881 GenBank	Graphics	Vext Match	🛦 Previous Match 🔺 First Match
Score		Expect	Identities	Gaps	Strand
52.8 bit	ts(28)	0.005	28/28(100%)	0/28(0%)	Plus/Plus
Query	3333873	GGCAGCGGCA	GCGGCAGCGGCAGCGGCA	3333900	
Sbjct	812854	GGCAGCGGCA	GCGGCAGCGGCAGCGGCA	812881	

• Chromosome 4

Alignment	s 🗄 Dowi	nload <u>~ G</u>	enBank <u>Gra</u>										
			Descr	iption				Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Streptomy	vces himast	atinicus ATC	C 53653 super	rcont1.1. wh	ole genome	shotgun seq	uence	154	154	0%	10-33	76.22%	NZ GG65775
ments													
Bownload	→ <u>Gen</u> B	Bank <u>Graph</u>	ics							V N	ext 🔺 F	Previous	A Description
Streptomyo	ces hima	statinicus A	ATCC 5365	3 superco	nt1.1, who	le genom	e shotgu	n seque	ence				
Range 1: 534	82817 to 5	5383118 Ger	Bank Graphic	5	er of materie	V Ne	xt Match 🔺	Previous	Match				
Score 154 bits(83	3)	Expect 1e-33	Identities 234/307(76	9%)	Gaps 10/30/(3%)	Strand Plus/Pl	us	-				
Query 56	52194	ттесссте	CAGGGTCGT	CAAGTTCC	CAGTTGAG	GTAGCGT-	ттесссе	GAAAGA	AGGGGG	5622	252		
Sbjct 53	882817	TTGCCCGG	CCGGGTCGT	TGAGCTTO		GTA-CGTC	TTGCCGG	GGAAGT.	ACGGGC	5382	2875		
Query 56	52253	AGGCGTCG		ATGGTGAT	GACGATGT	C-GCTGGC	CTGGACA	GCATCG		5623	311		
Sbjct 53	882876	AGGCGTCG	CCGCAGCCC	ATGGTGAT	GACGACGT	CCGAC-GC	стосасо	GCTTCC	GTGGTC	5382	2934		
Query 56	52312	AGAATCTT	CGGTTT-TT	SGTCAGTG	ATGTCGAT	GCCTTCTT	CACG-CA	TGGCTT	CGACCA	5623	869		
Sbjct 53	882935	AGCACCTT	GGGGATCTC	TG-CGGCG	ATGTCGAT	GCCGACTT	C-CGCCA	TCGCTT	CACCA	5382	2992		
Query 56	52370	CGACGGGG	TTGATGGAG	TCGGCAGG	CGCTGAGC	CAGCGGAG	CGGACCT	CAACAG	са-тсе 	5624	28		
Sbjct 53	382993	CGGCGGGG	TTGACGTGT	TCGGCGGG	GGCCGAAC	CGGCGGAG	CGGACTT	ĊGÁĊ-Ġ	ĊGGŤĊĠ	5383	8051		
	52429	CCTGCGAG	GTAGGTGAG	STAGCCAG	CGGCTATT	TGAGAACG	GCCGGCG	TTATGA	ATGCAG	5624	88		
Query 56		CCCGCGAG	GTGGGTGAG	SAAGGCGG	CGGCCATC	TGGGAGCG	GCCCGCG	TTGTGG.	ACGCAG	i 5383	3111		
Query 56 Sbjct 53	383052	CCCGCGAG	GIGGGIGAG										
Query 56 Sbjct 53 Query 56	383052 52489		562495										

- B) Pairwise alignment with P. sclerotiorum strain 113 genome: No Significance
- C) Pairwise alignment with A. terreus NIH2624 genome:

o aac	: ref NZ_	GG03//3	4.1								
(De: Molec Quer	RID Query ID scription cule type y Length	AMC85868 NZ_GG65 None dna 10901646	<u>8014</u> (Expire 7754.1	es on 04-09 01:1	9 am)	Da	itabase Desc Pi	e Name cription rogran	e nr n Nuc n BLA	leotide co STN 2.9.0	llection (nt))+ ▶ <u>Citation</u>
Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer] Sequences producing significant alignments: Select: All None Selected:0											
Sequer Select:	All None	Selected: 0	nt alignments	s:	trop of regults						
Sequer Select:	All None	sing significa Selected:0 Download →	nt alignments GenBank Q Descrip	s: Graphics Distance	tree of results	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Sequer Select:	All None sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce sproduce spr	significa Selected:0 Download ~	nt alignments GenBank C Descrip TP synthase be	s: Graphics Distance otion . eta chain, mitochondri	tree of results	Max Score 191	Total Score 191	Query Cover 0%	E value 4e-44	Per. Ident 74.12%	Accession XM_001218237.
Select:	nces produce All None s proments approximation of the second pergillus terre pergillus terre	sing significat Selected:0 Download ~ us NiH2624 A us NiH2624 al	nt alignments GenBank O Descrip TP synthase be dehyde dehydr	s: Graphics Distance otion ata chain, mitochondri rogenase (ATEG_050	Iree of results al precursor (ATEG_ 20) partial mRNA	Max Score 191 87.9	Total Score 191 87.9	Query Cover 0% 0%	E value 4e-44 6e-13	Per. Ident 74.12% 77.78%	Accession XM_001218237. XM_001214198.
Select: Alig	All None 3 nments in pergillus terre pergillus terre pergillus terre	Selected:0 Download ~ us NIH2624 A us NIH2624 a us NIH2624 a	nt alignments GenBank (Descrip TP synthase be dehyde dehydr ialate synthase	S: Graphics Distance otion ta chain, mitochondri rogenase (ATEG_050 (ATEG_07126) partia	tree of results al precursor (ATEG_ 20) partial mRNA al mRNA	Max Score 191 87.9 63.9	Total Score 191 87.9 63.9	Query Cover 0% 0%	E value 4e-44 6e-13 1e-05	Per. Ident 74.12% 77.78% 84.38%	Accession XM_001218237. XM_001214198. XM_001209812.

Aspergillus terreus NIH2624 ATP synthase beta chain, mitochondrial precursor (ATEG_09616) partial mRNA Sequence ID: XM_001218237.1 Length: 1242 Number of Matches: 1 Related Information

							Related Information
Range	1: 703 to 1	1200 GenBank G	iraphics	\	Next Match	A Previous Match	Gene - associated gene details
Score	ite(103)	Expect	Identities 378/510(74%)	Gaps 21/510(4%)	Strand Dluc/M	inuc	
1910	105(105)	40 44	576/510(7470)	21/310(470)	Flus/Pl	inus	
Query	3599517	CTTGTACTTCTG	GAGGATCGACTT-CACGCGC	GAGGCGCAG - TCGTAGTG	ATCCTGCG-	3599572	
Sbjct	1200	cttgtactcctg	GAGGGT-GCGTTGCACGCGC	-A-TCGCAGTATCATAGTG	ctcct-ccc	1145	
Query	3599573	AGATG-TAGCGCO	GGGTCCAGGATCCGGGACGT	CGAGTCCAGCGGGTCCACC	GCCGGGTAG	3599631	
Sbjct	1144	CGACGATA-CGT	GGTCCATGAGCCGAGACTT	 CGATCCCAGCGGGTCTACA	 GCAGGGTAT	1086	
Query	3599632	ATGCCCTTCTCC	AGATCGGCCGGGAGAGCAC	сөтөөтсөсөтсөлөөтөс	GCGAAGGTG	3599691	
Sbjct	1085	ATACCCAGCTCA	GAGATACCACGGGACAGTTC	GGTCGTGGCATCCAAGTGG	ATGAAGGTC	1026	
Query	3599692	etcecceeeecc	Genterenterenterenteren	бееечсетнентсесстес	ATCGAGGTG	3599751	
Sbjct	1025	GTTGCGGGGGCA	GGATCAGTCAGATCGTCAGC	 GGGCACGTACACGGCCTGC	ACGGAGGTA	966	
Query	3599752	ATCGAGTGACCG	CGGGTCGAGGTGATGCGCTC	CTGGA-GGATGCCCATCTC	GTCGGCCAG	3599810	
Sbjct	965	ATAGAGCCCTTG	GTGGTCGTGGTGATACGCTC	LIIIIIIIIII CTGCATGGCT-CCCATGTC	GACCGCGAG	907	
Query	359981 1	GTTCGGCTGGTA	SCCCACCGCGGAGGGCATAC	GOCCOAGCAGOGTCGAGAC	CTCGGAACC	3599870	
Sbjct	906	TGTGGGCTGGTA	TCCCACTGCAGAAGGAATCC	 GGCCAAGGAGTGCAGAGAC	TTCGGATCC	847	
Query	3599871	GGCCTGGGTGAA	GCGGAAGATGTTGTCGATGA	ΑGAACAGCACGTCCTG-C-	TTCTGCACA	3599928	
Sbjct	846	AGCCTGGGTGAA	GCGGAAGATGTTGTCGATGA	AAAGCAGGACATCCTGGCC	TTC-GT-CA	789	
Query	3599929	TCGCGGAAGTAC	I CCGCCATGGTCAGACCGGC	CAGGGCCACGCGCAG-ACG	бетессее	3599987	
Sbjct	788	-CG-GAAA-TAT	I II IIIIII III TCAGCAATGGTCAAGCCGGT	 GAGAGCAACGCG-AGCACG	GGCACCAGG	733	
Query	3599988	GGGCTCGTCCAT	TGGCCGAAGACCAGCGC	3600017			
Sbjct	732	AGGCTCATTCAT	TGGCCGAAAACCAGCGC	703			

Aspergillus terreus NIH2624 aldehyde dehydrogenase (ATEG_05020) partial mRNA Sequence ID: $\underline{XM_001214198.1}$ Length: 1494 Number of Matches: 1

sequer		_001214190.1	Length: 1494 Nun	nder of Matches: 1		
Range	1: 1115 to	1257 GenBank	Graphics		🔻 Next Match 🔺 Previous	Match
Score 87.9 b	oits(47)	Expect 6e-13	Identities 112/144(78%)	Gaps) 2/144(1%)	Strand Plus/Plus	<u>Gene</u> - a:
Query Sbjct	9974364 1115	CCGACGGGTACTT CCGAGGGCTACTT	CATCGAGCCGACCGTG	GCTCACCGGGCTCACCAACGA	CACCCGCACCG 9974423 CATGAAGATCG 1174	
Query Sbjct	9974424 1175	CCCAGGAGGAGAT	CTTCGGCCCGGTCATC CTTCGGCCCGGTCGTC	ACCGTCATCGAA - TTCGACG ACCATCCT - GAAGTTCAAGG	ACGTGGCGGAC 9974482 	
Query Sbjct	9974483 1234	GCCCTCGCCCTCG GCCATCAAGATCG	CCAACGACACC 997 GCAACGACACC 125	4506 7		

🖥 Download 🗸	<u>GenBank</u> <u>Graphi</u>	<u>2S</u>			▼ Next ▲ Previous
Aspergillus terr	eus NIH2624 m	alate synthase (ATE	EG_07126) partial r	mRNA	
Range 1: 268 to 3	_001209812.1 Le	ngth: 1587 Number o	f Matches: 1	Next Match 🔺 Previous M	Related Ir
Score 63.9 bits(34)	Expect 1e-05	Identities 54/64(84%)	Gaps 0/64(0%)	Strand Plus/Plus	
Query 2724983 Sbjct 268	GACCGCCGGGTGGAC	ATCACCGGTCCCACCGACC	GCAAGATGACGATCAACG	CGCTCAAC 2725042 	
Query 2725043 Sbjct 328	TCGG 2725046 TCGG 331				
Bownload 🗸	GenBank Graphi	<u>CS</u>			Vext 🔺 Previous
Aspergillus terr	eus NIH2624 ar	ninomethyltransfera	ise, mitochondrial p	precursor (ATEG_00	271) partial mRNA
Range 1: 835 to 8	367 GenBank Graph	ics	V	Next Match 🔺 Previous M	Related Ir
Score 62.1 bits(33)	Expect 3e-05	Identities 33/33(100%)	Gaps 0/33(0%)	Strand Plus/Plus	Gene - assoc

Range	1: 835 to	367 GenBank Grapt	NICS	1	Next Match 🔺 Previous Match	Cono
Score		Expect	Identities	Gaps	Strand	<u>Gene</u> -
62.1 t	oits(33)	3e-05	33/33(100%)	0/33(0%)	Plus/Plus	
Query	3440746	CGCACCGGCTACAC	CGGCGAGGACGGCTTCGAG	3440778		
Sbjct	835	CGCACCGGCTACAC	CGGCGAGGACGGCIICGAG	867		

2. NH08_RS0115770 putative gamma-butyrolactone biosynthesis enzyme

[Streptomyces rimosus subsp. rimosus] Accession No: NZ_JNYR01000009.1

A) Pairwise alignment with M. purpureus YY1 genome: No significance

- B) Pairwise alignment with P. sclerotiorum strain 113 genome: No Significance
- C) Pairwise alignment with A. terreus NIH2624 genome:

Job title: NZ_JNYR0100009:Streptomyces rimosus subsp.... RID AMCDEUGE014 (Expires on 04-09 01:21 am) Query ID NZ_JNYR0100009.1 Database Name nr Description None Description Nucleotide collection (nt) Molecule type dna Program BLASTN 2.9.0+ Citation Query Length 194584 Sequences producing significant alignments: Select: All None Selected:0 Alignments Bownload ٥ tree of res Max Total Query Е Per. Description Accession Score Score Cover value Ident Aspergillus terreus NIH2624 conserved hypothetical protein (ATEG_02223) partial mRNA 89.8 89.8 0% 2e-14 80.15% XM_001211401.1 **Alignments**

Sequence	D: XM_001211401	<u>.1</u> Length: 1800 Number	of Matches: 1	uzzza) partiai mikin	л	
Range 1: 4	20 to 545 GenBank	Graphics		Next Match 🔺 Previous	Match	Related Information
Score	Expec	t Identities	Gaps	Strand		Gene - associated gene detai
89.8 bits	48) 2e-14	105/131(80%)	10/131(7%)	Plus/Plus		
Query 17	160 астаатсатса	CCGCGCCGTGCA-GGGCGTCGG	CGGCGGTGGCCTGATGGTCA	CCG-CG 17217		
Sbjct 42	0 GCTGATCGTCTT	CCGCGCC-TGGACCGGCGTCGG	CGGCGGCGGCCTGATGA	CCGTCG 475		
Query 17	218 ATGGCGCTGATC	G-C-G-GACGTCATCCCGCTGC	GCGAGCGCGGCAAGTACCAG	GGCGCG 17274		
Sbjct 47	6 -CGCAGATGATC	GTCAGCGACGTCGTGCCGCTGC	GCGAGCGCGGCAAGTACCAG	GGGATT 534		
Query 17	275 CTGGGCGCCGT	17285				
shict 53	5 CTGGGCGCCGT	545				

3. AQJ27_RS33045 gamma-butyrolactone biosynthesis enzyme [Streptomyces

olivochromogenes] Accession no: NZ_KQ948465.1

Gene symbol AQJ27_RS33045 Gene description gamma-butyrolactone biosynthesis enzyme Locus tag AQJ27_RS33045 Gene type protein coding Organism Streptomyces olivochromogenes (strain: DSM 40451, culture-collection: DSM:40451) Lineage Bacteria; Actinobacteria; Streptomycetales; Streptomycetaceae; Streptomyces Old locus tag AQJ27_33020 ATGTCGTACACCACCTCCACCCGTGTGTCCGTCGAAGACGACGTCGCGACGGTGGGCACCAGCGCCTCAC GAACCGCCTCACGACCCGCTGCTCGACACGCTGTTCAACCCGCCGTTCAGACCTCCGCCCAGACCTCTGC CCAGGTCTCCGCCCAGGCCTCGGTCCAGGCCTCCGTCCAGACCGCCGCGCAACTCTGACGCCGCGGCTG ACCACGACGGTTCCGCGCGAGTACGTCCATCGCGCCGCCGTCTCGGAGGTGTTGCTGACCGGCTGGGAGG CCGCCGCCGAACCAGCCGGACCCGACCCGGACGAATTCGCGGTCAGCGCCCAATGGCCGCGCAGCCACTC CTGCTCGCGCATGCGGAGTTCGGCGTCCCCTTCGGGCACCAGTTCCTGATGTGGGACATGTTCTTCAGCA CCTCCCCCGAACTGCTCGTCGCGGACGCCGTCCCCACGGAGGTCGAACTGCGCACGGTCTGCCGCGACAT CGTCCGCAGGGGCCGGGTGCTCGGCGGTATGCGCTACGACGTCACCGTGCTGCGCGACGGAAGGGCGCTG CCACCAGCGACCGCGTCGTCGCCGACCGCGATCGACCCGGCCGTGGGGCCACTCCGACGACCGGCACGT GCTGCTCGCCGAGCCCGGATCGGACTCCGGCACCGGCGACCGCTGGGAACTGCGCGTCGACACCGCCCAC CCCACTTTCTTCGACCACCCCGTCGATCACATACCAGGCATGGTCCTGCTGGAGGCCGCCCGGCAGGCCG CCCTCGTGTCGACCGGGATGCCGGACGCCCTCCTGGTGGGTCTCAAGAGCAACTTCGCGCGCTACGCCGA

GTTCGACGCGCCCTGCTGGATCGAGCCGAGGCCGAACCGCACGGTACGGAGGGCGGCGTACTGGTGCGG GTGCGCGGTACGCAGCACGCTGAGACCGTCTTCACCGCCGAGCTCGTCTTGAGCCCGCGCGACCGCTGA

A) Pairwise alignment with *M. purpureus* YY1 genome: No significance

B) Pairwise alignment with P. sclerotiorum strain 113 genome: No Significance

C) Pairwise alignment with A. terreus NIH2624 genome:

Job title: NZ_KQ948465:Streptomyces olivochromogenes...

RID	AMCM6XW3014 (Expires on 04-09 01:25 am)		
Query ID	NZ_KQ948465.1	Database Name	wgs (WGS_VDB://AAJN01)
Description	None	Description	▶ See details
Molecule type	dna	Program	BLASTN 2.9.0+ Citation
Query Length	303545		

Other reports:
Search Summary [Distance tree of results] [MSA viewer]

Sequences producing significant alignments:

11	Alignments 📳 Download 👻 GenBank Graphics Distance tree of results						0
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	Aspergillus terreus NIH2624 cont1.170, whole genome shotgun sequence	427	427	0%	3e-115	75.62%	AAJN01000170.1
	Aspergillus terreus NIH2624 cont1.10, whole genome shotgun sequence	156	156	0%	1e-33	81.25%	AAJN01000010.1
	Aspergillus terreus NIH2624 cont1.177, whole genome shotgun sequence	65.8	65.8	0%	3e-06	83.82%	AAJN01000177.1
	Aspergillus terreus NIH2624 cont1.4, whole genome shotgun sequence	56.5	56.5	0%	0.002	96.97%	AAJN01000004.1

Range	1: 85597 t	o 85788 GenBank	Graphics		Next Match 🔺 Previous Match
Score 156 bi	its(84)	Expect 1e-33	Identities 156/192(81%)	Gaps 0/192(0%)	Strand Plus/Plus
Query Sbjct	3716154 85597	ATCGACAAGGCTC ATCGACAAGGCTC	CTGAGGAGCGCCAGCGCGGT CTGAAGAGCGTAAGCGTGGT	ATCACCATCTCCATCGCG	CACGTCGAG 3716213 CACATCGAG 85656
Query	3716214 85657	TACCAGACGGAGA	ACCGTCACTACGCCCACGTC ACCGTCACTACGCCCACGTC	GACTGCCCCGGTCACGCG	GACTACATC 3716273 GATTACATC 85716
uery bjct	3716274 85717	AAGAACATGATCA AAGAACATGATTA	CGGGTGCGGCGCAGATGGAC CTGGTGCCGCCAACATGGAT	GGCGCCATCCTCGTGGTC GGTGCTATTGTTGTCGTT	GCCGCGACC 3716333 GCCGCTTCC 85776
uery bict	3716334 85777		3716345		
Dowr Sequen	nload v (gillus terr nce ID: AAJ	GenBank Graph eus NIH2624 (IN01000177.1	hics cont1.177, whole ge Length: 382630 Numb	nome shotgun seq er of Matches: 1	uence
Dowr JDowr Jange Score	nload v (gillus terr nce ID: AAJ 1: 324909	GenBank Graph eus NIH2624 (IN01000177.1 1 to 324976 GenB: Expect 3e-06	hics cont1.177, whole ge Length: 382630 Numbr ank Graphics Identities 57/68(84%)	nome shotgun seq er of Matches: 1 Gaps 0/68(0%)	Uence
Down Asper Gequen Lange Score 65.8 b Juery bjct Juery	nload v (gillus terr nce ID: AAJ 1: 324909 Dits(35) 727860 324976 727920 324916	GenBank Graph eus NIH2624 (comparing the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second s	hics cont1.177, whole ge Length: 382630 Numbr ank Graphics Identities 57/68(84%) AGATCACCGGTCCCACCGAC IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	nome shotgun seq er of Matches: 1 Gaps 0/68(0%) CGCAAGATGACCATCAAC CGCAAGATGGTTGTCAAC	Uence Next Match Previous Match Strand Plus/Minus GCGCTCAA 727919 GCTTTGAA 324917
Dowr Aspersteequen Bange Score 55.8 b bjct bjct bjct	nload v (gillus terr nce ID: AA, 1: 324909 oits(35) 727860 324976 727920 324916 nload v (gillus terr	GenBank Grapi eus NIH2624 (IN01000177.1 1 to 324976 GenBa Expect 3e-06 cGACCGCGGGGGG CGACCGCGGGGGGG CTCGGGCG 7279 IIIIIIII CTCGGACG 3249 GenBank Grapi eus NIH2624 (hics cont1.177, whole ge Length: 382630 Numbrank Graphics Identities 57/68(84%) AGATCACCGGTCCCACCGAC AGATCACCGGTCCCACCGAC 27 09 hics cont1.4, whole geno	nome shotgun seq er of Matches: 1 Gaps 0/68(0%) CGCAAGATGACCATCAAC IIIIIIII CGCAAGATGGTTGTCAAC	Uence Vext Match Previous Match Strand Plus/Minus GCGCTCAA 727919 GCTTTGAA 324917
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Aspergillus terreus NIH2624 cont1.170, whole genome shotgun sequence Sequence ID: AAJN01000170.1 Length: 900750 Number of Matches: 1

Range	1: 875172	to 876082 GenBan	Graphics	V Ne	ext Match	🔺 Previous Ma
Score 127 b	its(231)	Expect 3e-115	Identities 698/923(76%)	Gaps 33/923(3%)	Strai Plus,	nd /Plus
Query	4404525	GGTGCAGGCCACTGC			CTCGTA	4404584
Sbjct	8/51/2	ĠĠĊĠĊĂĊĠĊĊĂĊĊĠĊ	ĊĠŀĠĂĊĊĂĠĊŀŀĠĠĊĂĊĊĠ	h chí cácá a accác com	TİĊĠ-G	875230
Query	4404585	CGAACGC-CCCACCA	TGAAGCCGGAGATGTTCTG		сесест	4404643
Sbjct	875231	ĊĠŦĊĠĠĊĠĊĊĠĂĊĊĂ	TGAAGCCGGAGATGTTCTG	CAGGAAGACTAGGGGGGATTI	тасаст	875290
Query	4404644	GGTCGCACAGCTCGA	TGAAGTGGGCGCCCTTCTG	GGCGGATTCGGAGAACAGGA	тессет	4404703
Sbjct	875291	GGGCGCAGAGCTCAA	TAAAGTGTGCGCCCTTCAG	GGAGGACTCGGAGAAGAGAG	ATGCCGT	875350
Query	4404704	TGTTGGCGACGATGC	CGACCGGATGGCCGTGGAG	ATGGGCGAAGCCGGTGAC	CAGCGT	4404761
Sbjct	875351	TGTTGGCAACGATGC	CCACCTGGTGCCCGAAG	ATGCGCGCAAAGCCTGTCAC	CAGCGT	875408
Query	4404762		CCTTGAACTCCGCGAAG-C	GCGAACCGTCCACGAGGCGG	iGCGATC	4404819
Sbjct	875409	GGAGCCGTAGTCGCG	-CTTGAACTCGGCGAACTC	GCTG-CCGTCGACGATGCGG	GCGATG	875466
Query	4404820	ACCTCGCGCACGTC-	ATAGGGCGTACGGGAGTCC	GCGGGCACCACGCCGTAGAG	SCCCGGC	4404878
Sbjct	875467	ACTTCGTGCACGGGG	ATCTGTCGG CGGAGGTTG	GTGCCCACGATGCCGTTGAG	TTCGTT	875525
Query	4404879	CGGATCGGCCTTGGG	CTCCTCGACGGT CCGC-	-ACCG-TCCA-CGGCA-GC-	GG-CG-	4404928
Sbjct	875526	CGGGTCGTAGAGCGG	CTCTTTGATGGTGTCCGCG	GAGAGCTGCAGCGGCACGC1	GGTCTT	875585
Query	4404929	сесестсесселее	TGGAGACG-ATGGTGCGCA	ссабесесаетесатебесе	тсетсс	4404987
Sbjct	875586	CGGG-TAGTTGAGGT		L I I I I I I I I I I I I I CGAGGA LAA I GGCG I G I GCC		875643
Query	4404988	TCGGCGAGATGGTCG			AGCTCC	4405047
Sbjct	875644	ACGGCCAGATAGTCC	GTCACGCCGGAGATGGTGC	төтөсаөстөөссөссөссө	GAGGTCC	875703
Query	4405048				GAAGAT	4405106
Sbjct	875704	TCGGCGGAGACCTCT	TCGCCGGTAGCTGCCTTGA	CGAGCGGCGGGGCCCGCA-AG	GAAGAT	875762
Query	4405107	CGTGCCCTGGTTGCG	GACGATCACGGCCTCGTCG		GCCGCC	4405166
Sbjct	875763	GGTGCCCTGGTTCTC	GACGATGATGGTCTCGTCG	CTCATGGCGGGGGGGCGTAGGC	сстсс	875822
Query	4405167	CGCCGTGCAGGAACC	GAGGACGGCGGCGATCTGC	GGGATACCGGCGCCGGA	CATCCG	4405223
Sbjct	875823		CATGACGACGGAGATCTGG	GGTATGCCGA-GGGAACT	CATCCG	875879
Query	4405224	CGCCTGGTTGTAGAA	GATCCGCCCGAAGTGCTCC	CGGTCGGGGGAAGACCTCGTC	CTGCAT	4405283
Sbjct	875880	CGCCTGGTTGAAGAA	TATACGGCCGAAGTGTTCC	TTGTCCGGGAAGACGTCGG	стосто	875939
Query	4405284	CGGCAGGAAGGCGCC	GCCGGAGTCCACGAGATAC	AGACAGGGCAGCCGGTTCTC	CAGGGC	4405343
sbjet	875940	GGGGAGGTTTGCGCC	GCCGGAGTCGACGAGGTAG	AGACAAGGGAGC⊤TGTTCTC	TTGGGC	875999
Query	4405344	GATCTCCTGCGCCCG	CAGATGCTTCTTGACCGTC	ATCGGGTAGTACGTACCGCC	CTTGAC	4405403
Sbjct	876000		CAGGTGCTTCTTCACCGTG	ATCGGGTAGTACGTGCCACC	CTTGAC	876059
Query	1105101	CGTGGCGTCATTGGC	GACGATCA 1105126			
Sbjct	876060	GGTACTGTCGTTGGC	CACAATCA 876082			

4. SU9_RS04625 gamma-butyrolactone biosynthesis enzyme [Streptomyces auratus

AGR0001] Accession no: NZ_JH725387.1

 Gene symbol
 SU9_RS04625

 Gene description
 gamma-butyrolactone biosynthesis enzyme

 Locus tag
 SU9_RS04625

 Gene type
 protein coding

 Organism
 Streptomyces auratus AGR0001 (strain: AGR0001)

 Lineage
 Bacteria; Actinobacteria; Streptomycetales; Streptomycetaceae; Streptomyces

 Old locus tag
 SU9_04731

A) Pairwise alignment with *M. purpureus* YY1 genome: No significance.

B) Pairwise alignment with A. terreus NIH2624 genome:

Job title: NZ_KQ	948465:Streptomyces olivochromogenes		
RID	AMCM6XW3014 (Expires on 04-09 01:25 am)		
Query ID	NZ KQ948465.1	Database Name	wgs (WGS VDB://AAJN01)
Description	None	Description	See details
Molecule type	dna	Program	BLASTN 2.9.0+ Citation
Query Length	303545		

Sec Sele	Sequences producing significant alignments: Select: <u>All None</u> Selected:0									
AT AT	Alignments EDownload 👻 GenBank Graphics Distance tree of results						0			
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession			
	Aspergillus terreus NIH2624 cont1.19, whole genome shotgun sequence	54.7	54.7	0%	3e-04	96.88%	AAJN01000019.1			

Bownload - GenBank Graphics

Aspergillus terreus NIH2624 cont1.19, whole genome shotgun sequence Sequence ID: <u>AAJN01000019.1</u> Length: 312518 Number of Matches: 1

Range	1: 21211	4 to 212145 GenBank	Graphics	V	Next Match 🔺 Previo	ous Match
Score 54.7 b	oits(29)	Expect 3e-04	Identities 31/32(97%)	Gaps 0/32(0%)	Strand Plus/Plus	
Query	144925	GTCACCAGGATGTCGTCC	TCGATGCGCACGCC	144956		
Sbjct	212114	GTGACCAGGATGTCGTCC	TCGATGCGCACGCC	212145		

C) Pairwise alignment with *P. sclerotiorum* strain 113 genome:

RID Query ID Description Molecule type Query Length	AJ4VNGP5114 (Expires on 04-08 05:00 am) <u>KV784220.1</u> Penicillium sclerotiorum strain 113 unplaced genomic scaffold scaffold1 dna 4666273	Sub Desc Molecu Subject Pr	ject ID ription le type Length ogram	Non Non dna 553 BLA	<u>JH725</u> e 0280 STN 2	.9.0+ Þ	Citation
Other reports: Þ	Search Summary [MSA viewer]						
Other reports: D	Search Summary [MSA viewer] g significant alignments:						
Other reports: D Sequences producin Select: <u>All None</u> Se	Search Summary [MSA viewer] g significant alignments: lected:0						
Other reports: D Bequences producin Select: <u>All None</u> Se Ali Alignments D	Search Summary [MSA viewer] g significant alignments: lected:0 wnload & GenBank Graphics						
Other reports: Description of the sequences producing select: All None Set Alignments and the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second	Search Summary [MSA viewer] g significant alignments: lected:0 wmload <u>v GenBank Graphics</u> Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession

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 $\label{eq:sequences} \begin{array}{l} \text{Streptomyces auratus AGR0001 Scaffold1, whole genome shotgun sequence } \\ \text{Sequence ID: } \underline{\text{NZ JH725387.1}} \quad \text{Length: 5530280 } \\ \text{Number of Matches: 2} \end{array}$

Range 1: 5275770 to 5276141 GenBank Graphics Vext Match 🗼 Previous Match									
Score 117 bits	s(63)	Expect 1e 22	Identities 275/378(73%)	Gaps 12/378(3%)	Str: Plu	and s/Plus		
Query	1820562				GG-TAGCCAC			GTCA	1820620
Sbjct	5275770	CTCAAGO	GCGAGGACCTCA	ACCACACO	CGGCTCG-CAC	AAGATCAAG	CAACGTGCTGC	GCCA	5275828
Query	1820621	GATCCTT	атсоссалосот	CTGGGCAA	AGACCCGCATC		SACTGGTGCTG	GCCA	1820680
Sbjct	5275829	GGCGCTG		ATGGGCAA	AGACCCGGGTC	ATCGCCGAG	GACCGGCGCCG	GCCA	5275888
Query	182068 <mark>1</mark>	GCACGGT	GTCGCTACTGCT		GTGCCAAGTTT		GTGCACTGTCT	TCAT	1820740
Sbjct	5275889	GCACGGC	GTCGCCACCGCC		SCGCCCTCTTC	GGCCTCGA	CTGCACCATCT		5275948
Query	1820741	GGGTGCC	GAG-GATGTTCG		GCTCTCAACGT	CTTCCGCA	GAAGCTTCTG	GGCG	1820799
Sbjct	5275949	GGGCGA-	GATCGACACCCA	GCGGCAG	GCCCTCAACGT	CGCCCGGA	GCGGATGCTC	GGCG	5276007
Query	1820800	CCGAGGT	төтсөссөтсөА	тө-стөөс	AGCCGCACCC	TTCGTGAT	GCAGTCAACGA	GGCC	1820858
Sbjct	5276008	CCGAGGT	CATCGCCGT-GA	AGTCCGG			GCCATCAACGA		5276066
Query	1820859	CTCCGCT	сстеееттасте	ATCTCGAC	ассастсаст	ΑςΑτρατρ	GG-ATC-T-GC	CATT	1820915
Sbjct	5276067	TTCCGCG		ACGTCGAC			GGACCGTCGC	C	5276123
Query	1820916	бетссто	Αςςςςττςςςς	1820933	3				
Sbjct	5276124			5276141	L				

Range 2	: 5288415 to	5288494 GenE	ank Graphics	🔻 Next Match	🔻 Next Match 🔺 Previous Match 🥻 First Match		
Score 76.8 bits(41)		Expect	Identities	Gaps	Strand		
		2e-10	68/81(84%)	2/81(2%)	Plus/Minus		
Query	4583661	CCGGTCATG	TACTCACAGCCGT				4583720
Sbjct	5288494	CCGGTCATG	TACTCACAGCCGT	GGTCACCGACGCCCT	CGGAGACCACCGTGG	CACCGGAG	5288435
Query	4583721	TTACGCACA	GC-GAAACGCTC	4583740			
Sbjct	5288434	TTGCGCAC-	GCAGAAGCGCTC	5288415			





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