Reduction of trimethylamine N-oxide to trimethylamine by the human gut microbiota: supporting evidence for 'metabolic retroversion'

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Reduction of trimethylamine *N*-oxide to trimethylamine by the human gut microbiota: supporting evidence for '*metabolic retroversion*'

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Introduction

Dietary sources of methylamines such as choline, trimethylamine (TMA), trimethylamine *N*-oxide (TMAO), phosphatidylcholine (PC) and carnitine are present in a number of foodstuffs, including meat, fish, nuts and eggs. It is recognized that the gut microbiota is able to convert choline to TMA in a fermentation-like process (Fig. 1).[1] Similarly, PC and carnitine are converted to TMA by the gut microbiota. It has been suggested that TMAO is subject to '*metabolic retroversion*' in the gut (i.e. it is reduced to TMA by the gut microbiota, with this TMA being oxidized to produce TMAO in the liver).[2] However, to date the role of the gut microbiota in TMAO degradation has not been investigated.

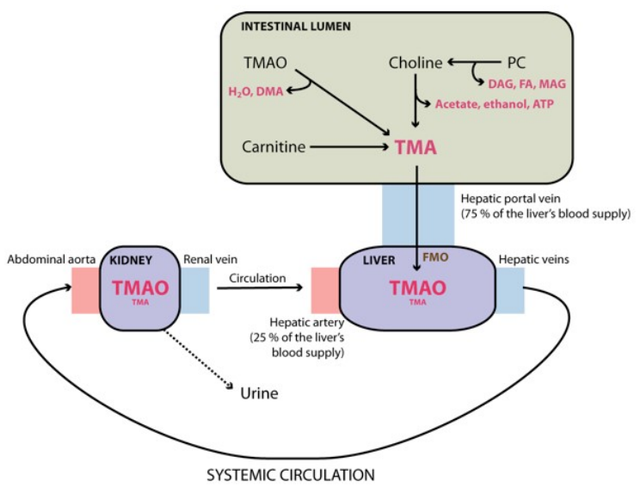

Fig. 1. Proposed methylamines' pathway, an example of the microbial–mammalian co-metabolic axis. TMA is derived from microbial degradation of dietary methylamines such as TMAO, choline, PC and carnitine in the intestinal lumen. TMA is absorbed by the host to be *N*-oxidized into TMAO by hepatic flavin mono-oxygenases (FMO) and demethylated into dimethylamine (DMA) and monomethylamine (MMA) by cytochrome P450s (CYP) in the liver during first-pass metabolism. TMAO, and a trace amount of unconverted TMA, can be readily detected in human blood and urine by NMR. DAG, diacylglycerols; FA, fatty acids; MAG, monoacylglycerols.

Methods

Screening of bacteria for ability to reduce TMAO. Sixty-six strains of human faecal and caecal bacteria (in-house collection) were screened anaerobically for their ability to utilize TMAO using liquid minimal media with and without 1% (w/v) TMAO. Metabolites in spent media were profiled by Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy.[3]

In vitro fermentation systems. Anaerobic, stirred, pH-controlled, batch culture fermentation systems were performed using minimal broth with and without 1% (w/v) TMAO and inoculated with faecal homogenates prepared from freshly voided stool samples (three healthy human; one male, two females; age range 20–31). Samples were taken at 0, 1, 2, 3, 4, 5, 6 and 9 h for microbiological (fluorescence *in situ* hybridization; FISH) and metabolite (^{1}H NMR) profiling.

Table 1. Gut bacteria screened for their ability to utilize TMAO

Genus	Species included	No. of strains	Source
Actinomyces	*odontolyticus*, *viscosus*	2	Caecum
Bacteroides	*fragilis*, *vulgatus*	3	Caecum/faeces
Bifidobacterium	*adolescentis*, *animalis* subsp. *lactis*, *bifidum*, *breve*, *dentium*, *gallicum*, *longum*, *longum* subsp. *infantis*, *longum* subsp. *longum*, *pseudocatenulatum*, unknown	17	Caecum/faeces/ intestine
Citrobacer	*gillenii*, *koseri*	2	Caecum
Clostridium	*bifermentans*, *innocuum*, *ramosum*, *paraputrificum*, *perfringens*, *sporogenes*	6	Caecum/faeces
Enterococcus	*faecalis*, *faecium*, *gallinarum*	5	Faeces
Escherichia	*coli*	16	Caecum/faeces
Fusobacterium	*ulcerans*	1	Caecum
Hafnia	*paralvei*	1	Caecum
Klebsiella	*pneumoniae* subsp. *pneumoniae*	1	Caecum
Lactobacillus	*fermentum*, *rhamnosus*	2	Caecum
Parabacteroides	*johnsonii*	1	Caecum
Pseudomonas	*aeruginosa*	1	Caecum
Staphylococcus	*hominis*	1	Faeces
Streptococcus	*anginosus*, *gallolyticus*, *oralis*, *sanguinis*, unknown, *vestibularis*	7	Caecum/faeces

Results

Screening cultures for TMAO reductase activity. The presence of TMAO in media increased the growth rate of *Enterobacteriaceae*; while it did not affect the growth rate of lactic acid bacteria, TMAO increased the biomass of these bacteria (Fig. 2).

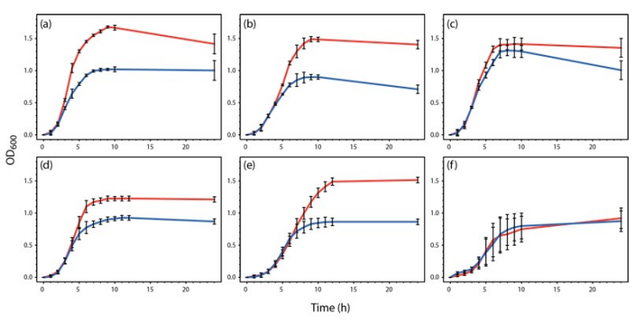

Fig. 2. Representative growth curves for strains grown with (red) or without (blue) TMAO. (a) *Escherichia coli* D1(2); (b) *Citrobacter gillenii* L26-FAA1; (c) *Klebsiella pneumoniae* L26-FAA1; (d) *Enterococcus gallinarum* D6(5); (e) *Streptococcus anginosus* D5(12); (f) *Clostridium perfringens* L20-BSM1. Data are shown as mean ± SD (*n* = 3).

Enterobacteriaceae produced the greatest amount of TMA from TMAO (38.79 ± 11.08 mM [14.92–53.91 mM]; *n*=20). Members of other families of bacteria produced low levels of TMA from TMAO (0.02–4.52 mM). Caecal/small-intestinal isolates of *Escherichia coli* produced more TMA from TMAO than their faecal counterparts (Fig. 3). Lactic acid bacteria (LAB) did not convert TMAO to TMA, but their production of lactate was greatly increased when they were grown in the presence of TMAO (Fig. 4).

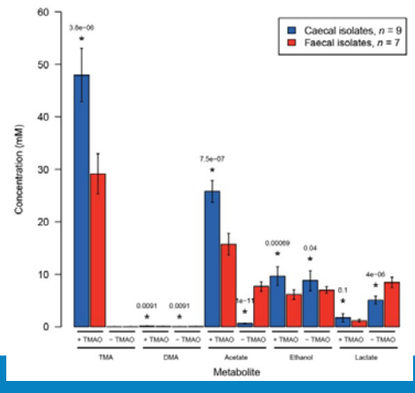

Fig. 3. Caecal *Escherichia coli* produce more TMA from TMAO than faecal isolates. Significant adjusted *P* values (Benjamini–Hochberg[4]) shown above bars in the graph indicate the caecal isolates were significantly different from faecal isolates for a particular metabolite.

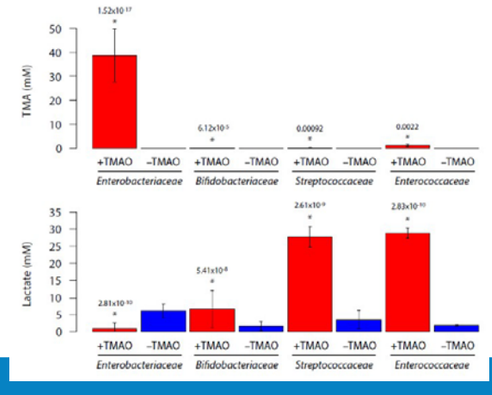

Fig. 4. Increased lactic acid production by LAB in the presence of TMAO. *Enterobacteriaceae*, *n* = 20; *Bifidobacteriaceae*, *n* = 17; *Streptococcaceae*, *n* = 7; *Enterococcaceae*, *n* = 5. *, Significantly different from its negative control (adjusted *P* values shown).

Effect of TMAO on gut bacteria within a mixed system. Abundance of *Enterobacteriaceae* (probe Ent) significantly increased in the presence of TMAO (Fig. 5). Huge variability was seen in the amounts of TMA and DMA produced, though levels of both steadily increased throughout the 9 h fermentation. No significant differences were seen between the lactate and acetate levels of TMAO and control systems (possibly due to cross-feeding).

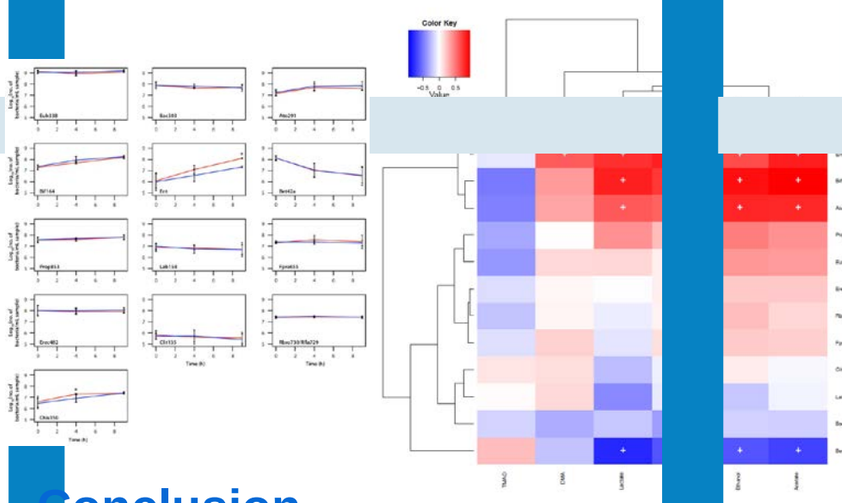

Fig 5. (L) Growth of *Enterobacteriaceae* (Ent) is promoted in the presence of TMAO. Red lines, TMAO-containing systems; blue lines, negative controls. Data are shown as mean ± SD (*n* = 3). *, Significantly different (*P* < 0.05) from the control at the same time point. (R) Bidirectional clustering of correlation matrix of FISH data and data for the six metabolites found in highest amounts in the NMR spectra from the batch-culture samples. +, Significant (*P* < 0.05).

Conclusion

Enterobacteriaceae made the greatest contribution to the conversion of TMAO to TMA, both in pure culture and in a mixed microbiota. This work clearly demonstrates different metabolic activity of strains of the same bacterial species from different gut niches.

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