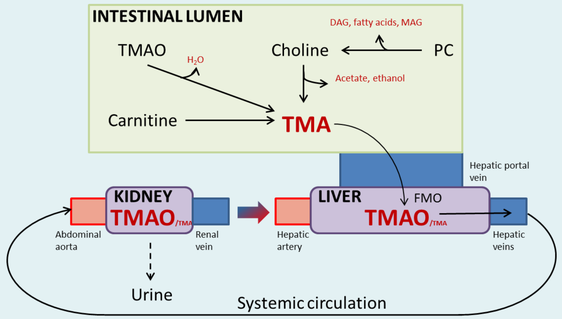
Bioinformatic exploration of trimethylamine *N*-oxide metabolism in human gut bacteria

Samuel Dawson and Lesley Hoyles
Antimicrobial Resistance, Omics & Microbiota Research Group, Nottingham Trent University

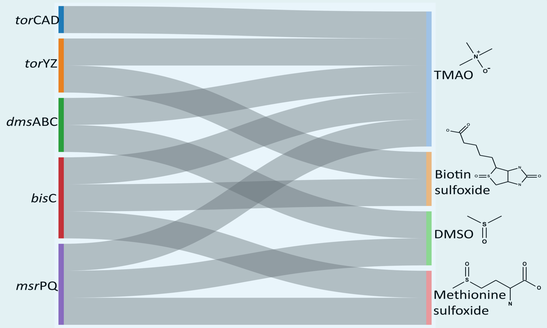
Introduction

- Trimethylamine *N*-oxide (TMAO) is an osmolyte found in fish, and in other foods such as red meat and eggs
- Different groups of gut bacteria can reduce TMAO, leading to the production of trimethylamine (TMA)[1]
- This can affect levels of TMAO in the human body
- Important as varying levels of TMAO potentially have both positive and negative effects on human health
- Previous work[2] has examined the prevalence of TMAO metabolism across different genera of gut bacteria, highlighting the TMAO reductase TorA as of relevance to *Escherichia coli* and *Klebsiella* spp. in particular, but it may have missed several key metabolic pathways
- This work aims to show the diversity of TMAO metabolism pathways among the human gut microbiota, as well as showing that the ability to reduce TMAO to TMA is limited to members of the family *Enterobacteriaceae*



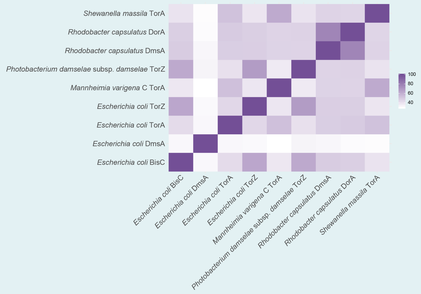
Metabolic pathways associated with TMAO metabolism

- An extensive survey of the literature was undertaken to identify all known pathways associated with TMAO metabolism
- *tor*CAD[3] is the most studied pathway with regard to TMAO metabolism
- *tor*YZ[4] is similar to *tor*CAD but is constitutively expressed
- *dms*ABC[5] may play a bigger role in TMAO metabolism than previously thought
- MsrP[6] has been shown to exhibit reductase activity on TMAO
- While BisC has not been shown to reduce TMAO its similarity to TorZ suggests that it may be able to
- All of these pathways should be considered when examining TMAO metabolism, not just *tor*CAD
- The literature suggests these pathways are mostly associated with *Enterobacteriaceae*

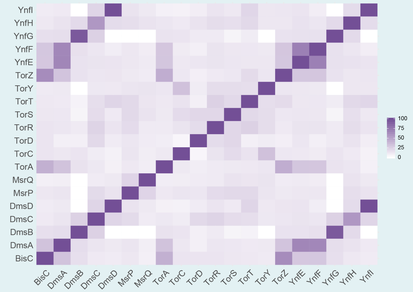


Alignments of TMAO protein sequences

- Previous work[2] was based on generating a single consensus sequence (referred to as TorA) from a range of bacteria, mostly of marine origin, whose TMAO metabolism genes share little homology with those of the gut bacterium *E. coli*

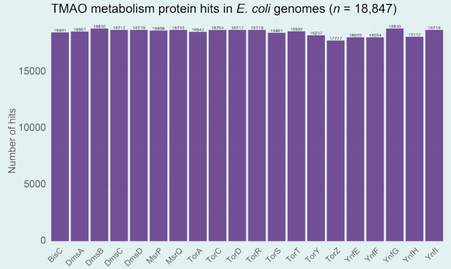


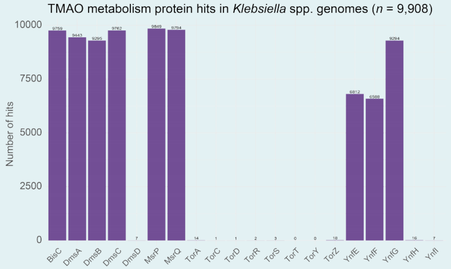
- Comparison of representative *E. coli* sequences for all TMAO pathway proteins identified above shows *Enterobacteriaceae* TMAO metabolism genes share little homology, with the exception of known (YnfE/F vs DmsA, YnfG vs DmsB, YnfH vs DmsC) or potential (TorZ vs BisC) homologues



Prevalence of TMAO metabolism proteins in *E. coli* and *Klebsiella* spp. genomes

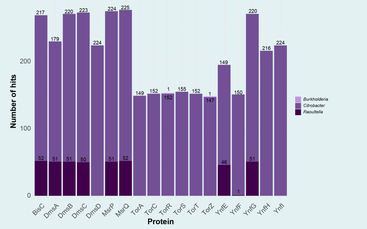
- Protein sequences encoded in RefSeq genomes of *E. coli* and *Klebsiella* spp. were screened (BLASTp) against the database of *Enterobacteriaceae* TMAO metabolism proteins
- As expected, all pathway genes were found in *E. coli* genomes but few *tor* genes were detected in *Klebsiella* spp.





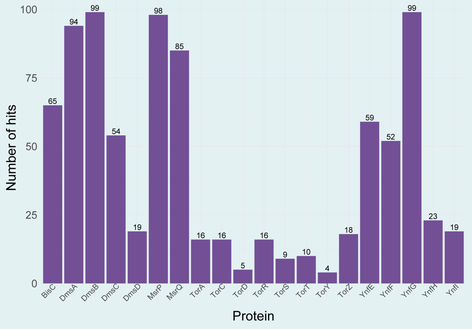
Prevalence of TMAO metabolism proteins in other Proteobacteria

- Protein sequences encoded in other RefSeq proteobacteria genomes were screened (BLASTp) against the database of *Enterobacteriaceae* TMAO metabolism proteins
- Genera screened were picked based on previous work [2]
- Only *Citrobacter* and *Raoultella* spp. (both *Enterobacteriaceae*) were found to encode TMAO metabolism proteins
- A small number of *Burkholderia* genomes also encoded TMAO metabolism proteins



Extending the search for TMAO metabolism proteins in human gut bacteria

- 4,644 human gut bacteria reference genomes and metagenome-assembled genomes[6] were screened vs the TMAO protein database
- 59% of the 4,644 hits were from a wide range of *Enterobacteriaceae* genera, including but not limited to *Citrobacter*, *Enterobacter*, *Escherichia* and *Proteus* spp.
- Still very few *tor* hits but *dms*A and *msr*P appear in some clostridia (*Clostridium* spp., *Anaerococcus prevotii*, *Blautia hansenii*) and lactic acid bacteria (*Enterococcus* spp., *Streptococcus* spp. and *Vagococcus teuberi*)
- Work is still being done to confirm how many of these hits correlate with full operons, providing further support that they are functional in the human gut



Summary

- Work presented here suggests that *tor*CAD may not be the most prevalent TMAO metabolism pathway present in the human gut in individuals whose *Enterobacteriaceae* populations are predominated by *Klebsiella* spp.
- Preliminary work done with intestinal isolates of *Klebsiella* spp. has confirmed these bacteria do not encode TorA and associated proteins
- It has also shown that *Klebsiella* spp. encode BisC, which may be capable of converting TMAO to TMA via an unknown mechanism
- Future work will focus on characterizing the microbial pathways that make the greatest contributions to conversion of TMAO to TMA in the human gut

1. Hoyles et al. (2018) *Microbiome* 6:73.
2. Jameson et al. (2016) *Microb Genom* 2:e000080.
3. Mejéan et al. (1994) *Mol Microbiol* 11:1169-1179.
4. Bilous et al. (1988) *Mol Microbiol* 2:785-795.
5. Loschi et al. (2004) *J Biol Chem* 279:50391-50400.
6. Almeida et al. (2021) *Nat Biotechnol* 39:105-114.

Contact
N0629512@my.ntu.ac.uk