**Supplementary Material**

# *Ex vivo* study on prebiotic & choline combination to modulate gut bacteria, enhance choline bioavailability, and reduce TMA production.

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## Section S1: Tables

| Subject | Gender | Age | Race | BMI Calculation | Have you suffered from any severe gastrointestinal |
|---|---|---|---|---|---|

| | | | | | **diseases in the past 1 year?** |
|---|---|---|---|---|---|
| AEM01 | Male | 69 | Chinese | 23.18 | No |
| AEM02 | Male | 68 | Chinese | 22.84 | No |
| AEM03 | Male | 66 | Chinese | 22.27 | No |
| AEM04 | Male | 72 | Chinese | 23.80 | No |
| AEF01 | Female | 71 | Chinese | 19.40 | No |
| AEF02 | Female | 65 | Chinese | 26.52 | No |
| AHF01 | Female | 55 | Chinese | 22.51 | No |
| AHM01 | Male | 47 | Indian | 28.58 | No |
| AHF02 | Female | 25 | Indian | 22.27 | No |
| AHM02 | Male | 32 | Chinese | 27.44 | No |
| AHF03 | Female | 56 | Chinese | 22.68 | No |
| AEM05 | Male | 70 | Chinese | 22.84 | No |
| AHM03 | Male | 40 | Chinese | 25.47 | No |
| AHF05 | Female | 41 | Indian | 24.89 | No |
| AEF03 | Female | 61 | Chinese | 20.20 | No |
| AEM06 | Male | 69 | Chinese | 24.39 | No |
| AHF04 | Female | 45 | Chinese | 19.31 | No |
| AEM07 | Male | 69 | Chinese | 24.22 | No |
| AHM04 | Male | 49 | Chinese | 26.29 | No |
| AHF06 | Female | 55 | Chinese | 18.61 | No |
| AEM08 | Male | 72 | Indian | 25.96 | No |
| AEM09 | Male | 69 | Chinese | 26.56 | No |
| AHM05 | Male | 50 | Chinese | 24.39 | No |
| AHF07 | Female | 55 | Chinese | 0.00 | No |
| AEF04 | Female | 69 | Chinese | 23.94 | No |
| AHM06 | Male | 54 | Chinese | 28.31 | No |
| AHM07 | Male | 50 | Chinese | 24.86 | No |
| AEM10 | Male | 67 | Chinese | 19.49 | No |

**Table S1.** Demographics of participants involved in the various analysis conducted in the study.

| **Pathway ID** | **Pathway Name** |
|---|---|
| PWY-6121 | 5-aminoimidazole ribonucleotide biosynthesis I |
| PWY-7851 | coenzyme A biosynthesis II (eukaryotic) |
| COA-PWY-1 | coenzyme A biosynthesis I (bacteria) |
| PWY-6163 | chorismate biosynthesis from 3-dehydroquinate |
| PWY-7197 | pyrimidine deoxyribonucleotide phosphorylation |
| SER-GLYSYN-PWY | superpathway of L-serine and glycine biosynthesis I |
| ARO-PWY | chorismate biosynthesis I |
| HISTSYN-PWY | L-histidine biosynthesis |
| COMPLETE-ARO-PWY | Super pathway of aromatic amino acid biosynthesis |
| GLYCOGENSYNTH-PWY | glycogen biosynthesis I (from ADP-D-Glucose) |
| PWY-6609 | adenine and adenosine salvage III |
| PWY-6703 | preQ0 biosynthesis |
| PWY-7221 | guanosine ribonucleotides de novo biosynthesis |
| PWY7222 | guanosine deoxyribonucleotides de novo |
| TRPSYN-PWY | L-tryptophan biosynthesis |
| GLUCUROCAT-PWY | superpathway of hexuronide and hexuronate ... |
| PWY-7977 | L-methionine biosynthesis IV |
| COA-PWY | coenzyme A biosynthesis I (bacteria) |
| PWY-5695 | inosine 5'-phosphate degradation |
| COBALSYN-PWY | Super pathway of adenosyl cobalamin salvage |
| NONMEVIPP-PWY | methylerythritol phosphate pathway I |
| PWY-7111 | pyruvate fermentation to isobutanol (engineered) |
| NONOXIPENT-PWY | pentose phosphate pathway (non-oxidative ) |
| PWY-5188 | uroporphyrinogen-III I (from glutamate) |
| PWY-8178 | pentose phosphate pathway (non-oxidative branch) |
| PWY-6892 | thiazole biosynthesis I (facultative anaerobic bacteria) |
| SERSYN-PWY | L-serine biosynthesis |
| PWY-6353 | purine nucleotides degradation II (aerobic) |
| PWY-6700 | queuosine biosynthesis |

| | |
|---|---|
| PWY-6124 | inosine-5'-phosphate biosynthesis II |
| PWY0-1061 | superpathway of L-alanine biosynthesis |
| GLUTORN-PWY | L-ornithine biosynthesis I |
| PWY-7560 | methylerythritol phosphate pathway II |
| CALVIN-PWY | Calvin-Benson-Bassham cycle |

**Table S2.** Table shows the pathway terms and its full annotation. HUMAnN pipeline was used with MetaCyc database was used for pathway analysis.

## Section S2: Figures

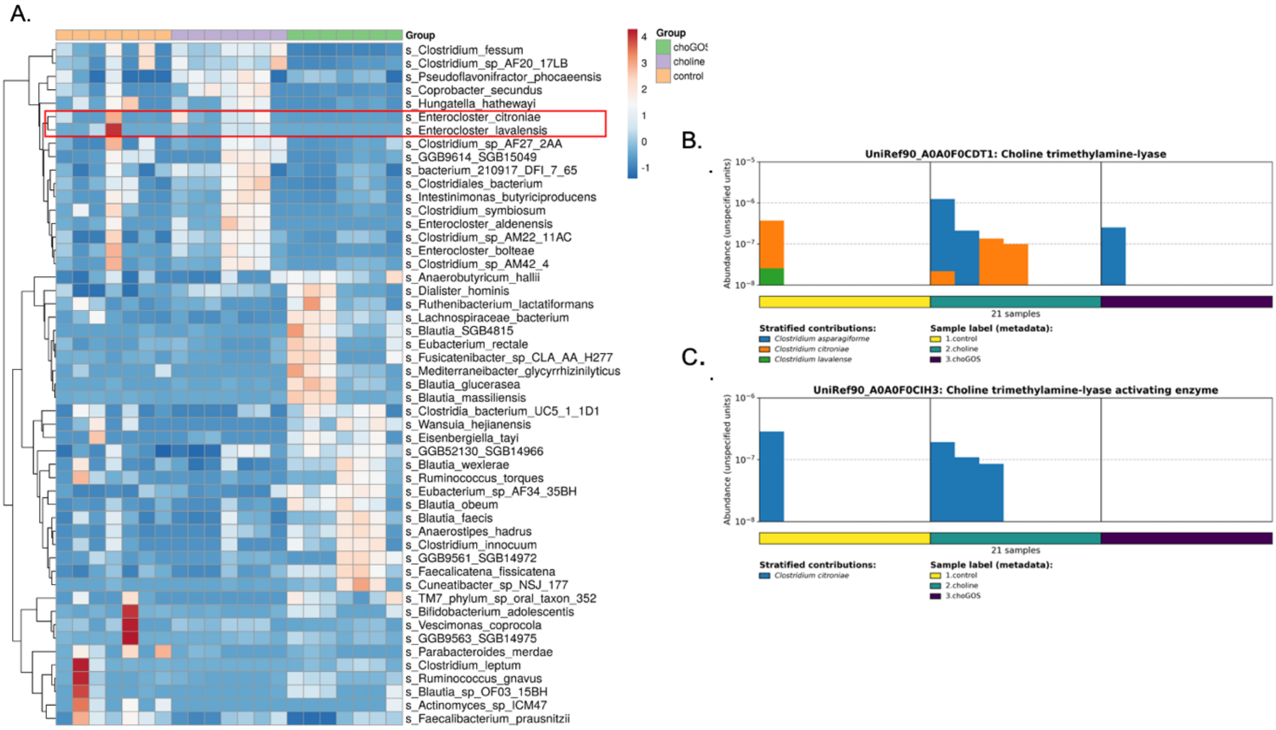
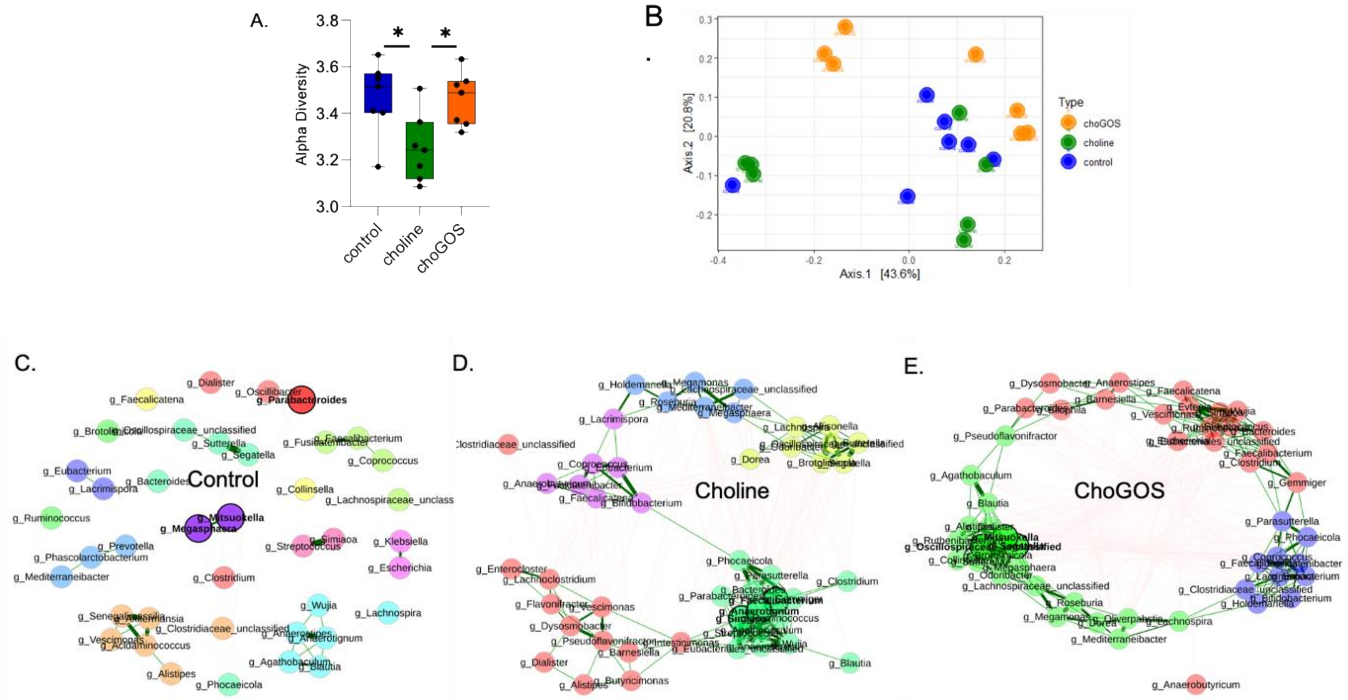


**Figure S1.** Reduction in Clostridium species encoding cutC and cutD in Choline + GOS group. (A) Heatmap showing list of bacteria species significantly different across the 3 groups. High abundance is indicated by red, and low abundance by blue. Heatmap shows only those pathway with significant difference at *P value* < 0.05.(B) Stratified bar plot showing abundance of *Clostridium* species which encode choline trimethylamine-lyase *(*cutC*)* and choline trimethylamine-lyase activating enzyme (CutD) gene across the 3 groups indicated in yellow (control), green (choline) and purple (choline+GOS).



**Supplementary Figure S2.** Microbial diversity and correlation of microbiota in each group. (A) Shannon’s alpha diversity among three dietary groups. (B) Microbial community differences across the three groups indicated by the Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarity distance. Single correlation network analysis at the genus level for (C) Control(D) Choline and (E) ChoGOS. NetCoMi , with netconstruct function with the following parameters, measure= “pearson”, filtTax = "highestFreq", filtTaxPar = list(highestFreq = 50), zeroMethod = "pseudo", zeroPar = list(pseudocount = 0.5), normMethod = "clr", sparsMethod = "threshold", thresh = 0.8) was used to generate the correlation networks. Nodes represent genus, edges the correlation. Green edges indicate positive correlations and red edges indicate negative correlations, with edge thickness representing correlation strength.

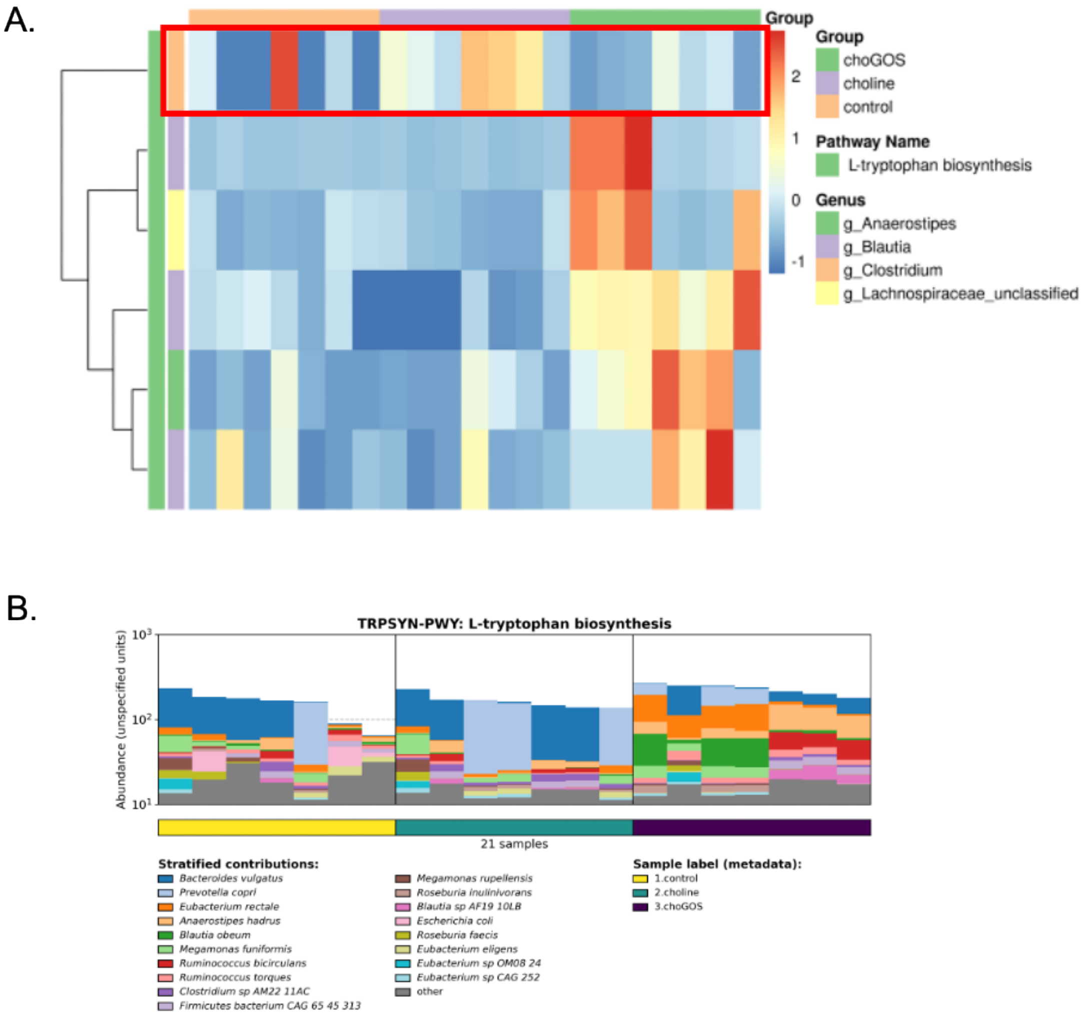


**Figure S3.** Microbiota- specific Tryptophan biosynthesis across 3 groups. (A) Heatmap illustrating the enrichment of different microbiota for tryptophan biosynthesis pathway across three groups. The red rectangle shows the pathways related to clostridium. The heatmap only includes pathways that show statistically significant differences ($p < 0.05$). (B) Stacked bar plot showing the stratified contribution of each microbe involved in the L-tryptophan biosynthesis pathway in each group.