

GUT ARCHAEA in Estonian population

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INTRODUCTION

ARCHAEA: Unique Microbial Life

Archaea represent a unique domain of life, distinct from bacteria and more closely related to eukaryotes at the molecular and genetic levels.

DIVERSITY IN GUT: 27 species are known

The latest version of the Unified Human Gastrointestinal Genome (UHGG v2.0.2) database includes 27 archaeal species

METANOGENESIS: unique metabolite path

Archaea are capable to metanogenesis - unique metabolism produced methan - one of the main greenhouse gas.

METHODS

Sequencing: shotgun paired-end 150 bp reads with deep coverage (56M reads/sample in average).

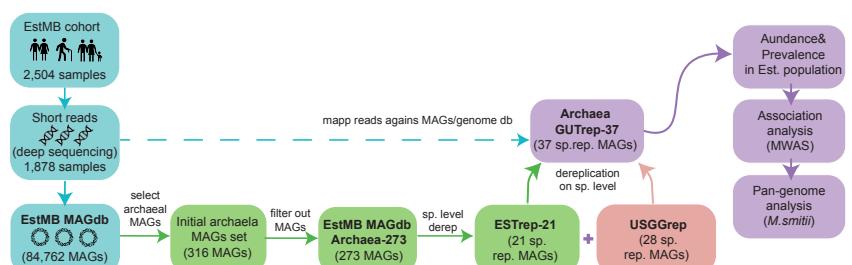
MAGs Assembly: Multi-binning metagenome assembly (VAMB, MaxBin, MetaBAT), refined with DAS-Tools. MAG quality assessed using CheckM2, GUNC, and SeqKit. MAGs were cluster with dRep. Taxonomic annotation performed with GTDB-Tk. Abundance & prevalence estimated with CoverM.

Phylogenetic Tree: Multiple sequence alignment was performed with GTDB-Tk, the tree was calculated using FastTree, and visualization was done with iTOL.

MWAS: Association analysis was performed using a linear model between CLR-transformed relative abundances of species (prevalence >1%) and dietary, disease, or metadata variables. Significance levels were adjusted using Bonferroni correction.

Pan genome: Anvio pangome pipeline.

Pipeline visualization



RESULTS & CONCLUSIONS

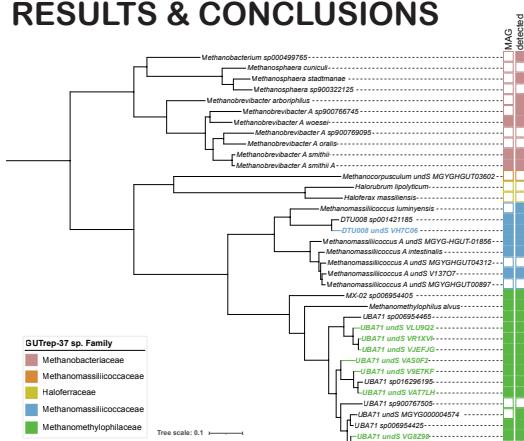


Fig.1 Phylogenetic tree of human gut archaea species, including newly assembled species from Estonian population (highlighted with a color)

Expanded the known archaeal diversity by 30%

Out of 21 assembled species, 12 were classified under the orders Methanobacterales and Methanomassiliicoccales. Among the Methanomassiliicoccales, **9 species were identified as novel**. Notably, 7 of the 9 new species belonged to the *UBA71* genus. The latest version of the Unified Human Gastrointestinal Genome (UHGG v2.0.2) database includes 27 archaeal species, we expanded the known archaeal diversity at the species level by 30% (Fig. 1).

New “Archaea GUTrep-37” MAGs Collection

A comprehensive collection of representative MAGs at the species level has been established, accompanied by an additional set of MAGs designed for potential strain-level analysis.

Almost half of Estonians harbor Archaea in their gut

Methanobrevibacter_A smithii was detected in 37% of individuals, while *Methanobrevibacter_A smithii*_A was found in 30%. Notably, the *UBA71* genus exhibits far greater diversity in the Estonian population compared to findings from earlier studies in other populations (Fig. 2).

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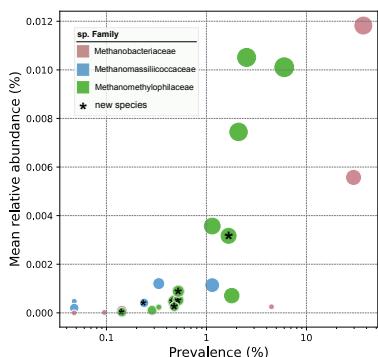
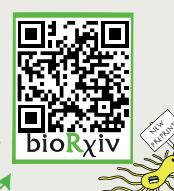


Fig. 2 Prevalence and relative abundance of Archaea Species in the Estonian Population (Species Colored by Family).

Significant correlations between archaeal species and diet, stool type, and BMI

Methanomethylphilus alvus show significant association with coffee consumption & TakenSupplements. *UBA71_sp905187815* with Boiled vegetables consumption. *Methanobrevibacter_A smithii*, *Methanobrevibacter_A smithii*_A and *Methanomassiliicoccus_A intestinalis* has correlation with stool type, but only *Methanobrevibacter_A smithii*_A from them also correlated with BMI & Porridge/muesli consumption (Fig. 3).

Pan-genome analysis reveals a distinct set of genes that differentiate *Methanobrevibacter_A smithii* from *Methanobrevibacter_A smithii*_A (Fig. 4)

Metabolite pathway reconstruction reveals differing abundances across three metabolic pathways. One pathway was classified under cofactor and vitamin metabolism, while the other two were related to fatty acid metabolism (Fig. 5).

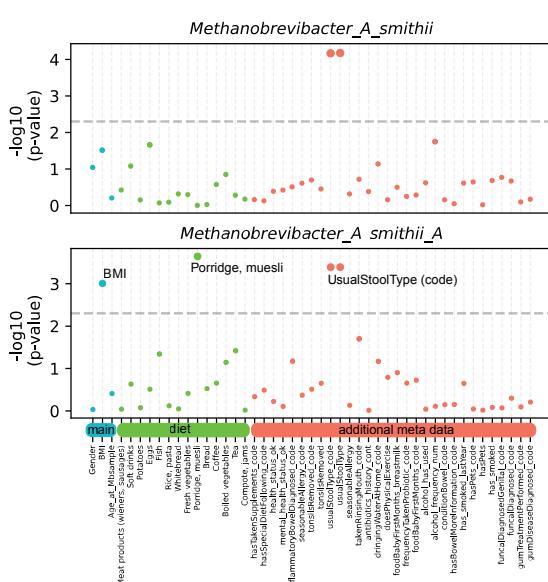


Fig.3 Microbes wide association study (MWAS) of archaeal species.

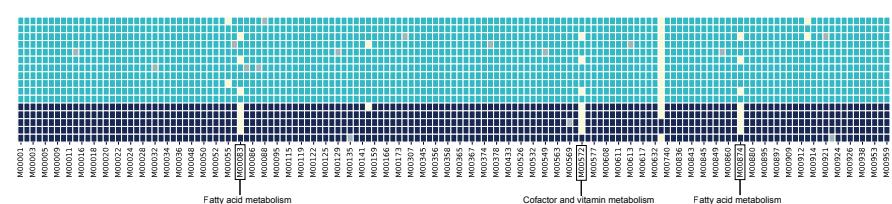


Fig.5 Comparative analysis of metabolite pathways in MAGs belonging to *Methanobrevibacter_A smithii* and *Methanobrevibacter_A smithii*_A species.

