Archaea in Mammalian Gut Microbiomes

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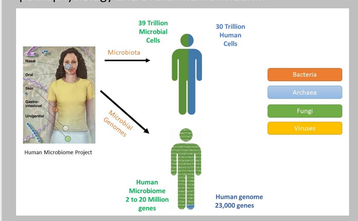
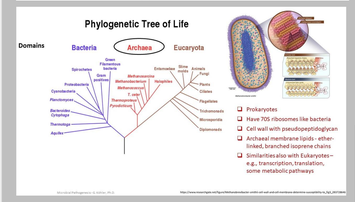
INTRODUCTION

Humans are supraorganisms consisting of about 30 trillion human cells and about 39 trillion microbial cells. There are about 23,000 human genes but human microbiomes make up about 2 to 20 million genes. Microbiomes can be described as complex communities made up of microorganisms like bacteria, archaea, fungi, and viruses. The human body provides these microorganisms with nutrition and a suitable environment while the microorganisms play an essential role in human health and disease, for example as a defense against harmful pathogens. Most of the microorganisms colonize the digestive tract. Approximately 100 trillion microorganisms exist in the human gastrointestinal tract (1). The gut microbiota interacts with the environment and host genetics to influence the health status of the host. The composition of gut microbiota is commonly determined using DNA-based methods, such as next-generation sequencing of 16S ribosomal RNA genes or whole-genome shotgun sequencing (2, 3).

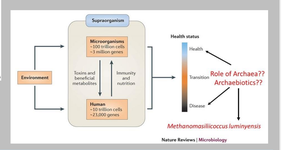
Archaea are the most enigmatic domain of the three domains of life. Archaea are unique in that they share some characteristics with bacteria and others with eukaryotes while they are also distinct from these two domains. Most archaea are extremophiles that are found in highly acidic, high-salt, or high-temperature environments. However, recent microbiome research has revealed that these prokaryotes are also a part of the gut microbiota, albeit their functional roles in gut health or disease are unclear. Archaea are naturally occurring components of the human gut microbiota and their biological significance has been recently reevaluated (4). Archaea could be keystone species in the gut that engage in important syntrophic relationships with other gut microbes. The recent proposal of archaeal strains as a new class of probiotics (archaebiotics) could be of interest for improving gut pathophysiology and overall human health.



Microbiome = Microbiota + Microbial genomes



Archaeal Domain. Obtained from Carreon et al., 2005 and Gottlieb et al., 2016. Alimentary Pharmacology and Therapeutics.



Potential role of Archaea in Health and disease.

OBJECTIVES

The main aim of this study is to mine extant microbiome data sets in our laboratory for the presence of archaeal sequences.

METHODS

Animal care and handling
All animal procedures used in this study were performed under a protocol approved by the OSU-CHS Institutional Animal Care and Use Committee (IACUC).

Fecal DNA isolation
Fecal DNA was isolated using ZymoBIOMICS DNA Miniprep kit.

Library preparation, Sequencing, and microbiota analysis
The V4 hypervariable region of the 16S rRNA gene was amplified using the method of Kozich et al., 2013 (7). The 16S rRNA gene-based NGS was performed with an Illumina MiSeq system. Whole-genome shotgun metagenomics was performed at OSU Stillwater Genomics Core Facility.

Metagenomic data sets from mammalian microbiome studies (e.g. Mouse, rats, Prairie voles) based on 16S rRNA amplicon or whole-genome shotgun sequencing approaches were screened for archaeal sequences. Taxonomic profiling workflows in the Qiagen CLC Genomics Workbench and other bioinformatics software such as MetaPhlan were used to elucidate the relative abundances of these enigmatic microorganisms.

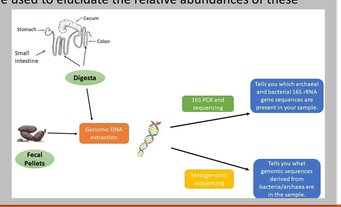
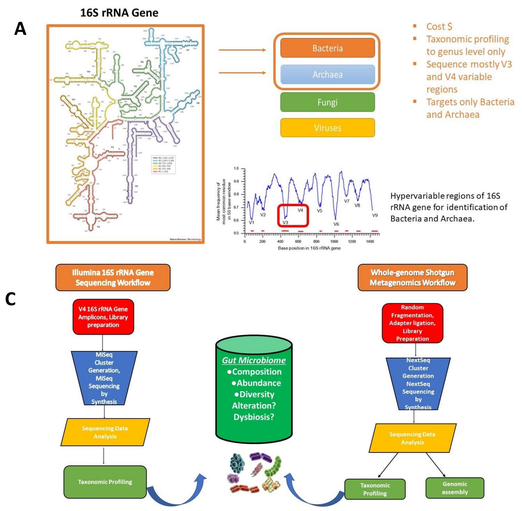


Fig 1. Schematic illustration showing DNA extraction, sequence library preparation, and sequencing.

METHODS cont.



- Genomes fragmented randomly
- Small DNA fragments sequenced
- Sequence reads assembled into contigs
- Genomes can be assembled using a reference database or de novo assembly for the unknown genomes
- Sequence reads can also be used for taxonomic profiling

- Cost $$$
- Allows for assembly of microbial genomes
- Taxonomic profiling to strain level, functional profiling possible
- Computational analysis more challenging

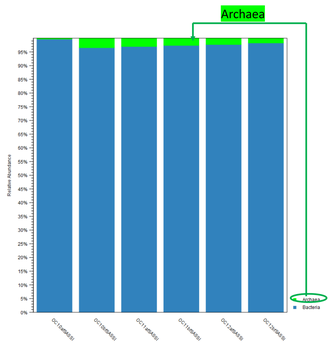
Fig. 2 16S rRNA gene amplicon sequencing and Whole-genome Shotgun Metagenomics workflow. A) Secondary structure and hypervariable region of 16S rRNA. B) Whole-genome shotgun metagenomics sequencing workflow. C) Workflow for bacteria and Archaea sequence data analysis.

RESULTS

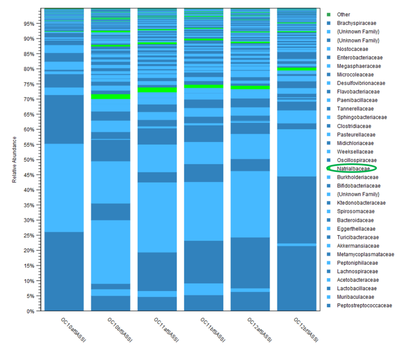
Relative abundance of Archaea in Different Rodents

Animal	Sample Collected	16S (Relative abundance)	WGS Metagenomics
Prairie vole	Small Intestine	0 - 0.01%	tbd
	Colon	0 - 0.01%	tbd
	Fecal	0 - 0.01%	Tbd
Mouse	Small Intestine	0 - 0.01%	tbd
	Colon	0 - 0.01%	tbd
	Fecal	0 - 0.01%	tbd
Rat	Small Intestine	tbd	0-3%
	Colon	tbd	< 1%
	Fecal	tbd	< 1%

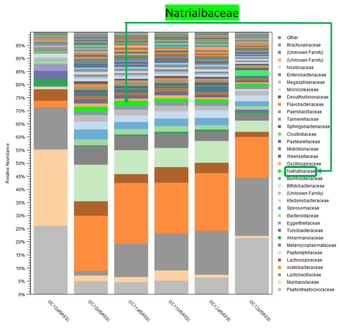
Relative abundance of Archaea and Bacteria at Kingdom and Family level



Relative abundance of Bacteria and Archaea at Kingdom level from 6 rat small intestine samples



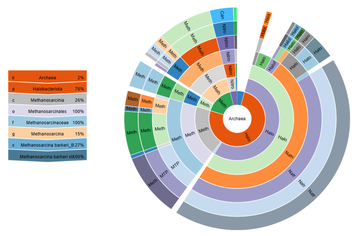
Relative abundance of Archaea at the family level



Relative abundance of Bacteria and Archaea at the Family level

- Only 2% Archaea

Sunburst's chart displaying Bacterial and Archaeal diversity



Sunburst's chart focused on Archaea

CONCLUSION

Our studies indicate that Archaea can be found in samples from the small intestine and colon however, in much lower abundance than bacteria.

Both 16S rRNA and metagenomic studies showed the presence of low quantities of archaea in samples; however, whole-genome Shotgun (WGS) metagenomics appeared more reliable to detect archaea.

Future studies include confirmation of the presence of archaea using qPCR, determination of the role of archaea in microbial communities in health and disease. Further research will also include analysis of human samples.

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