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Archaea: Essential inhabitants of the human digestive microbiota

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ABSTRACT

Prokaryotes forming the domain of Archaea, named after their first discovery in extreme environments, are acknowledged but still neglected members of the human digestive tract microbiota. In this microbiota, cultured archaea comprise anaerobic methanogens: *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanobrevibacter massiliense*, *Methanosphaera stadtmanae*, *Methanobrevibacter arboriphilus*, *Methanobrevibacter millerae* and *Methanomassiliicoccus luminyensis*; along with the non-methanogen halophilic Archaea *Halopherax massiliense*. Metagenomic analyses detected DNA sequences indicative of the presence of additional methanogenic and non-methanogenic halophilic Archaea in the human intestinal tract and oral cavity. Methanogens specifically metabolize hydrogen produced by anaerobic fermentation of carbohydrates into methane; further transforming heavy metals and metalloids into methylated derivatives, such as trimethylbismuth which is toxic for both human and bacterial cells. However, the role of Archaea as pathogens remains to be established. Future researches will aim to increase the repertoire of the human digestive tract Archaea and to understand their possible association with intestinal and extra-intestinal infections and diseases including weight regulation abnormalities.

Contents

Introduction . . . 2
Methods for studying methanogens of the human digestive microbiota . . . 2
Culture . . . 2
Fluorescent microscopy . . . 2
Molecular methods . . . 2
16S rRNA and methyl coenzyme-M reductase (*mcrA*) PCR-sequencing . . . 2
Metagenomic . . . 2
Quantitative real-time PCR (qPCR) . . . 3
Repertoire of archaea in human digestive microbiota . . . 3
Intestinal methanogens . . . 3
Oral methanogens . . . 3
Non-methanogenic archaea . . . 3
Diversity of methanogens in the human gut with age . . . 4
Human-associated methanogens and carbohydrates degradation . . . 5
Prospective role of human-associated methanogens . . . 5
Methanogens as probiotics . . . 5
Role in microbial ecological interactions . . . 5
H_2 consumption . . . 5
Heavy metal transformation . . . 5
Methanogens as fecal pollution indicators . . . 5

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Methanogens and diseases . . . 5
Potential for implication in infectious diseases . . . 6
Potential for implication with intestinal disorders . . . 6
Potential for implication in physiologic disorders . . . 6
Potential for implication with malnutrition . . . 6
Conclusions and perspectives . . . 6
References . . . 7

Introduction

Specificities in the ribosomal RNA (rRNA) subunits and other macromolecules allowed to group some prokaryotes into one life domain named Archaea [1,2]. These unicellular microorganisms which morphologically look like bacteria have been initially detected in extreme environments. Further investigations detected archaea in human microbiota [3–6] of the oral cavity and in the gut microbiota [7–9]. Archaea include a wide variety of organisms that share properties with both members of Eukarya (similar machineries for DNA replication, RNA transcription and protein translation, histones packaging chromosomal DNA) and members of Bacteria (various morphologies, presence of one single circular chromosome, lack of introns, similar post-transcriptional modifications). They also present unique characteristics such as lacking peptidoglycan in the cell wall and a membrane formed by L-glycerol ethers/isoprenoids chains instead of D-glycerol esters/fatty acids as in the two other domains [10–13]. Methanogens are unique archaea in processing methanogenesis with methane being both a source of energy (biogas) produced in bioreactors [13] and a greenhouse gas emitted from natural and anthropic environments, including livestock [14,15]. Methanogens are strict anaerobes found in freshwater, marine sediments, soils [16–18] and the gut of many animals and humans [8,14,18–22]. In humans, methanogens were found in the intestinal mucosa [8,20,21]; the oral cavity [3,22,23] and the vaginal mucosa [24] and skin [6]. Interestingly, archaea are *in vitro* susceptible only to antimicrobials which are active against both Bacteria and Eukarya members, meaning that most antibiotics commonly used to fight bacteria are inactive against archaea [25,26]. The repertoire of human-associated archaea has rapidly expanded from only three archaea known for 30 years [27–30] to 26 currently documented archaea, including 20 species in the human gastrointestinal tract [5,6,8,29,31,32]. Only two human gastrointestinal tract methanogens were cultured in 1982 and 1985 whereas six methanogens and two halophilic archaea have now been isolated from human stool specimens [20,27–31]. During the meantime, more archaea genomes were released, including eleven human-associated genomes.

We here review the state of knowledge regarding the methanogens and halophilic archaea associated with the human gastrointestinal tract microbiota.

Methods for studying methanogens of the human digestive microbiota

Culture

Culture of methanogens was first developed by Hungate [35,36]. Balch and colleagues further developed culture in pressurized atmosphere-based systems containing a gas mixture of 80% H_2 and 20% CO_2 reducing the growth of contaminants [37,38]. Roll-tube techniques have been then used to isolate methanogens on agar [39]. Recently, we developed a new two-chamber method for aerobic cultivating of methanogens in the presence of hydrogen-producing *Bacteroides thetaiotaomicron* [29]. Indeed, methanogens are obligate anaerobes producing methane by combining H_2 with CO_2, or methylated C1 compounds (methanol, methylamines, methylthiols) or acetate [40,41]. Although some of the H_2/CO_2-consumers are capable of utilizing formate, acetate is consumed by a limited number of *Methanosarcina* spp. and *Methanosaeta* spp. which are not able to use formate. Methylamine is used for methanogenesis by *Methanomassiliicoccales* members in addition to some *Methanosphaera* spp. and *Methanomicrococcus blatticola* which are also H_2/CO_2-consumers.

Fluorescent microscopy

Auto-fluorescence of methanogens is linked to the coenzyme F420 emitting a blue green light, detected by fluorescent microscope [42]. However, this method is not suitable for *Methanosaeta* members due to low F420 content and for *Methanosarcina* members which form aggregates [43,44]. Fluorescence *in situ* hybridization (FISH) [45–49] combining 16S rRNA-targeted oligonucleotide probe Arc915 and confocal laser scanning microscopy was used to detect methanogens along the intestinal mucosa [5]; and in sub-gingival pockets and dental calculus collected from individual samples dating from the 14th to the 21th centuries [47,48].

Molecular methods

16S rRNA and methyl coenzyme-M reductase (mcrA) PCR-sequencing

Combining 16S rRNA and *mcrA* gene sequencing [49–51], many studies of the human gastrointestinal and oral cavities allowed the detection of *Methanosarcina* and *Methanoculleus* sequences, in addition to *M. smithii*, *Methanosphaera stadtmanae, Methanobrevibacter arboriphilus, Methanomassiliicoccus luminyensis, M. oralis* and *Methanobrevibacter massiliens* sequences, and sequences related to uncultured methanogens, *Crenarchaeota* spp., *Halobacteriales* spp. and *Traumarchaeota* spp. [3,5,8,23,33,34,52]. In addition, by analyzing 16S rRNA clone libraries, Eckburg et al. [53] reported that all of the 1524 archaeal sequences examined from three healthy human fecal samples belonged to *M. smithii*. Similar to the results from the 16S rRNA clone libraries, a microbiome analysis revealed that *M. smithii* was the only archaeal species from two healthy human feces [54]. Data from *mcr*A clone libraries from six patients also yielded *M. smithii* in 95.73% of the total clones [46]. Clone sequences of *mcrA* closely related to *Methanoculleus chikugoensis*, a hydrogenotrophic methanogen of the Methanomicrobiales order were found associated to the intestinal mucosa [55]. Vianna and colleagues used terminal restriction fragment length polymorphism to study the prevalence and distribution of methanogens and possible associations with bacteria in oral biofilms, and they found the dominance of *M. oralis* along with one unique phylotype [56].

Metagenomic

Metagenomic studies detected *M. smithii* as the major human gut archaea [57,58]. A study of the human gut microbiome based on the correlation of the long-term and short-term dietary data diet with bacterial residents (*Methanobrevibacter* spp., *Methanosphaera* spp., *Thermogymnomonas* spp., *Thermoplasma*