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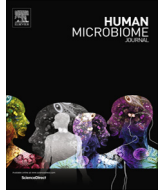
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## Archaea: Essential inhabitants of the human digestive microbiota



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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.

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## 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<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> with CO<sub>2</sub>, or methylated C1 compounds (methanol, methylamines, methylthiols) or acetate [40,41]. Although some of the H<sub>2</sub>/CO<sub>2</sub>-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<sub>2</sub>/CO<sub>2</sub>-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].

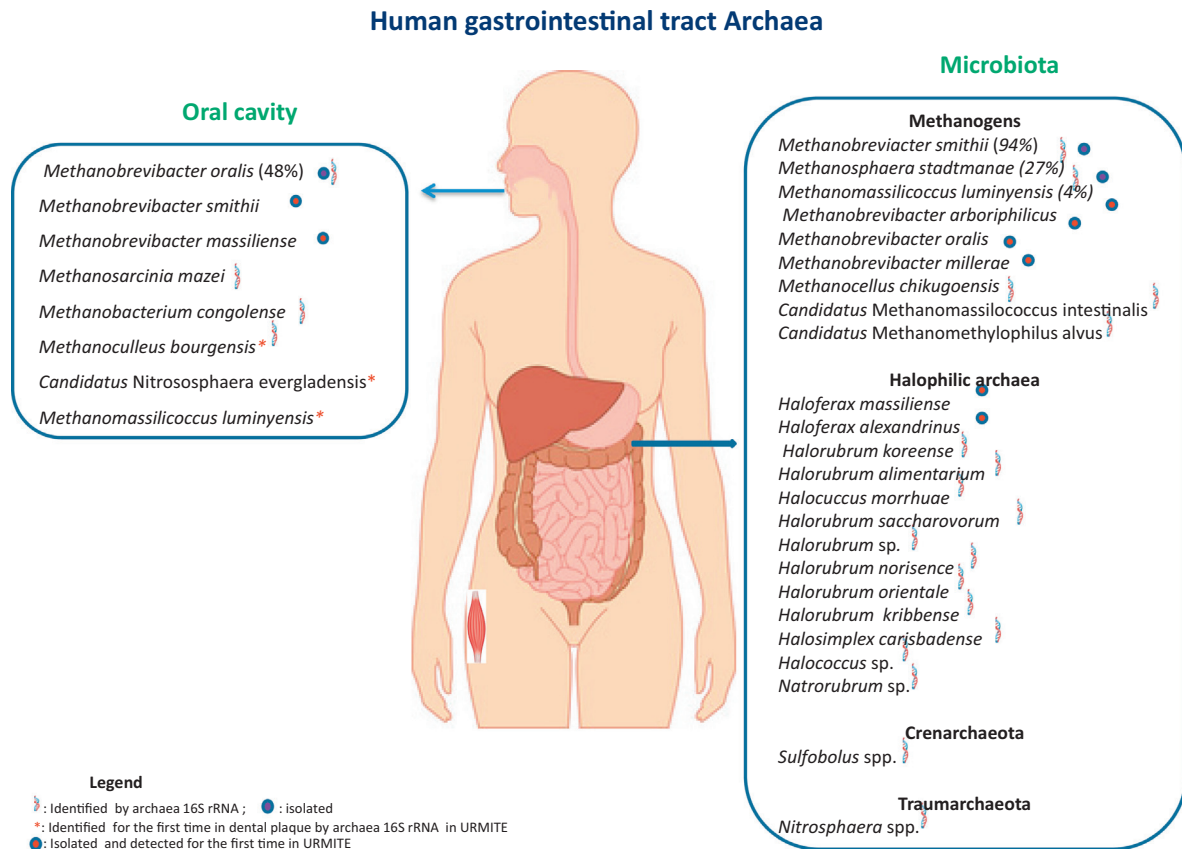
### Molecular methods

#### 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 massiliensis* 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 *mcrA* 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*



**Fig. 1.** Repertoire of human-associated methanogens.

spp. and *Nitrosphaera* spp.) was conducted and sequences were detected. The study also showed that several lineages co-varied with *Methanobrevibacter*-positive samples, including the commonly encountered reductive acetogenic bacteria *Ruminococcus*, and rarer lineages such as *Oxalobacter* and *Papillibacter*. A higher intake of carbohydrates correlated with *Methanobrevibacter*-positive samples [59].

#### Quantitative real-time PCR (qPCR)

A few studies combining 16S rRNA or *mcrA* gene qPCR confirmed that *M. smithii* was the predominant methanogen in the intestinal tract [9,60–62]. Combining 16S rRNA, *mcrA* and heat-shock protein *cnp06* gene detection allowed to find that *M. oralis* was the major archaea found in the oral cavity and its presence correlated with the severity of periodontal diseases [22,3,62–64]. qPCR targeting *rpoB* encoding the RNA polymerase  $\beta$ -subunit showed higher specificity than qPCR 16S rRNA for detecting methanogen [9].

#### Repertoire of archaea in human digestive microbiota

##### Intestinal methanogens

The most frequently isolated methanogens in the human gastrointestinal tract belong to the  $H_2/CO_2$ -consumer *Methanomicrobiales* order [20,29,31,33]: *M. smithii* with a reported prevalence of up to 97.5% and *M. stadtmanae* with a prevalence of up to 23% [9]. Also isolated in the gut are *M. oralis* [65], *M. arboriphilus*, an archaeon previously isolated in rotting trees, *Methanobrevibacter millerae* and *Methanobrevibacter ruminantium* which are archaea known to colonize the digestive tract of cows and ruminants

[35,66–68]. The isolation of *M. ruminantium* remains doubtful because it was obtained after the addition of non-sterilized rumen fluid without any negative control. Methylamine-consuming methanogens have also been found in intestinal microbiota such as *Methanomassiliococcus luminyensis* with a prevalence of up to 4% [7]. *M. luminyensis* is the only isolated species of this group. In addition, sequences of two cultured but not yet isolated strains *Candidatus* “*Methanomethylophilus alvus*” and *Candidatus* “*Methanomassiliococcus intestinalis*” which are also methylamine-consuming methanogens have been founded in human intestine [8,20,30]. The actual repertoire of human intestinal methanogens comprises five isolated methanogens among the eight which are known (Fig. 1).

##### Oral methanogens

*M. oralis*, *M. smithii* and *M. massiliense* have been isolated and identified from subgingival plaques [3,64,65]. Additionally, 16S rRNA gene amplicon sequencing studies detected methanogens related to *Methanomassiliococcales* [69] as well as members of the *Methanobacterium* [70] and *Methanosarcina* genera [71] in modern dental plaques. Our recent investigation of ancient human dental calculus from over five centuries [48] found methanogens related to *M. massiliense* (44.6%) and *M. oralis* with a prevalence of up to 19.6% and *M. luminyensis*-like with a prevalence of up to 12.5% and *Methanoculleus bourgensis*.

##### Non-methanogenic archaea

Sequences specific to halophilic *Crenarchaeota* and *Traumarchaeota* archaea have been detected in human intestinal mucosa specimens (Fig. 1). Among representatives of the *Crenarchaeota*

phylum, one amplified SSU rRNA gene sequence (AY887079) exhibits a 97% sequence similarity with *Sulfolobus solfataricus* while another sequence (AY887074) exhibits a 99% sequence similarity with *Candidatus "Nitrososphaera gargensis"* [72]. These two latter organisms are hyperthermophilic species and it is very unlikely that they inhabit the human gastrointestinal tract. Until cultured representatives of the *Crenarchaeota* members are obtained in independent studies, the presence of the this archaeal phylum in the human gastrointestinal tract remains questionable [73]. Oxley et al. have succeeded in identifying the DNAs of various halophilic archaea from colonic mucosal biopsies and fecal samples of patients suffering from intestinal bowel diseases in Germany [5]. Nam et al., obtained 16S rDNA sequences similar to *Halorubrum koreense*, *Halorubrum alimentarium*, *Halorubrum saccharovorum* and *Halococcus morrhuae* from stools collected in Korean individuals [52]. These observations all point out to salty food as the source of intestinal halophilic archaea. *Halopherax massiliense* was recently isolated in a stool sample collected in a Senegalese individual [36]. Sequences of halophilic archaea were also found in the intestinal mucosa as well as sequences of *Traumarchaeota* which were found in ancient human dental plaques [48]. Halophilic archaea are salt-loving organisms that inhabit hypersaline environments where salt concentrations exceed 100–150 g l<sup>-1</sup>, such as salt lakes, solar salterns and subterranean salt deposits [74,75]. These organisms have also been described from refined salts and an array of manufactured food products where large quantities of salt are employed in the preservation process such as salted fish, hides, pork, sausages and fish-based sauces [76]. Examples include 'jeotgal', traditional Korean fermented seafood, the Japanese 'fugunoko nukazuke' prepared by fermentation of salted puffer fish ovaries in rice bran, and 'nam-pla', a Thai fish sauce. In some cases, the salt concentration during the fermentation process is sufficiently high for the development of

*Halobacteriaceae* archaea. Accordingly, *Halococcus thailandensis*, *Natrinema gari*, *Haloarcula salaria* and *Haloarcula tradensis* were isolated from a Thai fish sauce called "nam pla" [76–78] and *Halalkalicoccus jeotgali* from the Korean shrimp jeotgal [78].

### Diversity of methanogens in the human gut with age

Methanogens in the human digestive tract account for 10% of all gut anaerobes and help increase the efficiency of digestion [79]. Studies using quantitative PCR techniques reported that methanogens colonize the human intestine early after birth until old age (Fig. 2). The infant gut microbiota is more variable in its composition and less stable over time. In the first year of life, the infant intestinal tract progresses from sterility to extremely dense colonization, ending with a mixture of microbes that is broadly very similar to that found in the adult intestine [80]. Some studies suggest that there may be an increase in the concentration of methanogens in the human colon during the ageing process, as for the rat, in which the fecal concentrations of methanogenic Archaea increase with age [81]. Using qPCR analysis, Palmer and colleagues [82] described that among 14 healthy babies, seven babies aged from 3-days to 5-months presented subdominant levels of methanogens in their stool during their first year of life, with 10<sup>3</sup>–10<sup>6</sup> 16S rRNA gene copy numbers recovered per gram of stool and also indicated that in four of these babies methanogens were detected in only a single sample. They indicated that this presence of methanogens may be transient and almost exclusively in the first few weeks of life, as methanogens were detected in only one of the several samples collected at different ages in four newborns and only one infant after the fifth week of life. A limited analysis of archaeal sequences amplified from the three maternal stool samples that were tested positive for archaea revealed a predominance

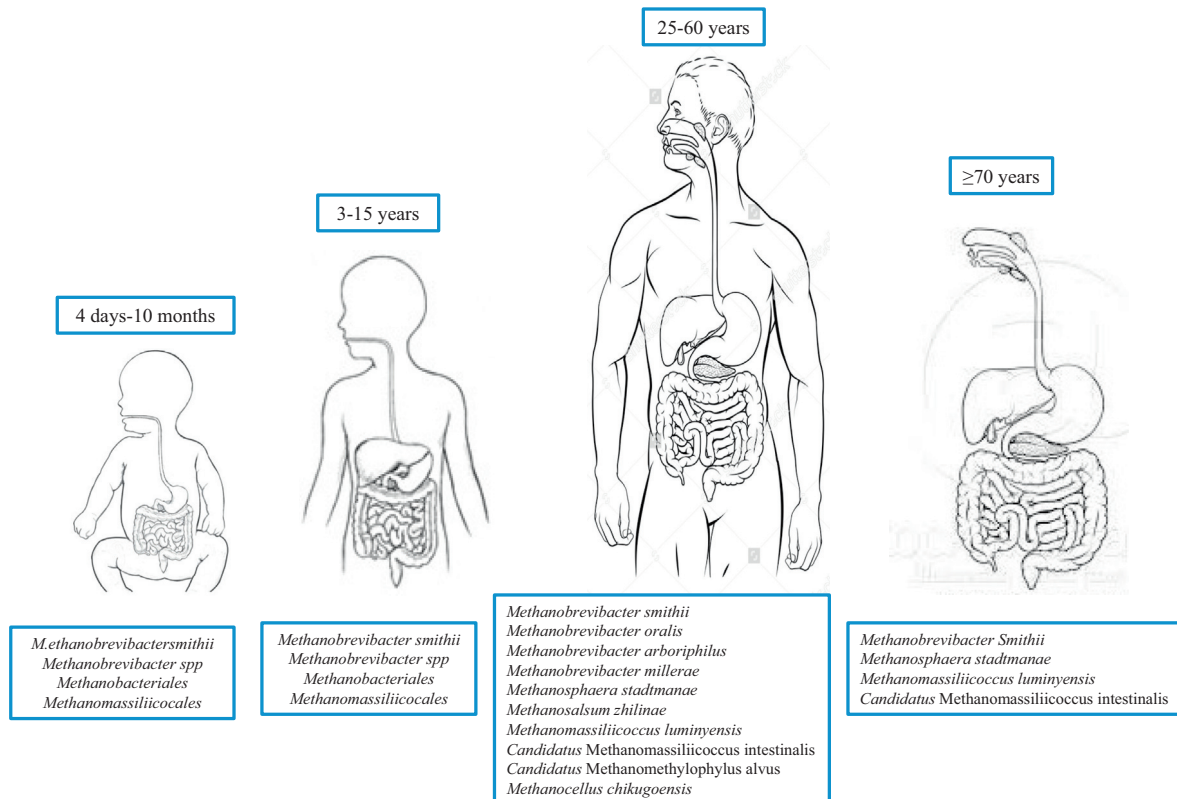


Fig. 2. Diversity of human methanogens in the gut with age.

of *M. smithii* (7/8 archaeal clones identified, including at least one clone from each mother) [83]. Methanogens 16S rRNA gene sequences were also detected in the meconium [84]. These findings suggested that mother's vaginal and intestinal microbial are major sources for the colonization of the infant gut [84]. Moreover, studies in families [85] and in twins [86] suggested that environmental factors are associated with methanogenic flora transmission, independently of genetical predisposition. Mihajlovski et al. [50] characterized the diversity and the distribution of methanogens among healthy subjects of different ages including 23 newborns, 20 adults and 20 elderly subjects using a molecular approach associating the analysis of a specific molecular metabolic marker of methanogenesis. They described the presence of *M. smithii*, *M. stadtmanae*, *Methanomassiliicoccales* sp. and non-*Methanobacteriales* in stools samples of adults and elderly individuals; while only one sample of the newborn's group harbored a sequence of *Methanomassiliicoccales* sp. They also found uncharacterized archaea that are closely related to *Thermoplasmatales* which was frequently associated to the gut microbiota of the elderly and indicated that age is a determinant factor of methanogen-carriage for newborns versus the two other groups, but is not a significant factor between adults and elderly people [50]. Studies based on PCR approaches also revealed a significantly lower proportion of methanogens among babies [50]; on the contrary, we observed that the prevalence of *M. luminyensis* increased with age [7] and was mainly detected in healthy elderly individuals (Drancourt M, unreported data).

Studies based on PCR approaches also revealed a significantly lower proportion of methanogens among babies [63] and children than among adults. Recently, an association between age and breath methane was described in the German population age range of: 4–95 [87]. Studies based on molecular approaches support this relationship and provide supplemental information thanks to a lower detection limit of methanogens and the access to their diversity.

### Human-associated methanogens and carbohydrates degradation

The fact that the human diet contains numerous complex carbohydrates (oligosaccharides and polysaccharides) correlates with the fact that the digestive microbiota contains many microbial species that have evolved in a very large number and variety of enzymes to break down these molecules to simple sugars [88]. In this sugar-rich environment, methanogens stand out by their almost complete lack of enzymes for complex carbohydrate deconstruction. Thus, the genomes of *M. smithii*, *M. oralis*, *M. arboriphilus*, *M. millerae* and *M. stadtmanae* encode no enzyme whatsoever for the breakdown of glycosidic bonds, while *M. luminyensis* only encodes two enzymes, presumably for its own intracellular trehalose cycle. Although these methanogens have little (if any) capacity to breakdown external glycans to monosaccharides, their genomes do contain numerous glycosyltransferases, the enzymes that can link monosaccharides to a variety of acceptors to build glycoconjugates. Coutinho & Henrissat [89] have reported this paradox whereby the methanogens appear to be unable to recycle the carbohydrate structures that they assemble.

### Prospective role of human-associated methanogens

#### *Methanogens as probiotics*

Due to their very specific metabolism leading to trimethylamine depletion for methanogenesis, the members of the *Methanomassiliicoccales* might have a positive effect on health by limiting gut TMA concentration, without the bad side effects of

limiting choline inputs through diet [90]. The members of the order *Methanomassiliicoccales*, by limiting trimethylamine production and absorption in the intestine, are a means to reduce plasmatic trimethylamine-*N*-oxide level, and its deleterious effects on blood vessels; it could be used as probiotics to prevent cardiovascular diseases, thus reducing the amount of the pro-atherogenic TMAO produced by the liver and therefore its plasma level. The *Methanomassiliicoccales* could also be a simple way to limit trimethylaminuria, by reducing the TMA concentration in the gut and in consequence, its olfactory impact on the body. In both pathological cases, it will be possible to avoid a drastic diet, thus limiting nutritional deficiencies risks. This concept of probiotics has recently been developed under the name of "archaeobiotics" as to show the innovative and positive contributions that we expect from archaeal strains to human health [91].

#### *Role in microbial ecological interactions*

##### *H<sub>2</sub> consumption*

In the human gut, the accumulation of hydrogen reduces the efficiency of microbial processes and reduces the energy yield. Methanogens such as *M. smithii*, are pivotal in the removal of this hydrogen in excess from the gut, by metabolizing the hydrogen generated during the fermentation of carbohydrates into methane gas. H<sub>2</sub> consumption promotes more ATP synthesis in anaerobic bacteria in the same habitat and subsequently indirectly promotes resident bacterial population growth including opportunistic pathogens. The accumulated evidence suggests that methanogens, through the production of methane, can directly influence intestinal transit; in fact, there seems to be a direct correlation between methane production and constipation [92].

##### *Heavy metal transformation*

Heavy metals or metalloids are transformed into methylated derivatives, which are more toxic compounds. *M. smithii* and *M. stadtmanae* were shown to be able to produce more trimethylbismuth, the main volatile derivative of bismuth produced in human feces [93]. This volatile bismuth has toxic effects not only on human cells but also on bacteria such as *B. thetaiotaomicron*.

#### *Methanogens as fecal pollution indicators*

*M. smithii* may be a useful indicator of sewage or human fecal pollution because of its host's specificity and high abundance in the human intestine [94,95]. Investigating sewage-associated *nifH* gene marker of *M. smithii* in recreational waters [96–99], showed a 29% sensitivity among individual human fecal samples, but a 93% sensitivity among sewage samples and a 100% specificity in tested samples of Mississippi and Missouri and concluded that this result appears more representative of real-world problems [100].

Another study reported in the USA the detection of *M. smithii* in 10<sup>-3</sup> and 10<sup>-4</sup> dilutions of sewage and in all sewage samples collected from south Florida, northwest Florida, and Mississippi and concluded that *M. smithii* is ubiquitous in sewage as a human-specific markers of fecal pollution [101]. However, it was reported that the application of the *nifH* marker alone may not be sensitive enough to provide evidence of sewage pollution; and that the *nifH* gene can be useful as an additional marker to the *Bacteroides* HF183 or viral markers for source tracking studies [102].

### Methanogens and diseases

Methane has previously been considered as an inactive gas that it is mainly excreted in flatus, while a certain amount is excreted by breathing. However, recently, methanogens have been associ-

ated with infectious diseases such as brain abscesses, sinus abscesses, as well as gastrointestinal disorders, mainly chronic constipation and metabolic diseases like obesity. *M. stadtmanae* and *M. smithii* have been recently shown to induce monocyte-derived dendritic cell maturation [103], and *M. stadtmanae* also induced a strong pro-inflammatory cytokine (TNF- $\alpha$ , IL-1  $\beta$ ) release from these cells and is more prevalent in patients with inflammatory bowel disease [104].

#### Potential for implication in infectious diseases

Recently in our laboratory, by combining a specific culture approach for methanogens, *in situ* hybridization, metagenomic approach, real-time PCR and PCR-sequencing, we detected and isolated for the first time *M. oralis* in a sample from a patient suffering from chronic sinusitis. We also isolated *M. smithii* from a sample of a patient with a paravertebral abscess [105]. In this way, we contributed to highlight for the first time the presence of methanogens in pathological situation in humans and suggested that methanogens are opportunistic or emerging pathogens.

#### Potential for implication with intestinal disorders

A strong association between methanogens' presence and chronic constipation in humans was shown. Methane was shown to slow intestinal transit by 59% [106], and thus may contribute substantially to constipation. In a study of 96 patients with chronic constipation and 106 controls, methane positivity was examined using glucose breath testing and colon transit time was measured using radiopaque markers, and methane positivity was detected in 75% of the slow transit patients, 44% of the normal transit patients and 28% of controls [107]. In another study, the amount of methane produced after a carbohydrate challenge and its prevalence were both higher in patients with slow-transit constipation compared with normal-transit constipation and non-constipated controls [108]. In contrast, methane production was not related to stool frequency or consistency in contrast to findings in constipation-IBS [109]. Methane was associated with the presence and degree of constipation both subjectively and objectively in a study on IBS patients, whom produced methane on lactulose hydrogen breath test [110,111]. There is only one case report of a patient with slow transit constipation and methane positivity whose stool frequency and consistency improved, and colon transit accelerated after 14 days of treatment. Moreover, glucose breath methane excretion 10 days after the end of treatment was low compared to the concentration before treatment, indicating that constipation improvement was related to methane eradication [55]. Taken together, these findings indicate that in individuals with already slow transit constipation, methanogens may bloom and promote further constipation. If methane has a direct or indirect impact on intestinal transit, attempting to manipulate methanogenic flora may be a viable therapeutic option. However, the future may include inhibitors of methanogenesis, targeted antimicrobial therapy, and even probiotics.

#### Potential for implication in physiologic disorders

Obesity is a physiological state that has emerged as a major health concern in populations that have adopted a Western diet and is closely tied to the microbiota [112].

Based on a gnotobiotic mouse model, a link between *M. smithii* and the development of obesity could be established. The colon of germ-free mice was colonized with a polysaccharide-degrading bacterium *B. thetaiotaomicron*, a sulfate-reducing bacterium *Desulfovibrio piger*, and *M. smithii* in different combinations.

*B. thetaiotaomicron* degraded polyfructose containing glycans more efficiently in the presence of *M. smithii*. In addition, mice colonized with these two microbes showed an increase in fermentation products and an increase in adiposity (increased storage of energy in fat cells). These effects were not observed after co-colonization with the pair *B. thetaiotaomicron* and *D. piger* (as alternative H<sub>2</sub>-consumer), suggesting that methanogens may play a role in caloric harvest and weight gain in humans [110]. In humans, it was shown that an increased methane production on breath tests is associated with a higher average BMI, both in a normal population and in obese subjects. In the obese population, methane was associated with a remarkably greater BMI compared to non-methane controls; further evidence has been provided by Zhang *et al.* who reported higher numbers of *Methanobacteriales* in obese humans together with enrichment of H<sub>2</sub>-producing *Prevotella* sp. and suggested that increased energy harvesting in obese individuals is related to hydrogen transfer between taxa as they observed a concurrent increase in both hydrogen-producing *Prevotellaceae* and hydrogen-utilizing methanogens [111]. In comparison to the previous study, recent studies reported opposite results; the number of *M. smithii* in 20 obese persons compared to 20 persons of normal weight was not significantly increased. Instead, authors found a significantly increased number of *M. smithii* in nine anorexia nervosa patients compared to persons with normal weight. The result, also produced by real-time quantitative PCR, shows that more research is needed to truly understand the ecology of the human gut flora and the role of archaea in intestinal disorders [112]. According to another study investigating the occurrence of methanogens in twins, the abundance and prevalence of *M. smithii* were not related to weight [113], while a German study based on body mass index even observed a reduced abundance of *Methanobrevibacter* sp. in obese subjects [111]. Million *et al.* [114] also demonstrated that *M. smithii* was lower in the GI tract microbiota of obese individuals.

#### Potential for implication with malnutrition

A recent study showed that *M. smithii* was not detected in the 20 stool samples of West African children suffering from severe acute malnutrition whereas it was detected in 40–75% of controls [115]. Because no causality was demonstrated, the study proposed a unifying theory linking gut environment oxidation to the depletion of anaerobes and methanogens in severe acute malnutrition [115].

## Conclusions and perspectives

The repertoire and the prevalence of human gut methanogens and their variations have been underestimated for a long time, undoubtedly because of the complicated nature of their culture and the fact that standard protocols for DNA extraction have not been optimized for such fastidious microorganisms. Therefore, it is conceivable that many more archaea from the four major Archaea phyla remain to be discovered in the future years in the intestinal tract of both animals and humans. Efforts will aim to more precisely estimate the variations of archaea along the digestive tract as well as the sources of the various archaeal species. While methanogens have been conclusively linked to only periodontitis [67], their role in physiology and pathology is largely unexplored [10,11,116]. As a consequence, the spectrum of antimicrobial agents with demonstrated anti-archaeal activity remains remarkably limited and will clearly require further imaginative studies in order to be ready to propose an effective treatment to patients with archaeal disorders.

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