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Methanogens as emerging pathogens in anaerobic abscesses

E. Sogodogo[1,2] · M. Drancourt[1,2] · Ghiles Grine[1,2]

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Abstract
Methanogens are strictly anaerobic archaea metabolising by-products of bacterial fermentation into methane by using three known metabolic pathways, i.e. the reduction of carbon dioxide, the fermentation of acetate or the dismutation of methanol or methylamines. Methanogens described in human microbiota include only Euryarchaeota, i.e. *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanobrevibacter arbophilus*, *Methanobrevibacter massiliensis*, *Methanomassiliicoccus luminyensis*, *Methanosphaera stadtmanae* and Ca. *Methanomethylophilus alvus* and Ca. *Methanomassiliicoccus intestinalis*. Methanogens are emerging pathogens associated with brain and muscular abscesses. They have been implicated in dysbiosis of the oral microbiota, periodontitis and peri-implantitis. They have also been associated with dysbiosis of the digestive tract microbiota linked to metabolic disorders (anorexia, malnutrition and obesity) and with lesions of the digestive tract (colon cancer). Their detection in anaerobic pus specimens and oral and digestive tract specimens relies on microscopic examination by fluorescence in situ hybridisation, specific DNA extraction followed by polymerase chain reaction (PCR)-based amplification of the 16S rRNA and *mcr*A gene fragments and isolation and culture in the supporting presence of hydrogen-producing bacteria. Diagnostic identification can be performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and can be further completed by genotyping through multi-spacer sequencing and, ultimately, whole genome sequencing (WGS). Ornidazole derivatives, fusidic acid and rifampicin are the compounds to be included in in vitro susceptibility testing to complete the clinical workflow. Clinical microbiology laboratories should work toward developing cheap and easy protocols for the routine detection and identification of methanogens in selected specimens in order to refine the diagnosis of infections, as well as to expand the knowledge about this group of intriguing microorganisms.

Introduction: methanogens in the environment

Methanogenic archaea (further referred to as methanogens), the only known methane-producing microorganisms in the environmental and human ecosystems, belong to the orders Methanococcales, Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanopyrales, Methanocellales and Methanomassiliicoccales (Fig. 1). Methanogens are strictly aero-intolerant organisms exhibiting fully anaerobic respiration. During aerobic respiration, O_2 is the final acceptor of electrons to form water, while in the case of methanogens, carbon present in small organic molecules is the final acceptor of electrons [1, 2]. According to the carbon substrates used for methanogenesis, methanogens are divided into three groups, hydrogenotrophs, methylotrophs and acetotrophs [1]. Two new phyla, Bathyarchaeota and Verstraetearchaeota, recently detected in deep-ocean, freshwater sediments, soils and hydrocarbon-rich environments by metagenomic approaches are both likely to belong to the methylotrophs [3].

Methanogens in natural human microbiota

Oral microbiota

Five genera of methanogens, namely, *Methanobrevibacter* spp., *Methanosphaera* spp., *Methanosarcina* spp., *Thermoplasma* spp. and *Methanobacterium* spp., have been isolated from subgingival dental specimens [4]. A review of the international literature concluded that *Methanobrevibacter oralis* was

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✉ Ghiles Grine
grineghiles@gmail.com

[1] IHU Méditerranée Infection, UMR MEPHI, 19–21, Bd. Jean Moulin, 13005 Marseille, France

[2] Aix-Marseille Université, MEPHI, IRD, IHU Méditerranée Infection, Marseille, France

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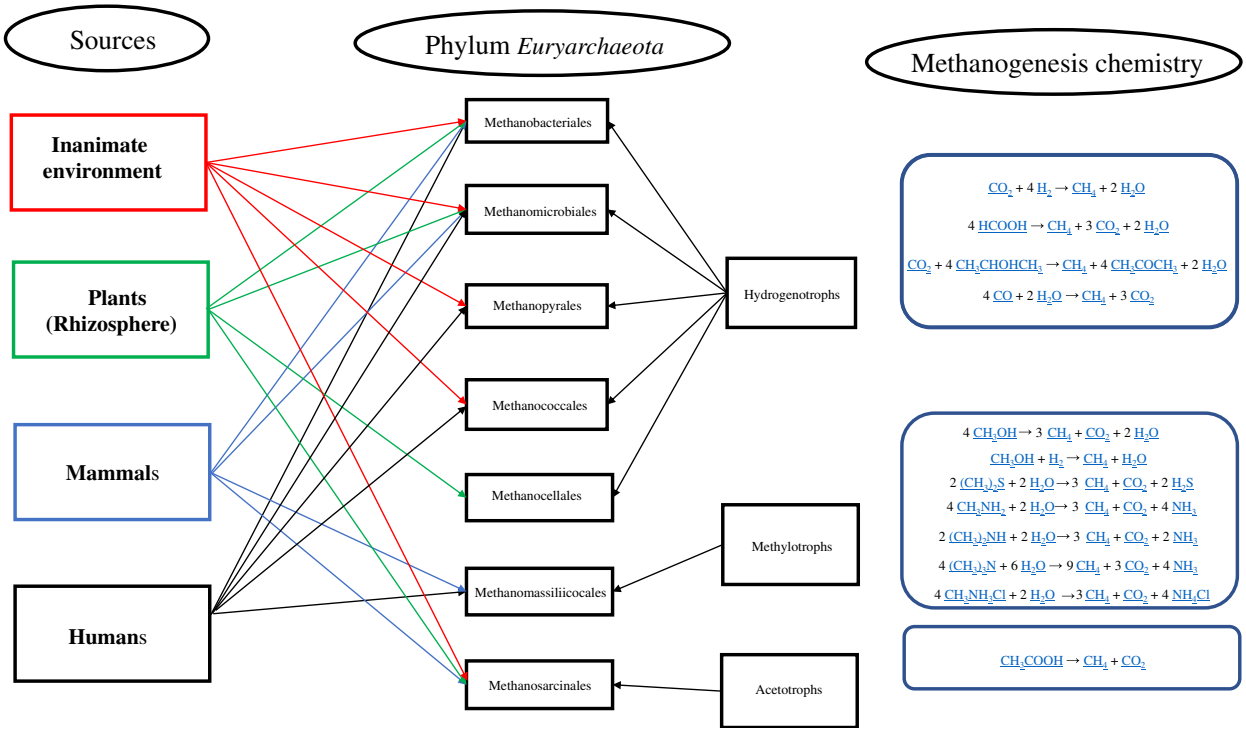

Fig. 1 Methanogens sources, biochemistry and phylogeny

significantly associated with periodontal disease based on comparisons of abundance between patients and controls and between diseased and healthy sites in the same patient [4]. Also, we recently observed that tobacco smoking increased the prevalence of detection of methanogens in the oral cavity, suggesting that environmental factors play a role in the repertoire variation of mucosa-associated methanogens [5].

Gastrointestinal tract microbiota

Only eight methanogen species have been cultured from the gastrointestinal microbiota, including *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanosphaera stadtmanae*, *Methanobrevibacter arbophilus* and *Methanobrevibacter massiliensis*, all from the order Methanobacteriales [1], and Ca. *Methanomethylophilus alvus*, Ca. *Methanomassiliicoccus intestinalis* and *Methanomassiliicoccus luminyensis* from the order Methanomassiliicoccales [6, 7]. The healthy human colon has extremely low oxygen concentrations and methanogens account for 10% of all gut anaerobes and actively support digestion efficiency [1]. Quantitative polymerase chain reaction (PCR)-based studies indicated that methanogens including *M. smithii* colonise the human digestive microbiota early after birth [8, 9], with the mother's gut or vaginal microbiota as probable sources of colonisation [10]. *Methanobrevibacter smithii* was first isolated in 1982 from faeces [11] and has a prevalence of up to 95.7%, while *M. stadtmanae* has a prevalence of up to 23% [12]. The latter has the lowest energy metabolism of all currently known methanogens, being totally dependent on acetate as a carbon source and showing methane production that requires methanol and hydrogen [13]. It has recently been shown that *M. smithii* and *M. stadtmanae* induce monocyte-derived dendritic cells [14]. Furthermore, *M. stadtmanae* also induces a strong pro-inflammatory cytokine release from monocyte-derived dendritic cells and is more common in patients with inflammatory bowel disease [14]. Another study on the newborn's stomach detected the presence of *M. smithii* in all newborns [9]. This observation indicates that *M. smithii* is an early inhabitant of the human stomach, colonising the gastric mucosa just after birth, with the gut microbiota of the mother being the likely source of colonisation.

Respiratory tract microbiota

Apart from a single metagenomic study demonstrating the presence of methanogen DNA sequences in lung biopsies [15], the presence of living methanogens along the respiratory tract remains to be studied in more detail.

Skin microbiota

The human skin microbiome consists of bacteria, archaea, eukaryotes and viruses. A study using archaea-specific 16S rRNA gene primers revealed the presence of archaea on the skin of 13/13 volunteers, with a relative abundance greater

than 4% in one individual. Thaumarchaeota sequences were detected on the palms of two individuals [16]. A recent study showed that archaeal signatures were more diverse and archaea also more abundant in individuals younger than 12 years of age or older than 60 years than in individuals from other age groups [17].

Vagina microbiota

A study by Belay et al. [18], performed on vaginal samples, showed that 2/12 patients cultured *M. smithii* in two patients diagnosed with bacterial vaginosis.

Detecting methanogens in the clinical microbiology laboratory

The clinical specimens

Saliva [5], oral cavity samples [4, 19], newborn gastric fluid samples [8, 9] and stools [1] have been sampled for the search of methanogens in microbiota. In addition, pus samples, including periodontitis pocket pus, muscular pus and brain abscess pus, have been collected for the search of methanogens in pathological situations [1] (Fig. 2). Clinical specimens can be collected at the patient's bedside directly into a sterile tube containing a transport SAB medium [20]. Upon reception in the laboratory, samples collected into routine sterile tubes will be inoculated into Hungate tubes containing SAB medium [20]. Hungate pioneered the culture of methanogens after having developed sealed tubes to preserve the anaerobic atmosphere: modern Hungate tubes feature a stream-sterilised screw cap with a 9-mm opening, a non-toxic, gas-impervious butyl rubber stopper and a disposable screw cap keeping an 80% H_2 and 20% CO_2 atmosphere [21]. Hungate tubes have a shelf life of 4 weeks when kept at 4 °C. Then, they have to be manipulated under strict anaerobic atmosphere and all further manipulations of Hungate tubes, including inoculation of specimens and preparation for subcultures, have to be performed in a strict anaerobic atmosphere.

Microscopic examination and morphology

Methanogens can be observed microscopically using a fluorescence in situ hybridisation (FISH) technique, combining an oligonucleotide probe targeting the 16S rRNA gene [22] or a probe targeting the methyl coenzyme-M reductase gene (*mcr*A) [23] and confocal laser scanning microscopy. FISH is a reliable method for the visualisation of methanogens in the oral [5] and gastric mucosa [9]. Although the oligonucleotide probes currently used for FISH are not species-specific, they do visualise a diplococcoid morphology for *M. smithii*

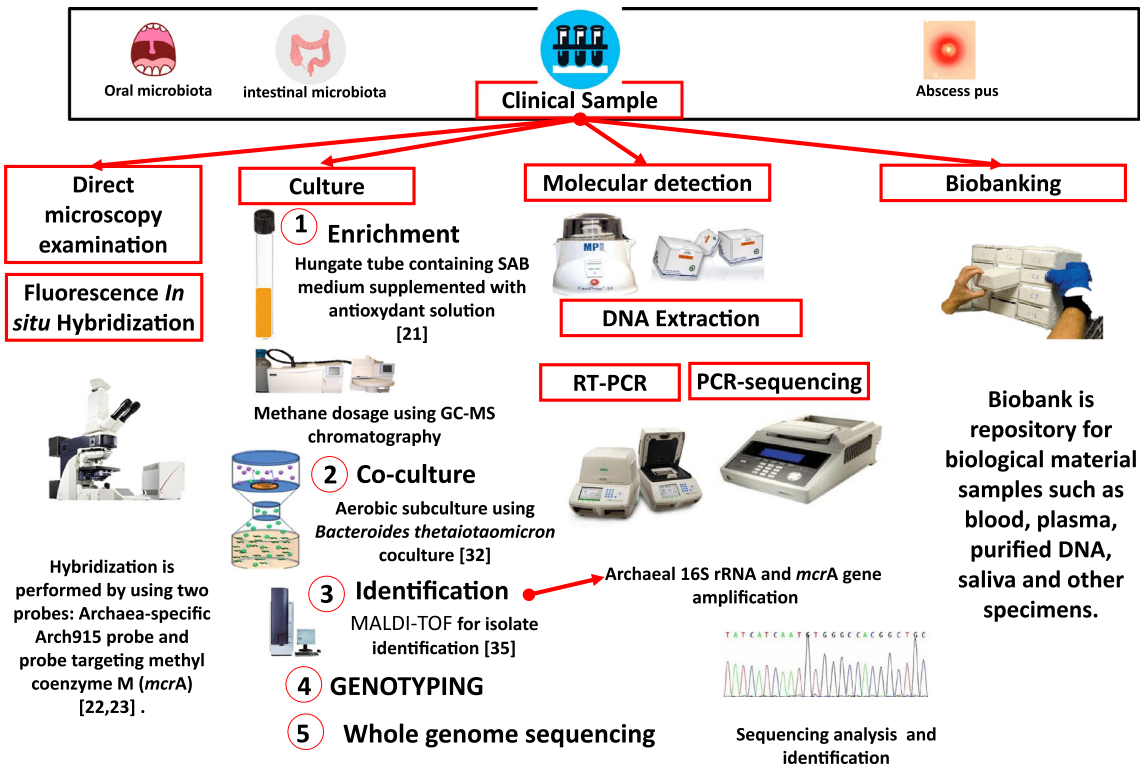

Fig. 2 Molecular techniques, culture techniques and microscopic techniques used in the microbiology laboratory for methanogens detection [21–23, 32, 35]

and a bacillary morphology for *M. oralis* [5]. The morphology of methanogens varies: coccoid forms are seen for members of the genera *Methanococcus* spp., *Methanosphaera* spp., *Methanococcoides* spp. and some *Methanobrevibacter* spp., the baton form is observed for *Methanobacterium* spp. and *Methanobrevibacter* spp., and the chain stick for *Methanopyrus* spp. [24]. Other methanogens may have a spiral shape (e.g. *Methanospirillum* spp. and *Methanoplanus* spp.), but these methanogens have not yet been detected in humans [25–27]. The irregular cocci forming sarcina (cell packages) is characteristic of *Methanosarcina* spp. [28]. *Methanolobus* spp., *Methanosarcina* spp. and *Methanobacterium* spp. can form cell aggregates as well [29].

DNA-based detection and identification

Molecular techniques are effective alternatives to culture for studying the prevalence of methanogens in human specimens and for studying the potential association of methanogens with human diseases, albeit they detect both living and dead methanogens [1]. DNA extraction is a crucial step upstream of PCR. One study compared different DNA extraction methods on 110 human stool samples and showed that a semi-automated protocol consisting of digestion with protein K coupled with a mechanical lysis step using glass powder was the fastest and most quantitative method [30]. There are two routinely used PCR-based detection systems for methanogens, one targeting the archaeal 16S rRNA gene [31] and the other targeting the *mcr*A gene [23]. Also, a real-time PCR protocol targeting the *rpo*B gene encoding the beta subunit of the RNA polymerase could be used for the detection of *M. smithii* and *M. stadtmanae* [12]. Numerous studies have detected *M. smithii*, *M. stadtmanae*, *M. arboriphilus*, *M. luminyensis*, *M. oralis*, *M. massiliense*, *Methanosarcina* and *Methanoculleus* sequences in the oral cavity and in the digestive tract [1, 5, 6, 12]. These studies have shown that members of the genus *Methanobrevibacter* spp. were dominant in the digestive tract and in the oral cavity [1, 4, 5].

Isolation and culture

The growth of any methanogen is detected in inoculated Hungate tubes through methane production. Gas chromatography (GC) makes it possible to detect methane release in the medium [24]. GC detects the presence of living methanogens but does not allow for more precise identification of methanogens [6, 9, 24]. Inoculated broth of Hungate tubes positive for methane production is subcultured onto solid SAB culture medium. In order to simplify the subculture process, we recently developed a new culture technique [32]. This technique uses two compartments for the aerobic culture of methanogens in the presence of *Bacteroides thetaiotaomicron* producing hydrogen, while the aerobic culture of methanogens is being made further possible by using antioxidants. In brief, the sample is seeded in the Hungate tube containing SAB broth supplemented with ascorbic acid, uric acid and glutathione as antioxidants and inoculated with *B. thetaiotaomicron* to produce hydrogen. Subcultures seeded on SAB medium supplemented with agar and deposited in the upper compartment and the lower compartment contain a culture of *B. thetaiotaomicron* [32] (Fig. 3). Methanogens are autofluorescent microorganisms and this feature could be used for the rapid detection of methanogen colonies (but not in clinical specimens) by epifluorescence microscopy [24]. Indeed, methanogens carry a factor 420, excited in green blue when exposed to UV light at a wavelength of 420 nm [21]. The factor 420 is a deazaflavin hydride carrier coenzyme comprising three cycles (Supplementary Fig. 1) [33]. F_{420} was purified and structurally characterised from the methanogen *Methanobacterium* sp. strain M.o.H. [33]. Methanogens use F_{420} for two reduction steps in methanogenesis going from methenyltetrahydromethanopterin to methyltetrahydromethanopterin, as well as for other hydride transfer reactions [33].

MALDI-TOF MS identification

The first-line identification of colonies can be done by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), a rapid technique for the identification of prokaryotes, eukaryotes and viruses [34]. To date, there is only one study on the identification of methanogens by MALDI-TOF MS [35]. The protocol consists of mechanically lysing a suspension of methanogen colonies with glass beads in an Eppendorf tube and two successive washes in water. A 1.5-µL volume of the suspension is deposited on the MALDI-TOF MS steel sample plate and covered with matrix, prior to analysis. Identification can be confirmed by detecting the 16S rRNA gene, the *mcr*A gene or the *rpo*B gene using qPCR or PCR sequencing as described above. Identification can be refined by genotyping colonies using multi-spacer typing, as previously described [36, 37]. Briefly, this technique relies on sequencing four PCR-amplified intergenic spacers in order to get a strain-specific fingerprint of the isolate. So far, 15 spacer types have been described for *M. smithii* [36] and nine for *M. oralis* [37]. Finally, the overall genetic information is obtained by whole genome sequencing (WGS), after appropriate DNA extraction and Illumina protocols as described above. *Methanobrevibacter smithii* genome sizes are between 1.8 and 2.1 Mb with a 30–32% GC value, whereas the *M. oralis* genome is between 2.12 and 2.15 Mb with a 27.7–28% GC value. These methanogens do not harbour plasmids or transposons.

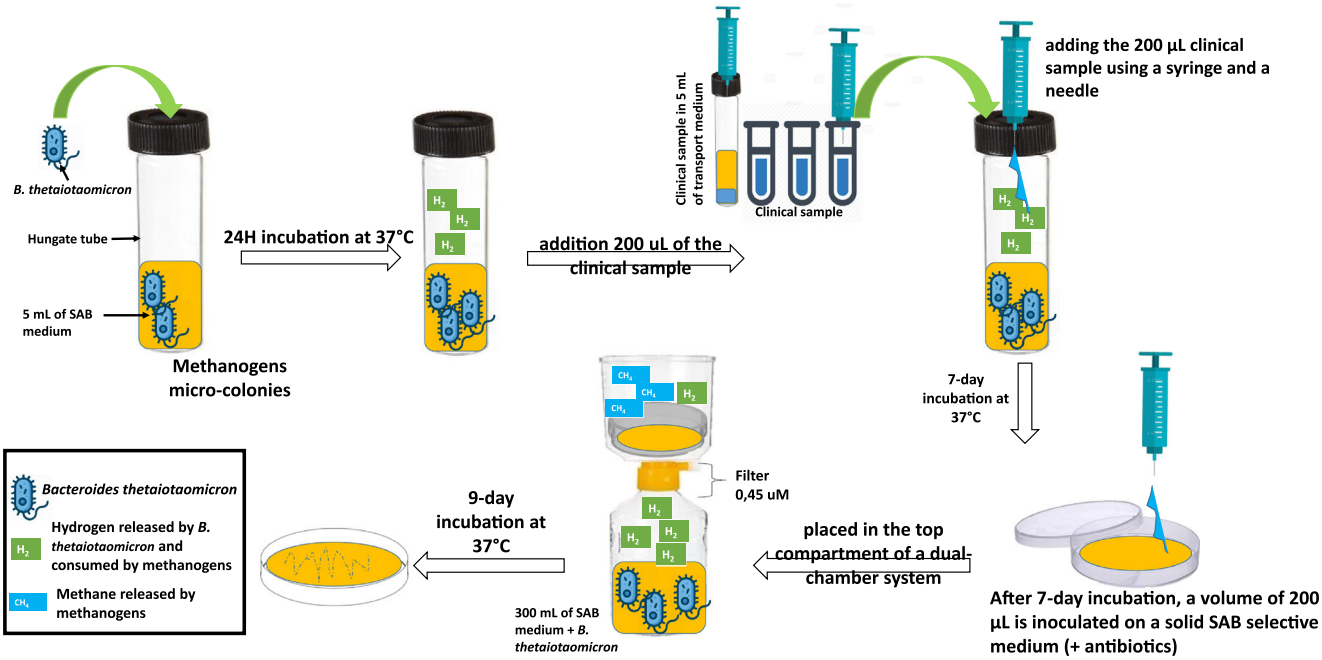

Fig. 3 Methanogens subculture method using *Bacteroides thetaiotaomicron* as the hydrogen source for methanogens strain. Adapted from reference [32]

In vitro antibiotic susceptibility testing

Most antibiotics used to fight bacteria are in vitro inactive against methanogens [38]. Indeed, intestinal *M. smithii* isolates are highly resistant to beta-lactams, aminoglycosides, glycopeptides, lincosamides and fluoroquinolones, and susceptible only to metronidazole, fusidic acid, rifampicin, bacitracin and squalamine [38, 39]. Lovastatin is a pro-drug, which needs to be metabolised by anaerobes before being active against *M. smithii* [1]. The in vitro susceptibility of methanogens to chloramphenicol is variable: *M. smithii*, *M. oralis* and *M. luminyensis* encode a chloramphenicol O-acetyltransferase and exhibit minimum inhibitory concentrations (MICs) of up to 25 mg/L, in contrast to *M. stadtmanae*, which exhibits an MIC of 4 mg/L [38].

Role of methanogens in diseases

Digestive tract dysbioses

Obesity

Gavage of *B. thetaiotaomicron*-inoculated germ-free mice with *M. smithii* led to weight gain, suggesting a role of the methanogen in weight gain and possibly obesity [40]. However, three clinical studies found exactly the opposite, that the prevalence of *Methanobrevibacter* spp., including *M. smithii*, was lower in obese individuals compared to controls, illustrating the limits of animal models in terms of human physiology and pathology [41].

Malnutrition

A recent study showed that *M. smithii* was not detected in the 20 stool samples of West African children suffering from severe malnutrition, while it was detected in 40–75% of the healthy controls. These findings provided important new insights into malnutrition, correlating altered redox metabolism with potentially irreversible disruption of host–archaeal–bacterial mutualism through anaerobic and methanogenic depletion [42].

Anorexia

Metagenomic studies revealed profound gut microbiome perturbations in anorexia nervosa, characterised by an unbalanced relative abundance between Gram-positive and Gram-negative bacterial species [41]. In addition, anorexia nervosa microbial communities were unexpectedly enriched in *M. smithii* in comparison with healthy subjects.

Digestive tract lesions

Some studies have shown the negative methanogen impact in different digestive tract lesions: colorectal cancer, inflammatory bowel diseases, irritable bowel syndrome and diverticulosis. In the case of colorectal cancer, the observed abundance and detection rate of species belonging to Methanobacteriales, *Fusobacterium* spp. and *Bacteroides* spp. may be a potential marker for the early detection of colorectal cancer [1]. A recent study showed a low incidence of methanogens in inflammatory bowel diseases using *mcr*A analysis [1]. The density of methanogens in patients with irritable bowel syndrome is

lower than in controls and the proportion of methane producers is significantly higher in patients with constipation-predominant bowel syndrome (58% methane producers) than in patients with diarrhoea-predominant bowel syndrome (28% methane producers) [43]. In 1986, a study of the incidence and concentration of methane-producing bacteria in enema samples of 130 specimens collected prior to sigmoidoscopy did not show any difference between the presence of methanogens in control patients and patients with diverticulosis [44]. However, a higher number of methanogen colonies was observed in the group with diverticulosis compared to controls [44]. One study found that patients with diverticulosis were more likely to be methane-producers and their mean breath methane concentrations were higher than in controls [45].

Oral cavity diseases

Methanogens were detected from subgingival samples of patients with periodontitis, peri-implantitis and infected root canals and in their saliva specimens. The diversity and abundance of methanogenic archaea in the oral cavity have been analysed by different methods, including the PCR sequencing methods targeting specific archaea, metagenomic methods and methanogen culture methods. According to those methods, the most abundant methanogen species in oral microbiota is the species *M. oralis*. In addition to *M. oralis*, several other methanogen species were detected, including *M. smithii*, *Methanosarcina mazei*, *Methanobacterium congolense* and *M. massiliensis*. Among these methanogens, only *M. oralis*, *M. smithii* and *M. massiliensis* have been cultivated [19]. Recently, using PCR sequencing of the 16S rRNA gene and quantitative PCR targeting the *mcr*A gene, the methanogens were described in peri-implantitis disease. Thirty peri-implantitis samples and 28 control samples were collected in 28 consenting peri-implantitis patients and the results showed the high prevalence of *M. oralis* in 31/58 (51%) samples, including 16/28 (57%) control samples and 15/30 (50%) peri-implantitis samples, followed by *M. massiliense* detected in 5/58 (8.6%) samples, including 3/28 (1%) control samples and 2/30 (6.7%) peri-implantitis samples. Fisher's exact test demonstrated that there was no difference in the prevalence of *M. oralis* or *M. massiliense* in peri-implantitis and control samples [46].

Brain abscess

Brain abscesses are focal infections that can present with a wide variety of symptoms and signs, depending on the number, location and size of the abscesses. The annual incidence of bacterial brain abscesses in the general population is as high as 0.3 to 1.3 per 100,000 population [47]. Brain abscesses are mainly caused by various microorganisms, such as pyogenic bacteria, *Mycoplasma* spp. or, less commonly, mycobacteria, fungi or protozoa.

A recent molecular and culture-based study has also shown the presence of methanogens in brain abscesses [47]. *Methanobrevibacter oralis* was co-cultivated with *Streptococcus intermedius* in the brain abscess samples. Further investigation of a series of 18 brain abscess specimens by RT-PCR detected *M. oralis* in 7/18 (38.9%) specimens, with an average gene copies/specimen of 1.01E+03 ± 1.14E+03/mL and *M. smithii* in 1/18 (5.6%) cases, with a gene copies/specimen of 1.88E+02/mL. The metagenomic study of 32 brain abscess samples showed that archaea formed 5–27% of the brain abscess microbiota. These archaea mostly belong to the Euryarchaeota (64.2%) and Crenarchaeota (29.7%); *M. thermoautotrophicus* and *M. marburgensis* were the most abundant species. Finally, *M. oralis* was shown to be pathogenic to neuronal tissues in a mouse model [47, 48].

Chronic paravertebral muscle abscess

A single study looked for methanogens by molecular testing by applying specific archaea 16S rRNA gene and methyl coenzyme M reductase (*mcr*A) PCR assays on ten human internal abscesses of various origins [49]. The authors detected *M. smithii* in one paravertebral muscular abscess from a 41-year-old man. This was the first time that *M. smithii* occurred in a paravertebral abscess [49].

Conclusions

Our review indicates that methanogens are linked to certain pathologies, but, thus far, the exact role of methanogens in these different pathologies remains unknown. Several questions remain to be addressed regarding the sources of methanogens and their dynamics in microbiota. The potential for inoculum effect in the induction of pathologies is unclear at this stage. The consortium effect of methanogens and anaerobes in pathologies needs to be better defined. Also, the mechanisms contributing to the development of a methanogenic disease, such as the potentially toxic role of methane, need to be investigated in much greater detail. So far, methanogens have always been co-isolated with anaerobic bacteria [47–49]. Efforts should be made to incorporate the detection, isolation and culture of methanogens in the routine practice of clinical microbiology laboratories, in order to increase knowledge on the repertoire of mucosae-associated methanogens, their infectious potential and to increase the knowledge on effective anti-methanogen drugs. The methods reviewed here could be easily implemented in modern clinical microbiology laboratories.

References

1. Nkamga VD, Henrissat B, Drancourt M (2017) Archaea: essential inhabitants of the human digestive microbiota. Hum Microb J 3:1–8
2. Lyu Z, Shao N, Akinyemi T, Whitman WB (2018) Methanogenesis. Curr Biol 28:R727–R732
3. Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, Hugenholtz P, Tyson GW (2016) Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nat Microbiol 1:16170
4. Nguyen-Hieu T, Khelaifia S, Aboudharam G, Drancourt M (2013) Methanogenic archaea in subgingival sites: a review. APMIS 121: 467–477
5. Grine G, Terrer E, Boualam MA, Aboudharam G, Chaudet H, Ruimy R, Drancourt M (2018) Tobacco-smoking-related prevalence of methanogens in the oral fluid microbiota. Sci Rep 8:9197
6. Dridi B, Fardeau ML, Ollivier B, Raoult D, Drancourt M (2012a) *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. Int J Syst Evol Microbiol 62:1902–1907
7. Nkamga VD, Drancourt M (2016) *Methanomassiliicoccus*. In: Whitman WB (ed) Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Chichester, vol 34, pp 1–7
8. Wampach L, Heintz-Buschart A, Hogan A, Muller EEL, Narayanasamy S, Laczny CC, Hugerth L, Bindl L, Bottu J, Andersson AF, de Beaufort C, Wilmes PW (2017) Colonization and succession within the human gut microbiome by archaea, bacteria, and microeukaryotes during the first year of life. Front Microbiol 8:738
9. Grine G, Boualam MA, Drancourt M (2017) *Methanobrevibacter smithii*, a methanogen consistently Colonising the newborn stomach. Eur J Clin Microbiol Infect Dis 36:2449–2455
10. Neu J, Rushing J (2011) Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. Clin Perinatol 38: 321–331
11. Miller TL, Wolin MJ, Conway de Macario E, Macario AJ (1982) Isolation of *Methanobrevibacter smithii* from human feces. Appl Environ Microbiol 43:227–232
12. Dridi B, Henry M, El Khéchine A, Raoult D, Drancourt M (2009) High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. PLoS One 4:e7063
13. Lurie-Weinberger MN, Gophna U (2015) Archaea in and on the human body: health implications and future directions. PLoS Pathog 11:e1004833
14. Bang C, Vierbuchen T, Gutsmann T, Heine H, Schmitz RA (2017) Immunogenic properties of the human gut-associated archaeon *Methanomassiliicoccus luminyensis* and its susceptibility to antimicrobial peptides. PLoS One 12:e0185919
15. Koskinen K, Pausan MR, Perras AK, Beck M, Bang C, Mora M, Schilhabel A, Schmitz R, Moissl-Eichinger C (2017) First insights into the diverse human archaeome: specific detection of archaea in the gastrointestinal tract, lung, and nose and on skin. MBio 8: e00824-17
16. Probst AJ, Auerbach AK, Moissl-Eichinger C (2013) Archaea on human skin. PLoS One 8:e65388
17. Moissl-Eichinger C, Probst AJ, Birarda G, Auerbach A, Koskinen K, Wolf P, Holman HN (2017) Human age and skin physiology shape diversity and abundance of Archaea on skin. Sci Rep 7:4039
18. Belay N, Johnson R, Rajagopal BS, Conway de Macario E, Daniels L (1988) Methanogenic bacteria from human dental plaque. Appl Environ Microbiol 54:600–603
19. Huynh HTT, Pignoly M, Nkamga VD, Drancourt M, Aboudharam G (2015a) The repertoire of archaea cultivated from severe periodontitis. PLoS One 10:e0121565
20. Khelaifia S, Raoult D, Drancourt M (2013a) A versatile medium for cultivating methanogenic archaea. PLoS One 8:e61563
21. Wolfe RS (2011) Techniques for cultivating methanogens. Methods Enzymol 494:1–22
22. Raskin L, Stromley JM, Rittmann BE, Stahl DA (1994) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. Appl Environ Microbiol 60:1232–1240
23. Luton PE, Wayne JM, Sharp RJ, Riley PW (2002) The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology 148:3521–3530
24. Dridi B (2012) Laboratory tools for detection of archaea in humans. Clin Microbiol Infect 18:825–833
25. Kurr M, Huber R, König H, Jannasch HW, Fricke H, Trincone A, Kristjansson JK, Stetter KO (1991) *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110°C. Arch Microbiol 156:239–247
26. Ollivier B, Cayol JL, Patel BKC, Magot M, Fardeau ML, Garcia JL (1997) *Methanoplanus petrolearius* sp. nov., a novel methanogenic bacterium from an oil-producing well. FEMS Microbiol Lett 147: 51–56
27. Zeikus JG, Henning DL (1975) *Methanobacterium arbophilicum* sp. nov. An obligate anaerobe isolated from wetwood of living trees. Antonie Van Leeuwenhoek 41:543–552
28. Kern T, Fischer MA, Deppenmeier U, Schmitz RA, Rother M (2016) *Methanosarcina flavescens* sp. nov., a methanogenic archaeon isolated from a full-scale anaerobic digester. Int J Syst Evol Microbiol 66:1533–1538
29. Kern T, Linge M, Rother M (2015) *Methanobacterium aggregans* sp. nov., a hydrogenotrophic methanogenic archaeon isolated from an anaerobic digester. Int J Syst Evol Microbiol 65:1975–1980
30. Khelaifia S, Ramonet PY, Bedotto Buffet M, Drancourt M (2013b) A semi-automated protocol for archaea DNA extraction from stools. BMC Res Notes 6:186
31. Vianna ME, Conrads G, Gomes BPFA, Horz HP (2006) Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol 44:1274–1282
32. Khelaifia S, Lagier JC, Nkamga VD, Guilhot E, Drancourt M, Raoult D (2016) Aerobic culture of methanogenic archaea without an external source of hydrogen. Eur J Clin Microbiol Infect Dis 35: 985–991
33. Allen KD, White RH (2018) Identification of the radical SAM enzymes involved in the biosynthesis of methanopterin and coenzyme F420 in methanogens. Methods Enzymol 606:461–483
34. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D (2009) Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49:543–551
35. Dridi B, Raoult D, Drancourt M (2012b) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identification of archaea: towards the universal identification of living organisms. APMIS 120:85–91
36. Nkamga VD, Huynh HT, Aboudharam G, Ruimy R, Drancourt M (2015) Diversity of human-associated *Methanobrevibacter smithii* isolates revealed by multispacer sequence typing. Curr Microbiol 70:810–815
37. Huynh HTT, Nkamga VD, Drancourt M, Aboudharam G (2015b) Genetic variants of dental plaque *Methanobrevibacter oralis*. Eur J Clin Microbiol Infect Dis 34:1097–1101
38. Dridi B, Fardeau ML, Ollivier B, Raoult D, Drancourt M (2011) The antimicrobial resistance pattern of cultured human methanogens reflects the unique phylogenetic position of archaea. J Antimicrob Chemother 66:2038–2044
39. Khelaifia S, Brunel JM, Raoult D, Drancourt M (2013c) Hydrophobicity of imidazole derivatives correlates with improved activity against human methanogenic archaea. Int J Antimicrob Agents 41:544–547

40. Samuel BS, Hansen EE, Manchester JK, Coutinho PM, Henrissat B, Fulton R, Latreille P, Kim K, Wilson RK, Gordon JI (2007) Genomic and metabolic adaptations of *Methanobrevibacter smithii* to the human gut. Proc Natl Acad Sci U S A 104:10643–10648
41. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D (2009) Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and methanogens in anorexic patients. PLoS One 4:e7125
42. Million M, Tidjani Alou M, Khelaifia S, Bachar D, Lagier JC, Dione N, Brah S, Hugon P, Lombard V, Armougom F, Fromonot J, Robert C, Michelle C, Diallo A, Fabre A, Guieu R, Sokhna C, Henrissat B, Parola P, Raoult D (2016) Increased gut redox and depletion of anaerobic and methanogenic prokaryotes in severe acute malnutrition. Sci Rep 6:26051
43. Triantafyllou K, Chang C, Pimentel M (2014) Methanogens, methane and gastrointestinal motility. J Neurogastroenterol Motil 20:31–40
44. Weaver GA, Krause JA, Miller TL, Wolin MJ (1986) Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. Gut 27:698–704
45. Yazici C, Arslan DC, Abraham R, Cushing K, Keshavarzian A, Mutlu EA (2016) Breath methane levels are increased among patients with diverticulosis. Dig Dis Sci 61:2648–2654
46. Belkacemi S, Mazel A, Tardivo D, Tavitian P, Stephan G, Bianca G, Terrer E, Drancourt M, Aboudharam G (2018) Peri-implantitis-associated methanogens: a preliminary report. Sci Rep 8:9447
47. Drancourt M, Nkamga VD, Lakhe NA, Régis JM, Dufour H, Fournier PE, Bechah Y, Scheld WM, Raoult D (2017) Evidence of archaeal methanogens in brain abscess. Clin Infect Dis 65:1–5
48. Nkamga VD, Lotte R, Chirio D, Lonjon M, Roger PM, Drancourt M, Ruimy R (2018) *Methanobrevibacter oralis* detected along with *Aggregatibacter actinomycetemcomitans* in a series of community-acquired brain abscesses. Clin Microbiol Infect 24:207–208
49. Nkamga VD, Lotte R, Roger PM, Drancourt M, Ruimy R (2016) *Methanobrevibacter smithii* and *Bacteroides thetaiotaomicron* cultivated from a chronic paravertebral muscle abscess. Clin Microbiol Infect 22:1008–1009