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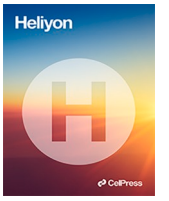

Review article

Methanobrevibacter smithii cell variants in human physiology and pathology: A review

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ABSTRACT

Methanobrevibacter smithii (*M. smithii*), initially isolated from human feces, has been recognised as a distinct taxon within the Archaea domain following comprehensive phenotypic, genetic, and genomic analyses confirming its uniqueness among methanogens. Its diversity, encompassing 15 genotypes, mirrors that of biotic and host-associated ecosystems in which *M. smithii* plays a crucial role in detoxifying hydrogen from bacterial fermentations, converting it into mechanically expelled gaseous methane. In microbiota in contact with host epithelial mucosae, *M. smithii* centres metabolism-driven microbial networks with *Bacteroides*, *Prevotella*, *Ruminococcus*, *Veillonella*, *Enterococcus*, *Escherichia*, *Enterobacter*, *Klebsiella*, whereas symbiotic association with the nanoarchaea *Candidatus* Nanopusillus phoceensis determines small and large cell variants of *M. smithii*. The former translocate with bacteria to induce detectable inflammatory and serological responses and are co-cultured from blood, urine, and tissular abscesses with bacteria, prototyping *M. smithii* as a model organism for pathogenicity by association. The sources, mechanisms and dynamics of *in utero* and lifespan *M. smithii* acquisition, its diversity, and its susceptibility to molecules of environmental, veterinary, and medical interest still have to be deeply investigated, as only four strains of *M. smithii* are available in microbial collections, despite the pivotal role this neglected microorganism plays in microbiota physiology and pathologies.

1. Introduction

Forty years ago, Blach and Wolfe successfully isolated a methanogenic archaea (herein referred to as methanogen) initially named "*Methanobrevibacter ruminantium* PS strain" from sludge [1]. This strain, later identified as *Methanobrevibacter smithii* (*M. smithii*) PS strain, was subsequently cultured from faecal samples obtained from four apparently healthy individuals under anaerobic conditions and observed using fluorescence microscopy [2]. These breakthroughs were followed by the indirect detection of *M. smithii* in the human digestive tract by measuring methane (CH_4) [3,4]. An enumeration method indicated that *M. smithii* was the most abundant methanogen in human feces [5] and was virtually present in 95.7 % of apparently healthy individuals [6]. Its certified absence in feces has been observed only in young patients suffering from life-threatening acute malnutrition [7]. Later observations underlined the key role of *M. smithii* in gut microbiota homeostasis, while further clinical studies implicated *M. smithii* in three lines of pathologies, including dysbiosis, abscesses, and archaemia (its presence in the blood) [8–10].

Over 40 years of cumulative reports related to *M. smithii* in mucosae-associated microbiota physiology and pathology, including

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notable contributions from our group of research to this field, incited us to comprehensively review data regarding the clinical microbiology of *M. smithii* in complex microbial networks. This work recently centred on symbiotic *Candidatus* Nanopusillus phoceensis, which may determine small and large cell variants exhibiting differential capabilities to act as opportunistic pathogens, always associated with bacteria.

1.1. Bibliography strategy

This review was based on the scientific literature published up to September 2023 available in Medline via PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar databases. To describe *M. smithii* and its presence in animals, the environment, and humans, the following query was generated and searched for in titles and abstracts: ("*Methanobrevibacter smithii*") AND ("Human" OR "Microbiota" OR "Environments" OR "Environment" OR "Environmental" OR "Sewer" OR "Sediments" OR "Sea" OR "Marine" OR "River" OR "Water" OR "Wastewater" OR "Waste" OR "Wastes" OR "Biomass" OR "Soil" OR "Soils" OR "Coal" OR "Bioreactor" OR "Reactors" OR "Plant" OR "Plants" OR "Rhizosphere" OR "Anaerobic digestion" OR "Halophile" OR "Halophilic" OR "Thermophilic" OR "Extremophile" OR "Extremophilic" OR "Rumen methanogens" OR "animals" OR "millipedes" OR "pigs" OR "Rumen" OR "Ruminants" OR "livestock" OR "protozoa" OR "Food" OR "Foodstuffs"). The initial screening of articles involved evaluating titles and abstracts against specific inclusion and exclusion criteria. To be included, articles had to meet certain conditions, such as being original research articles published in English and specifically addressing the detection and isolation of *M. smithii* in the context of human, animal, and environmental studies. Conversely, articles were excluded if they were published in languages other than English or if they were repetitions of previously included articles. Following the initial selection, the complete texts of chosen articles were thoroughly examined to gather pertinent information. Additionally, a manual search was conducted by exploring the references cited in the previously selected papers, aiming to identify and include any relevant articles that might contribute to the overall understanding of the subject. A total of 252 articles were retrieved from PubMed and 6260 articles from Google Scholar. After selection steps, based on titles and abstracts, exclusion of duplications, selection of original papers only written in English language, 166 papers were included in this review.

2. Bibliometry

According to the search results in the PubMed database, the first publication regarding *M. smithii* was in 1981 and publications have gradually increased since then up to 252. By means of control, this can be compared to work on *Staphylococcus aureus*, which showed a huge number of related papers (n = 63 899) (Supplementary Fig. S1). Accordingly, 95 genomes were available in GenBank (https://www.ncbi.nlm.nih.gov/genome) at September 2023, comprising in detail 34 genomes derived from *M. smithii* isolates and 61 genomes derived from metagenome-assembled genomes (MAGs). Moreover, only four genome sequences were complete, 22 at scaffold assembly level and 69 at contig level. In total, 76/95 (80 %) genomes derived *M. smithii* from the human digestive tract, especially the gut, while four extra-digestive tract *M. smithii* genomes (all made in our laboratory) included two human blood isolates, one brain abscess isolate, and one breast milk isolates. Fifteen extra-human genomes included ten genomes derived from pig guts, two from the digestive system of mice, two from monkey guts, and one reference ATCC 35061 genome from an anthropic sewage digester (Table 1).

3. Antiquity of *M. smithii* in human microbiota

Metagenomic studies detected *M. smithii* ancient DNA (aDNA) in 2/161 (1.24 %) human dental roots which were between 2223 and 3023 years old and one human dental calculus between 13 780 and 14 180 years old, collected from archaeological sites located in Maslomecz and Niemcza, both in Poland, and San Teodoro in Italy [11,12]. More recently, genomic investigations of eight human palaeofaeces which were older than 1000 years, derived from Boomerang Shelter (Utah, USA), the Arid West Cave (in Southwest USA, precise location unknown) and Zape (La Cueva de los Muertos Chiquitos, Mexico), yielded 181 MAGs, including two ancient *M. smithii* genomes from two Boomerang Shelter samples representative of two ancient *M. smithii* lineages which may have diverged around 85 000 years ago [13]. These data traced *M. smithii* as an ancient companion of the human digestive tract, suggesting its relative insusceptibility to environmental and population changes over such extensive spatial and temporal ranges (Fig. 1).

4. Sources for *M. smithii* microbiota colonisation

Little is known about the sources of *M. smithii* and its dynamics along the digestive tract during the human lifespan. In this paper, we review the environmental ecosystems of newborns, children and adults in whom *M. smithii* has been detected, which could constitute sources of contamination during the lifetime of the individuals.

4.1. Abiotic environment

The close connection between *M. smithii* and human faecal matter, demonstrated by its detection through PCR amplification in human sewage samples, has led to *M. smithii* being proposed as a biomarker for faecal contamination of recreational waters, rivers, beaches, and even rain water [14–23]. For example, water samples collected from Arroyo Burro (AB) (n = 6) and Mission Creek (MC) (n = 8) in Santa Barbara, California, and investigated using the *M. smithii nifH* gene standard PCR revealed four positive samples (AB: 1/6; MC: 3/8) and three positive samples (AB: 1/6; MC: 2/8) during the dry and wet weather seasons, respectively [18]. Of 21

Table 1
Summary of 95 *M. smithii* genomes available in Genbank till September 2023. The variant type, source, assembly accession, sequencing method, assembly level, genome length, number of coding sequences (CDS), number of CRISPR, coding ratio, GC%, and number of sequences were shown for each genome. (https://www.ncbi.nlm.nih.gov/genome/; https://dfast.ddbj.nig.ac.jp/dfc/).

M. smithii species	Variant type	Source	Assembly accession
ATCC 35061; PS; DSMZ 861	strain	Sewage digester	GCA_000016525.1
KB11	strain	Human gut	GCA_002813085.1
CE91-St68	strain	Human gut	GCA_022846175.1
CE91-St67	strain	Human gut	GCA_022846155.1
DSM 2375	strain	Human gut	GCA_000151245.1
DSM 2374	strain	Human gut	GCA_000151225.1
CAG:186	Metagenomics	Human gut	GCA_000437055.1
MGYG-HGUT-02163	isolate	Human gut	GCA_902384055.1
PRJEB30652	isolate	Human blood	GCA_900654205.1
L1_008_000M1	Metagenomics	Human gut	GCA_018379875.1
MCC662	strain	Human gut	GCA_018784305.1
B73_50.fa	Metagenomics	Human gut	GCA_021641085.1
MTG248_bin.32.fa	Metagenomics	Human gut	GCA_934879315.1
MTG240_bin.3.fa	Metagenomics	Human gut	GCA_934882215.1
MTG237_bin.37.fa	Metagenomics	Human gut	GCA_934854605.1
MTG238_bin.31.fa	Metagenomics	Human gut	GCA_934882835.1
MTG233_bin.9.fa	Metagenomics	Human gut	GCA_934841515.1
MTG232_bin.10.fa	Metagenomics	Human gut	GCA_934834405.1
MTG239_bin.1.fa	Metagenomics	Human gut	GCA_934882635.1
MTG246_bin.5.fa	Metagenomics	Human gut	GCA_934876175.1
MTG247_bin.44.fa	Metagenomics	Human gut	GCA_934881615.1
MTG235_bin.19.fa	Metagenomics	Human gut	GCA_934864325.1
MTG236_bin.38.fa	Metagenomics	Human gut	GCA_934864615.1
MTG234_bin.2.fa	Metagenomics	Human gut	GCA_934836805.1
S25M_St_metabat_50	Metagenomics	Monkey gut	GCA_030539805.1
S8M_St_25	Metagenomics	Monkey gut	GCA_030539535.1
TS145A	strain	Human gut	GCA_000189795.2
TS145B	strain	Human gut	GCA_000189815.2
TS146A	strain	Human gut	GCA_000189835.2
TS146B	strain	Human gut	GCA_000189855.2
TS146C	strain	Human gut	GCA_000189875.2
TS146D	strain	Human gut	GCA_000189895.2
TS146E	strain	Human gut	GCA_000189915.2
TS147A	strain	Human gut	GCA_000189935.2
TS147B	strain	Human gut	GCA_000189955.2
TS147C	strain	Human gut	GCA_000189975.2
TS94A	strain	Human gut	GCA_000189995.2
TS94B	strain	Human gut	GCA_000190015.2
TS94C	strain	Human gut	GCA_000190035.2
TS95A	strain	Human gut	GCA_000190055.2
TS95B	strain	Human gut	GCA_000190075.2
TS95C	strain	Human gut	GCA_000190095.2
TS95D	strain	Human gut	GCA_000190115.2
TS96A	strain	Human gut	GCA_000190135.2
TS96B	strain	Human gut	GCA_000190155.2
TS96C	strain	Human gut	GCA_000190175.2
ACE6	strain	Human brain abscess	GCA_000824705.1
WWM1085	strain	Human gut	GCA_002252585.1
C2 CSUR P5816	strain	Women breast milk	GCA_900650605.1
MGYG-HGUT-02446	isolate	Human gut	GCA_902387325.1
PRJEB32190	isolate	Human blood	GCA_901111125.1
COPD437	Metagenomics	Human gut	GCA_014871915.1
MAG004	Metagenomics	Pig gut	GCA_016294185.1
ERR260234-bin.14	Metagenomics	Human gut	GCA_905209735.1
SRR413754-bin.38	Metagenomics	Human gut	GCA_905207865.1
ERR1297821-bin.15	Metagenomics	Human gut	GCA_905206215.1
min17_bin38	Metagenomics	Human gut	GCA_928722215.1
SUG1019	Metagenomics	Pig gut	GCA_022784485.1
SRR14474043_bin.15_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945607175.1
SRR15732360_bin.19_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945868565.1
ERR1855546_bin.25_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945864075.1
SRR15057922_bin.11_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945928295.1
SRR17382103_bin.13_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945873965.1
SRR14812344_bin.14_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_946407825.1
SRR5240748_bin.9_metaWRAP_v1.3_MAG	Metagenomics	Pig gut	GCA_945830685.1
SRR19721633_bin.21_metawrap_v1.3_MAG	Metagenomics	Mouse digestive system	GCA_947002465.1

(*continued on next page*)

Table 1 (*continued*)

M. smithii species	Variant type	Source	Assembly accession	Assembly level	Method	Genome length	Country	CDS
VEG01_HAM_0019	Metagenomics	Human gut	GCA_025992465.1	Complete genome	WGS	1853160	United States	1789
VEG01_HIC_0070	Metagenomics	Human gut	GCA_025992335.1	Complete genome	WGS	1805545	South Korea	1756
SRR7721896_bin.58_CONCOCT_v1.1_MAG	Metagenomics	Human gut	GCA_938021325.1	Complete genome	WGS	1791155	Japan	1716
ERR688525_bin.5_CONCOCT_v1.1_MAG	Metagenomics	Human gut	GCA_938044845.1	Complete genome	WGS	1791153	Japan	1719
SRR17622767_bin.81_metawrap_v1.3_MAG	Metagenomics	Mouse digestive system	GCA_948471195.1	Scaffolds	WGS	1713248	United States	1679
SUG2190	Metagenomics	Pig gut	GCA_028722865.1	Scaffolds	WGS	1729275	United States	1682
ERR9354249_bin.30_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958349965.1	Scaffolds	MAG	1729973	Denmark	1707
ERR8263739_bin.32_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958346845.1	Scaffolds	WGS	1729275	United States	1682
ERR9578286_bin.9_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958428575.1	Scaffolds	WGS	1722603	France	1671
ERR9762707_bin.13_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958427485.1	Scaffolds	MAG	1850699	United States	1767
ERR9492527_bin.37_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958408655.1	Scaffolds	WGS	1785820	Republic of Ireland	1710
SRR18940298_bin.13_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958452655.1	Scaffolds	MAG	1532323	South Africa	1338
ERR9492520_bin.14_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958408285.1	Scaffolds	MAG	1715977	Australia	1690
ERR9322428_bin.27_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958404925.1	Scaffolds	MAG	1701208	Australia	1682
SRR18940299_bin.15_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958454305.1	Scaffolds	MAG	1733784	Australia	1697
SRR17382081_bin.21_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958450395.1	Scaffolds	MAG	1713615	Australia	1680
SRR22541668_bin.8_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958434505.1	Scaffolds	MAG	1732067	Australia	1692
ERR8263824_bin.44_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958406505.1	Scaffolds	MAG	1735187	Australia	1693
ERR9578260_bin.2_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958428305.1	Scaffolds	MAG	1738989	Australia	1693
ERR9610937_bin.21_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958441245.1	Scaffolds	MAG	1647299	Australia	1647
ERR9762677_bin.15_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_958367155.1	Scaffolds	MAG	1672903	Australia	1650
SRR20881984_bin.37_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959026905.1	Scaffolds	MAG	1725316	Australia	1680
SRR13494530_bin.17_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959022255.1	Scaffolds	MAG	1708876	Australia	1692
SRR20881978_bin.12_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959018705.1	Scaffolds	MAG	1690036	Australia	1587
SRR13494517_bin.23_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959026265.1	Scaffolds	MAG	1749155	Germany	1754
SRR16280099_bin.15_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959603635.1	Scaffolds	MAG	1472871	Germany	1406
SRR16244389_bin.33_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959605265.1	Contigs	WGS	1782421	United States	1775
SRR16244405_bin.3_MetaWRAP_v1.3_MAG	Metagenomics	Human gut	GCA_959596935.1	Contigs	WGS	1796940	United States	1863
N1173.2	Metagenomics	Human gut	GCA_030841835.1	Contigs	WGS	1791079	United States	1799
				Contigs	WGS	1793406	United States	1803
				Contigs	WGS	1947167	United States	2308
				Contigs	WGS	1712884	United States	1673
				Contigs	WGS	1951914	United States	1883
				Contigs	WGS	2008849	United States	1958
				Contigs	WGS	1964775	United States	1904
				Contigs	WGS	1972568	United States	2022
				Contigs	WGS	1888757	United States	1785
				Contigs	WGS	1885844	United States	1823

(*continued on next page*)

Table 1 (*continued*)

Assembly level	Method	Genome length	Country	CDS	Nb of CRISPR	Coding ratio %	GC%	Nb of sequences
Contigs	WGS	1910054	United States	1795	2	90.2	31	1
Contigs	WGS	1992013	United States	1954	1	89.5	31.2	1
Contigs	WGS	1972225	United States	1887	1	89.8	31.1	1
Contigs	WGS	1978848	United States	1865	1	89.9	31.1	1
Contigs	WGS	2011683	United States	1846	1	88.9	31.3	9
Contigs	WGS	1974662	United States	1842	1	89.5	31.3	10
Contigs	WGS	1869016	United States	1735	0	88.3	31.2	30
Contigs	WGS	1818091	United States	1735	1	89.5	31.3	10
Contigs	WGS	1718384	France	1644	1	89.8	31.2	14
Contigs	WGS	1926345	United States	1812	2	86.1	31	22
Contigs	WGS	1720664	France	1667				
Contigs	WGS	1926345	United States	1812				
Contigs	WGS	1712416	France	1660				
Contigs	MAG	1837225	Australia					
Contigs	MAG	1590692	China	1546				
Contigs	MAG	1807363	Sweden	1739				
Contigs	MAG	1793403	China	1740				
Contigs	MAG	2453823	Netherlands	1831				
Contigs	MAG	1636575	France	1670				
Contigs	MAG	1633763	Canada	1581				
Contigs	MAG	1599471	China	1564				
Contigs	MAG	1507832	China	1437				
Contigs	MAG	1306612	Germany	1259				
Contigs	MAG	1725827	France	1722				
Contigs	MAG	1712501	China	1676				
Contigs	MAG	1611557	Canada	1532				
Contigs	MAG	1306612	Germany	1259				
Contigs	MAG	1302826	United States	1161				
Contigs	MAG	1792275	South Korea	1749				
Contigs	MAG	1715912	South Korea	1663				
Contigs	MAG	1721518	China	1699				
Contigs	MAG	1929878	Austria	1810				
Contigs	MAG	1877634	Canada	1818				
Contigs	MAG	1191129	Canada	984				
Contigs	MAG	1857507	Denmark	1866				
Contigs	MAG	1607020	Netherlands	1557				
Contigs	MAG	1863041	Germany	1819				
Contigs	MAG	1787270	Sweden	1721				
Contigs	MAG	1894993	China	1787				
Contigs	MAG	1761494	Italy	1711				
Contigs	MAG	1789153	China	1710				
Contigs	MAG	1893122	Denmark	1799				
Contigs	MAG	1792464	Italy	1738				
Contigs	MAG	1550003	India	1402				
Contigs	MAG	1639372	China	1525				
Contigs	MAG	1673800	Netherlands	1484				
Contigs	MAG	1560492	Germany	1352				
Contigs	MAG	919190	China	774				
Contigs	MAG	886946	Sweden	748				
Contigs	MAG	1293539	United States	1176				
Contigs	MAG	1191129	Japan	984				
Contigs	MAG	1685532	United States	1639				
Contigs	MAG	1191129	Japan	984				
Contigs	MAG	1767959	Mongolia	1716				
Contigs	MAG	1809816	Malaysia	1763				
Contigs	MAG	1075103	Malaysia	823				
Contigs	MAG	1792464	United States	1738				

(*continued on next page*)

Table 1 (*continued*)

Nb of CRISPR	Coding ratio %	GC%	Nb of sequences
1	85.1	31.2	97
0	77	32	324
1	88.8	31.1	16
1	90.3	31.2	9
1	90.5	31.1	11
1	89.3	31.2	22
0	89.7	31.1	14
1	88.5	31.1	17
1	98.4	31.1	20
1	89.1	31.2	27
1	90	31.2	11
1	86.4	31.1	23
1	87.4	31.1	35
0	82.1	31.1	103
1	89.5	31.2	7
0	80.8	31.9	170
0	86.9	31.1	43
1	87.1	31.1	51
1	84.4	31.2	60
1	88.1	31.1	35
1	85.2	31.2	100
0	87.5	31.1	31
2	86.8	30.4	62
2	84.8	30.5	60
1	87	30.4	38
0	87.9	30.4	37
0	84.9	30.3	43
0	86.1	30.3	57
0	86.6	30.3	50
2	86.2	30.2	55
2	87.6	30.3	42
2	87.9	30.2	37
3	85.6	30.3	58
3	86.8	30.3	45
3	86.4	30.3	44
1	85.1	31.2	107
1	88.6	31.5	87
2	88.8	30.3	16
1	89.9	31.2	11
2	88.8	30.3	16
1	89.4	31.1	27
0		31.2	35
0	87.2	31.5	49
2	87	30.5	36
2	89.9	31.1	29
1	44.8	31.7	1328
3	87.3	31.2	31
0	85.8	31.4	43
0	85.8	31.4	71
0	82.2	31.7	142
0	83	32.2	200
2	87.4	31.2	33
1	85.2	31.2	51
0	83.2	31.4	94
0	83	32.2	200
2	79.5	32	220
1	90	31.1	1
1	89.5	31.3	1
1	86.8	34	34
2	86.3	30.2	22
0	83.8	31.1	117
0	72.7	31.5	234
1	89.3	31.2	27
1	89.7	31.4	49
4	88.7	31.1	14
2	88.7	31	13
2	87.9	30.3	19
3	89.6	31.1	15
3	88	31	21

(*continued on next page*)

Table 1 (*continued*)

Nb of CRISPR	Coding ratio %	GC%	Nb of sequences
2	86.1	30.3	38
2	87.8	31	20
0	77.6	31.2	136
2	80	31.4	158
1	78.9	30.8	194
1	72.9	30.3	209
0	68.1	31.7	203
0	66.6	31.5	197
0	80.3	31.6	199
0	72.7	31.5	234
0	89	31.3	64
0	72.7	31.5	234
3	89	31.1	24
3	90.1	31.1	16
0	63.2	30.9	253
2	87.8	31	20

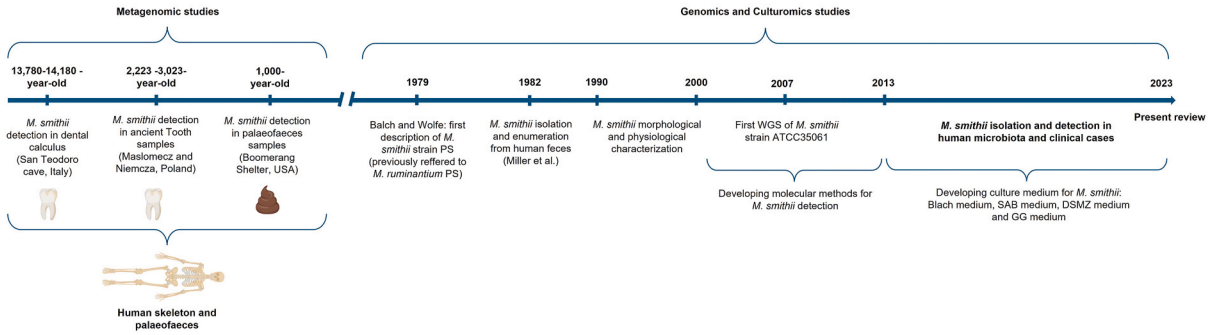

Fig. 1. *Methanobrevibacter smithii* from antiquity to present. Two parts of *M. smithii* investigation from human samples (dental calculus, feces) were shown. Metagenomics studies succussed to detect *M. smithii* DNA from ancient oral cavity and stool samples. Using genomics and culturomics approaches, first description and isolation of *M. smithii* was from human feces by Balch and Wolfe followed by several works about *M. smithii* enumeration in stools, microscopy observation, culture media development and genomic annotations.

environmental water samples collected from a Fitzgibbon (FG) stormwater drain, the Brisbane river (BR), Cabbage Tree Creek, Oxley Creek and Wivenhoe Dam in Australia, two samples derived from FG and BR were positive, using the same detection assay as above [20].

4.2. Animals

M. smithii inhabits the gastrointestinal tracts of various herbivore and ruminant mammals [24–28]. This archaeon plays a pivotal role in the digestive processes in these animals by aiding the breakdown of complex carbohydrates present in their diets [29]. In the stomach of ruminants, including cows and sheep, *M. smithii* thrives in the rumen, contributing to methane production during the fermentation of cellulose-rich plant material, later expelled by the animal through belching [30,31]. Similarly, *M. smithii* is found in the intestines of non-ruminant herbivores such as horses and rabbits, where it plays a comparable role in methane production during the digestion of fibrous plant material [27,32]. While essential for efficient energy extraction from cellulose-rich diets, methane generated by *M. smithii* and other methanogens in the digestive tract of animals also poses environmental challenges, due to its contribution to greenhouse gas emissions, preventing high atmosphere dissipation of energy from Earth [33,34]. As such, ongoing research seeks to balance the nutritional benefits of methanogens with strategies for methane reduction in livestock [35]. Accordingly, the study of *M. smithii* in animal models is pivotal to comprehending its role in the complex ecology of the gastrointestinal microbiome and its implications in health and disease. Rodent models, specifically germ-free and gnotobiotic mice, have been frequently used to identify *M. smithii* interactions with the host and other resident microbes [36–38]. Owing to their analogous anatomy and physiology to humans, pig models are also highly relevant, providing insights into the effects and interactions of *M. smithii* in the gastrointestinal environment [39–41].

4.3. Humans and the dynamics of acquisition

Emphasising the critical role of breastfeeding in promoting healthy infant gut microbiota, *M. smithii* was detected using real-time PCR (RT-PCR) in 27.3 % colostrum and 26.3 % milk samples [42], cultured from three colostrum and five milk samples, and a complete

genome sequence (Whole Genome Sequencing: WGS) was released for the breast milk strain C2 CSUR P5920 [42]. Accordingly, *M. smithii* was detected by PCR of the archaeal 16S rRNA gene followed by Sanger sequencing in 50/50 newborn gastric juice samples collected from day-old newborns [43]. These were further cultured in 35/50 samples in Hungate tubes containing SAB medium [44] and *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) as the source of H_2, including 34 collected from breastfed and one from formula milk-fed neonates [43]. Further detection of *M. smithii* in meconial samples from newborns, occurring prior to any exposure to external food sources and in the absence of any evidence of contamination, has sparked a re-evaluation of conventional notions surrounding foetal microbial colonisation [45]. The correlation between C-section delivery and meconial *M. smithii* (*P* value = 0.004) [45] suggested the prospect of *in utero* colonisation of *M. smithii* by transferring it from the maternal gut microbiota to the placenta through the bloodstream [46], and excluded the vaginal transfer of *M. smithii*, as the physiological vaginal microbiota does not harbour *M. smithii* with the exception of cases of vaginosis [47]. Moreover, the detection of *M. smithii* in the neutral stomach juices of one- or two-day old infants adds an intriguing layer to our understanding of early microbial colonisation and suggests that *M. smithii* may be encountered even before the establishment of a diverse diet or exposure to external sources of nutrition, strongly linking it to breastfeeding, as *M. smithii* has previously been detected in maternal breast milk [42,43]. The transition from a milk-based diet to a more varied diet including complex carbohydrates, fibres and proteins is associated with significant changes in the gut microbiota [48,49]. Conversely, antibiotics prescribed in childhood can imbalance the gut microbiota [7,50]. Moreover, dietary changes, illness, and other environmental influences can also affect the abundance and activity of *M. smithii* during these formative years [51–53] (Fig. 2). Moreover, pH plays a crucial role in the acquisition of *M. smithii* and its colonisation of the human digestive tract. *M. smithii* does not survive under pH 5.6 (G. Grine, personal data). Accordingly, the immature gastric environment of newborns, marked by a neutral pH, facilitates the acquisition of *M. smithii* [43]. In contrast, the mature gastric environment, which develops acidity as early as two to three months of age, may no longer favour the presence of *M. smithii* [54]. In summary, *M. smithii* prevalence was measured during different age ranges from birth to 60–100 years, a diagram showing this variation is presented in Supplementary Fig. S2 [55]. Chi-2 test between *M. smithii* prevalence in ages 0–10 years and 60–100 years yielded a stringency significant differences between the two values (*p*-value: 0.0016).

5. Human microbiota

5.1. Oral microbiota

M. smithii was isolated from the oral microbiota in the background of periodontal disease samples, after the detection of methane in 20/54 samples collected from oral cavities was revealed using gas chromatography, and *M. smithii* was shown in three samples by indirect immunofluorescence and electron microscopy [56]. An investigation of 200 oral samples collected from 52 tobacco smokers and 148 non-smokers revealed the PCR detection of the 16S rRNA methanogen gene in 64/200 samples (32 %), including 47 tobacco smokers (73 %) [57]. In this series, fluorescent *in-situ* hybridization (FISH) targeting the methanogen *mcr*A gene detected 19/48 positive samples (39.58 %), including nine samples co-infected by *M. smithii* and *M. oralis* exhibiting diplococcus and bacillus morphology, respectively [57]. Culturing these 64 PCR-positive samples yielded 46 growing samples, including an oral fluid sample from one tobacco-smoker growing *M. oralis* and *M. smithii* [57]. In conclusion, this study revealed a significant correlation between tobacco smoking and the presence of *M. smithii* in the oral microbiota, suggesting that tobacco-smoking modulates the methanogen population of the oral cavity [57].

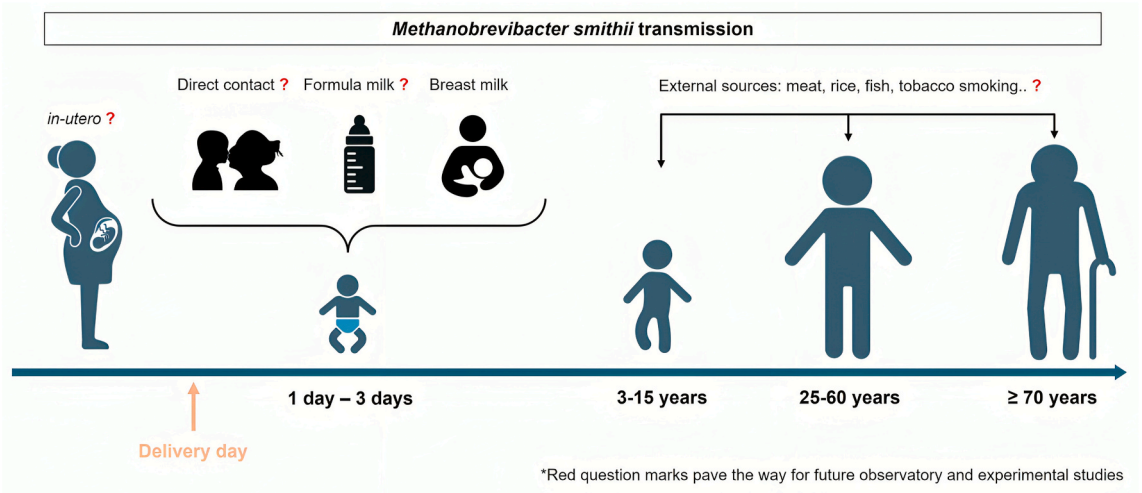

Fig. 2. Dynamics of *M. smithii* acquisition in human. *M. smithii* was transferred to newborn via different ways: *in utero* (by placenta) and after delivery (between 1 and 3 years through direct contact (kissing.), feeding style: formula milk or breastmilk. From three years and until elderly, human is exposed to several external sources that can control the presence of *M. smithii* such as diet style (meat, rice, fish.) and tobacco smoking.

5.2. Intestinal microbiota

Most knowledge about *M. smithii* in intestinal tract has derived from the study of feces, preventing an accurate localisation of *M. smithii* in the intestinal microbiota, which may depend on different conditions along different parts of the gut (the stomach, small intestine, and large intestine) such as pH, O_2 pressure, bacterial community, and mucus thickness. The acidic pH of the stomach ($2 < pH < 3.5$) [58], the presence of O_2 (pO_2-25 mmHg in mouse models) [59], low bacterial population [60], and a thin mucus layer make the stomach unfavourable for *M. smithii* colonisation [61]. In the small intestine, the transition from the duodenum to the ileum yields an increase in pH (from 6 to 7.5) [62], and a decrease in O_2 pressure (from 60 to 5 mmHg in mouse models) [59], which improves the growth of facultative anaerobic *Enterobacteriaceae* and *Streptococcaceae* [63] associated with *M. smithii* [64,65]. Further, the pH of the large intestine decreases from the ileum (pH = 7.5) to the caecum (pH = 6.3) [62], which does not constitute optimal conditions for *M. smithii* growth (G. Grine, personal data). In contrast, the increase once again in pH in the colon and rectum (pH = 6.5–7) [62], along with strong anaerobic conditions [59] and high mucus thickness in the colon [61] constitute ideal conditions for *M. smithii* to establish its niche and networks with members of *Bacteroidaceae* and *Ruminococcaceae* [9,66]. The prevalence of *M. smithii* in the human gut was verified by quantifying its 16S rRNA and *rpo*B gene copies in 700 faecal samples, 95.7 % of which were positive for *M. smithii*, with abundancy measuring between 100 and 7.43e+10 copies of 16S rRNA, and *rpo*B copies< 1.65e+10 [6]. *M. smithii* was also detected by 16S rRNA gene sequencing in 23/33 (69.69 %) pre-term neonate meconial samples, suggesting a colonisation of this methanogenic species during pregnancy [45].

In the intestinal microbiota, *M. smithii* is one among seven methanogens, comprising of *Methanosphaera stadtmanae* (*M. stadtmanae*) [67], *Methanomassiliicoccus luminyensis* [68], *Methanobrevibacter oralis* [44], *Methanobrevibacter arboriphilicus* [69], *Candidatus* Methanomassilococcus intestinalis [70] and *Candidatus* Methanomethylophilus alvus [71] whith only *M. stadtmanae* [67] and *M. luminyensis* [68] having been isolated by culture, while the knowledge regarding non-*M. smithii* methanogens mostly derives from non-culture observations by molecular detection, metabarcoding and metagenomics. Studies regarding human stool methanogens yielded a prevalence of 95.7 % for *M. smithii*, 29.4 % for *M. stadtmanae* [6] and 4 % for *M. luminyensis* [55]. This high prevalence of *M. smithii* in the human gut compared to other methanogenic species is attributed to its highly efficient enzymatic machinery for converting hydrogen and carbon dioxide into methane [72], and its ability to form beneficial relationships with other gut microbes by consuming hydrogen and promoting hydrogen-sensitive bacteria [73]. Additionally, diets rich in complex carbohydrates produce more hydrogen and carbon dioxide [74], which *M. smithii* effectively utilizes, supporting its growth. *M. smithii* has unique surface proteins and glycosylation patterns that may be less recognizable by the host's immune system which help the archaeon avoid detection and attack by immune cells [72].

5.3. Lower respiratory tract microbiota

M. smithii has been detected in the lower respiratory tract microbiota by analysing sputum, bronchoalveolar lavage, and bronchial aspirates [75]. PCR and RT-PCR amplification of the archaeal and *M. smithii* 16SrRNA gene confirmed *M. smithii* in two sputum and one bronchoalveolar lavage samples [75]. In addition, morphological examination by confocal microscopy after application of FISH revealed that only one bronchoalveolar lavage sample was positive for *M. smithii*, while there were no positive results for sputum samples [75].

5.4. Milk microbiota

Using improved DNA detection protocols and microbial culture techniques associated with antioxidants, *M. smithii* was isolated from three colostrum samples and five milk samples (day 10) [42]. *M. smithii* was detected in the colostrum or milk of 5/13 (38 %) and 37/127 (29 %) mothers by culture and qPCR, respectively [42]. The distribution of maternal body mass index according to the detection of *M. smithii* suggested an association with maternal metabolic phenotype [42]. In addition, previous studies demonstrated that breast milk contains a variety of bacteria that are also found in the maternal gut, also found that the composition of the gut microbiota in mothers and their breastfed infants showed significant overlap, suggesting that the gut microbiota, including *M. smithii*, could be transferred to breast milk [76,77]. All these findings suggest that breastfeeding may contribute towards the vertical transmission of these microorganisms and may be essential to seed the infant's microbiota with these neglected critical commensals from the first hour of life.

To clarify, *M. smithii's* activity in aerobic environments such oral cavity, blood and breast milk depends on its ability to find anaerobic niches or conditions that allow its survival and potential metabolic activity. In the oral cavity, for example, dental plaques can create microenvironments where *M. smithii* can thrive. In blood, its presence is usually indicative of an abnormal condition, suggesting that while it can be detected, it may not be actively contributing to normal physiological processes but rather involved in pathological conditions. The presence of *M. smithii* in breast milk could be transient or part of a maternal transfer of microbiota. The actual role of *M. smithii* in breast milk is still under investigation, and it remains to be seen whether it is active or simply a contaminant from the maternal gut or oral microbiota.

6. *M. smithii* in clinical microbiology

Different molecular and culture methods were performed to detect *M. smithii* in clinical samples, including RT-PCR, PCR, Sanger sequencing, and culture methods, followed by fluorescence and electron microscopy.

6.1. Microscopic detection

6.1.1. Direct microscopic examination

M. smithii vital factor 420 (F_{420}), producing a blue-green autofluorescence, could be detected when samples and viable cultured *M. smithii* cells were exposed to 420-nm wavelength UV light [78]. This feature was used to verify the presence and viability of *M. smithii* in culture using confocal microscopy [2,79]. As illustrated in Fig. 3C and 3D, autofluorescent *M. smithii* cells harbouring diplococcus shape with approximately size of 1.9 μm in length x 480 nm in width. The autofluorescent feature is not only produced by *M. smithii*, other methanogen species [80] and bacteria such *Clostridium* spp. [81] and *Cutibacterium* spp. [82] can emit autofluorescence. For this reason the contamination of other bacteria was minimized by using antibiotics cited below in the culture methods paragraph [83]. There is no specific staining for *M. smithii,* but Gram staining colonies demonstrates gram-positive fresh culture and gram-variable older culture *M. smithii* [2].

6.1.2. Indirect immunofluorescence

A library of rabbit antisera targeting a group of *Methanobacteriales* sp. demonstrated *M. smithii* PS and ALI strains by indirect immunofluorescence (using fluorescein isothiocyanate labelled goat immunoglobin) [84]. This method was used to detect and characterise *M. smithii* inside faecal samples and in cultures derived from the blood of febrile patients [2,10].

6.1.3. Fluorescent in-situ hybridization

FISH is a technique used for the detection of DNA or RNA sequences inside a sample by using a fluorescent probe specific to the target sequence [85]. Accordingly, probes were designed for the specific hybridization of methanogen at large targeting the 16S rRNA gene [86] and the *mcr*A gene [57] (Table 2). The protocol, including phosphate buffered saline (PBS) washing, paraformaldehyde treatment, and 16-h incubation with probes demonstrated *M. smithii* in oral fluid, sputum, meconial and vaginal samples [45,47,57,75].

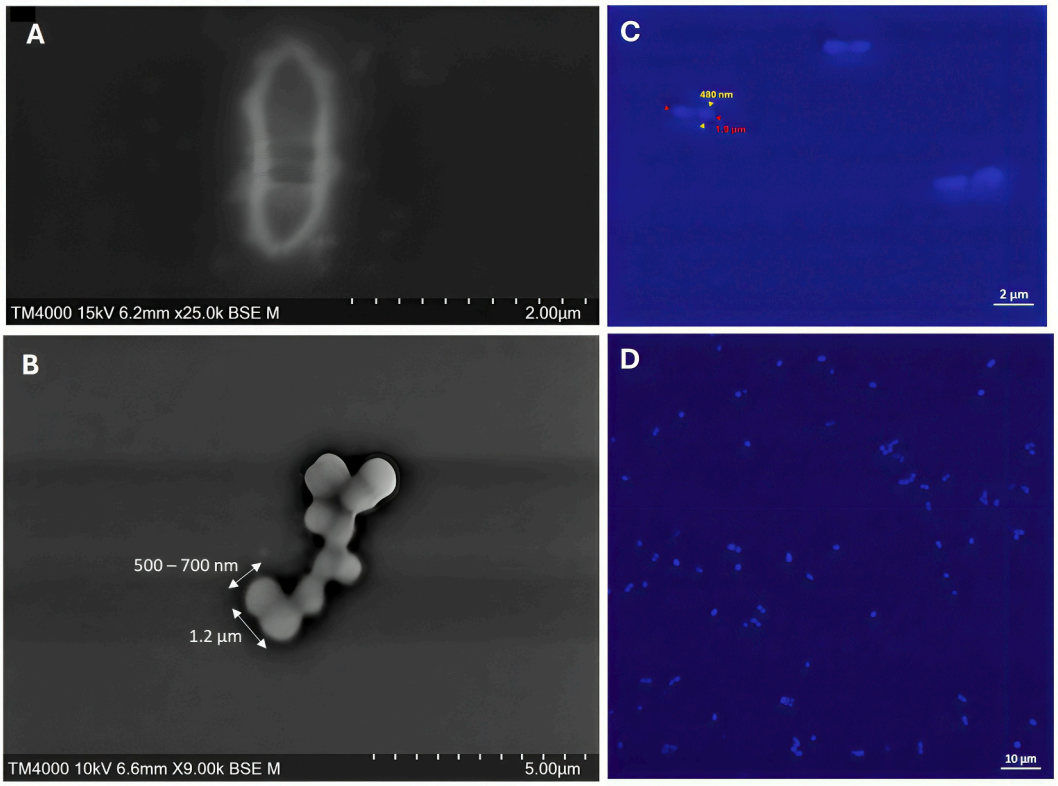

Fig. 3. *M. smithii* observed under microscope. A. Scanning electron microscopy showing *M. smithii* cell stained with PTA 10 %. B. Scanning electron microscopy showing diplococcus colonies of *M. smithii* with 500–700 nm diameter and 1.2 μm length. (TM4000 Plus tabletop, Hitachi, Tokyo, Japan) C. Three autofluorescent *M. smithii* cells exhibiting diplococcus shape with length 1.9 μm and width 480 nm approximately D. Autofluorescent *M. smithii* cells observed at 420-nm wavelength (C and D was observed with DAPI settings, 405 nm excitation, using light microscope LSM 900, Carl Zeiss Microscopy GmbH, Jena, Germany).

6.2. Molecular detection

Both preanalytical and analytical experimental steps have to be conducted to optimise the molecular detection of *M. smithii* in samples. It has been demonstrated that fresh faecal samples produced better results than older ones. One study reported that using fresh faecal samples improved the PCR detection of *M. smithii* and its correlating activity by methane detection, contrary to samples exposed to dioxygen (O_2) [87]. As for liquid stool samples, collected in cases of diarrhoea, an alternative protocol was proposed to improve DNA extraction: the application of a lyophilisation step before DNA extraction yielded 95.1 % positive samples for *M. smithii* versus 63.4 % of samples which were positive without a lyophilisation step (*P value* = 0.00043) [88]. Several PCR-based systems have been developed to amplify and detect *M. smithii* pieces of genomic DNA. Forward primer 300fEyAr and reverse primer 954rEyAr targeting the archaeal small subunit rDNA (SSU rDNA) were developed to detect *M. smithii* [89]. These two primers incorporated into PCR and applied to subgingival dental plaque DNA extracts, yielded 0.5–0.7-kb amplicons which were further purified and cloned for sequencing [89]. Of the 18 selected samples of 37/48 subgingival plaques samples which were positive for archaeal rDNA, 16/18 consisted in *Methanobrevibacter oralis* (*M. oralis*) and 2/18 consisted in *M. smithii* [89]. A few years later, new PCR assays targeting the *mcr*A of methanogens was described [90]. Faecal samples collected from healthy controls and different disease groups including Crohn's disease (CD), ulcerative colitis (UC), irritable bowel syndrome (IBS), colorectal cancer (CC) and polypectomised patients (PP) yielded 48 %, 30 %, 24 %, 48 %, 45 % and 50 % positivity in methanogens, respectively [90]. Further restriction fragment length polymorphism (RFLP) analysis of 558 *mcr*A library clones demonstrated that 510/558 (91.39 %) clones were identical to the *M. smithii mcr*A gene, confirming previous data on the abundancy of *M. smithii* in the gut [5,90]. Moreover, a PCR system targeting the *rpo*B gene of *M. smithii* was mentioned, resulting in 700 DNA faecal sample extracts showing the abundance of *M. smithii* in the gut, which was detected in 95.7 % of samples [6]. A DNA extraction and PCR protocol was applied to faecal sample after 0.8 μm filtration, overnight incubation with proteinase K, and DNA extraction using the NucleoSpin® Tissue Mini Kit (Macherey Nagel, Hoerdt, France) [6]. A comparison of results with DNA extracted through QIAamp Stool DNA Kit (Qiagen), showed *M. smithii* 16S rRNA amplification in 50/50 (100 %) vs 44/50 (90 %) and *M. smithii rpo*B gene amplification in 49/50 (98 %) vs 33/50 (66 %) samples (*P* value $\leq 10^{-5}$) [6]. In addition, a semi-automated extraction protocol including glass-powder crushing and overnight digestion by proteinase K following by EZ1® extraction using the DNA Tissue Kit (Qiagen, Courtaboeuf, France) demonstrated its efficiency compared to the fully automated method [91]. Of 110 faecal DNA extracts tested by RT-PCR, 82 % were positive for the *M. smithii* 16S rRNA gene using a semi-automated DNA method for extraction, while only 32 % were positive when a fully automated DNA extraction method was applied (*P* value = 0.001) [91].

Four real-time PCR systems and two standard PCR systems targeting *M. smithii* available in the literature are shown in Table 2.

6.3. Metagenomic detection

Metagenomics explores the genetic features of microbial communities, deepening our understanding of the organisms residing in complex environmental and human samples [92]. This method entails the extraction and sequencing of microbial DNA, followed by meticulous data analysis, generating datasets in which *M. smithii* can be precisely identified and quantified [92]. Metagenomic analysis of stool samples has revealed the predominance of *M. smithii* among archaea species [93–96]. Furthermore, thanks to metagenomics, *M. smithii* has been found inside gut paleofaecal and old dental calculus samples [11,12]. In addition, *M. smithii* was detected by the metagenomics approach inside the blood cultures of febrile patients [10]. Other studies underscored the prevalence and functional contributions of *M. smithii* within the human gut microbiome, noting its widespread presence and significant impact on gut health [97]. Detailed studies have examined its gene expression patterns and protein profiles, revealing its interactions with the environment and potential effects on host metabolism [6,98]. These findings enhance our understanding of *M. smithii*'s metabolic functions and suggest its role in shaping the composition and function of gut microbiota. Moreover, mathematical models and network analyses have been

Table 2
Primers and probes used for the detection of *M. smithii* by standard PCR and qPCR [6,14,17,88,151,163].

Target gene	PCR type	Primers and probes	Dye	Product size (bp)	References
16S rRNA	RT-PCR	Smit.16S-740F, 5′-CCGGGTATCTAATCCGGTTC-3′ Smit.16S-862R, 5′-CTCCCAGGGTAGAGGTGAAA-3′ Smit.16S FAM, 5′-CCGTCAGAATCGTTCCAGTCAG-MGB	FAM (MGB)	123	(88)
*rpo*B	RT-PCR	Ms_*rpo*BF, 5′-AAGGGATTTGCACCCAACAC-3′ Ms_*rpo*BR, 5′-GACCACAGTTAGGACCCTCTGG-3′ Ms_*rpo*BVIC, 5′-ATTTGGTAAGATTTGTCCGAATG-3′	VIC (MGB)	70	(6)
Repeat region	RT-PCR	*M. smithii*-F, 5′-ACCATAACyATCAGCAGCATTAT-3′ *M. smithii*-R, 5′-AGTATTGGTGAAGGATTTACTGT-3′ *M. smithii* probe, 5′-ACCyTTATCAGCTTTACCATTAATyAAAG-3′	FAM (MGB)	N/A	(151)
mcrA	PCR	MM_Mbs_Fw 5′-gCACCTAACAACACggACA-3′ MM_Mbs_Rv 5′-TTTggTCCTCTCATTTCgAg-3′	–	446	(163)
nifH	PCR	Mnif-342f 5′-AACAGAAAACCCAGTGAAGAG-3′ Mnif-363r 5′-ACGTAAAGGCACTGAAAAACC-3′	–	222	(14)
nifH	RT-PCR	Mnif 202F, 5′-GAAAGCGGAGGTCCTGAA-3′ Mnif 353R, 5′-ACTGAAAAACCTCCGCAAAC-3′ Mnif Probe, 5′-CGGACGTGGTGTAACAGTAGCTA-3′	FAM (MGB)	N/A	(17)

used to investigate its energy conservation mechanisms and interactions within microbial communities, which are essential for predicting how *M. smithii* responds to environmental changes, such as dietary shifts and alterations in microbial community dynamics [99,100]. Additionally, bioinformatics approaches utilizing metagenomic data have predicted its functional capabilities and implications for maintaining gut homeostasis [94,101]. These analyses highlight the complex relationships between *M. smithii* and other microbes, offering insights into its potential role in influencing gut health and metabolic disorders. Furthermore, systems biology approaches have been employed to quantify metabolic changes induced by diet, demonstrating how *M. smithii* adapts to dietary variations and its possible contributions to metabolic disorders [102]. This comprehensive perspective emphasizes the diverse roles of *M. smithii* in the gut ecosystem, from methane production to metabolic regulation, making it a key subject of research in microbiology and human health.

6.4. Isolation by culture

Despite several effective protocols being reported for the isolation and culture of *M. smithii*, only four strains (PS DSM 861; F1 DSM 2374; ALI DSM 2375, and B181 DSM 11975) are available in the German collection of microorganisms and cell cultures GmbH (DSMZ) (https://www.dsmz.de). The type strain PS is also available from the Japan Collection of Microorganisms (JCM) (https://jcm.brc.riken.jp) (Table 3). In addition, 15 isolates are available from the Collection de Souches de l'Unité des Rickettsies (CSUR) WDCM 875 (Table 3). *M. smithii* was initially isolated by culture by incubating faecal samples anaerobically in an H_2-CO_2 (80:20) atmosphere and 2-atm pressure after enrichment in the same conditions with agitation [2]. In these conditions, methane (CH_4) production could be monitored after seven-day incubation by gas chromatography (GC) as a proxy for *M. smithii* growth [2,44,47,103,104]. Serial dilutions of methane production-positive samples were then incubated in Balch medium 1 with 2 % Difco agar under a 1-atm H_2-CO_2 atmosphere, and colonies resulting from the most diluted culture were sub-cultured on agar Balch medium under anaerobic conditions to obtain pure cultures [2]. SAB medium was then proposed as a versatile medium to broaden isolation by culture of stool methanogens, sparing 1–3 days for *M. smithii* growth compared to DSMZ medium which is more specific for methanogens [44]. Moreover, specimens which were negative for *M. smithii* by PCR grew after three weeks of incubation in the SAB medium, whereas no growth was detected in the DSMZ medium [44]. Further co-incubation of faecal samples with H_2-producing *B. thetaiotaomicron* led to unprecedented aerobic culture of *M. smithii* [105]. In detail, the culture system consisted of one upper chamber containing a Petri dish with solid SAB medium inoculated with *M. smithii* and *B. thetaiotaomicron* co-culture and a lower chamber containing SAB broth inoculated with *B. thetaiotaomicron* [105]. Nine days of incubation at 37 °C yielded methane-producing colonies identified by quantitative real-time PCR (RT-PCR) targeting the *M. smithii* 16S rRNA gene [105]. Finally, a patented GG culture medium was designed for *M. smithii* isolation by adding formate and acetate without a source of H_2 or carbon dioxide (CO_2), maintaining the viability of *M. smithii* in samples transported at ambient atmosphere and temperature for 15 days, and greatly facilitating routine isolation and evading the use of exploding H_2 gas [83]. In summary, three culture media were available in literature: DSMZ medium, SAB medium and GG medium. These media share 27 components, while 17 components are common between the GG medium and the SAB medium, one component (sodium resazurin) is common between the SAB medium and the DSMZ medium, and there are no mutual components between the GG and DSMZ media (Venn diagram; Fig. 4). Moreover, the GG medium is the only one to contain glutathione, uric acid and ascorbic acid. In contrast, the DSMZ medium is the only one to contain hydrochloric acid (25 %), while no specific components were present in the

Table 3
Summary of *M. smithii* strains available in microbial collection databases [1,2,24,164].

Collection name	Strain designation	Isolation source	Storage conditions	reference
DSMZ/JCM	PS; DSM 861; JCM 30028	primary sewage digester, USA	Liquid medium	(1)
DSMZ	F1; DSM 2374	Human feces, USA	Liquid medium	(2)
DSMZ	ALI; DSM 2375	Human large intestine, USA	Liquid medium	(164)
DSMZ	B181; DSM 11975	Human feces, USA	Liquid medium	(24)
CSUR	Q5487	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5502	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5505	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5701	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5704	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5705	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5707	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5708	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5709	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5711	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5713	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5714	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5715	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5716	Human feces, France	Lyophilised; −80 °C	
CSUR	Q5718	Human feces, France	Lyophilised; −80 °C	

*DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) https://www.dsmz.de/.
*JCM: Japan collection of microorganisms https://jcm.brc.riken.jp.
*CSUR: Collection de Souches de l'Unité des Rickettsies https://csur.eu/.

SAB medium. All media were used in anaerobic conditions with N_2/CO_2 gases, while H_2 was only added to the SAB and DSMZ media (Fig. 4). As for identification of colonies, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI--TOF-MS) identified 28 cultured environmental species belonging to 12 Archaea families with reliable results [106]. Improvements in the protocol were made by adding a mechanical step for lysing methanogen cells which enlarged the spectrum of archaeal species tested, including human-associated archaea and *M. smithii* [107]. Interestingly, three *M. smithii* human gut isolates exhibited an identical spectral profile which differed from that derived from a sewage digester strain ATCC 35061, showing one additional 6050-Da peak (quality score 7). This illustrated the phenotypic diversity of *M. smithii* [107]. A further study, using a database based on the MS profiles of 16 methanogens, identified 21 human feces *M. smithii* isolates and clustered them within six *M. smithii* genotypes [108]. MALDI-TOF-MS was used to identify faecal *M. smithii* genotypes cultured anaerobically with *Bacteroides thetaiotaomicron* [109]. Taken as a whole, these studies demonstrate the practicability and usefulness of MALDI-TOF-MS for the rapid identification of *M. smithii* colonies, applicable for research and clinical microbiology. The culture, molecular, microscopic and metagenomic methods applied to detect *M. smithii* in clinical samples are shown in Fig. 5.

7. Microbiology of *M. smithii* cell variants

We recently discovered *Candidatus* Nanopusillus phoceensis associated with *M. smithii* in the human digestive tract microbiota after PCR detection of *M. smithii* in 87/110 (79 %) faecal samples and the co-detection of *M. smithii* and Nanoarchaeota in 17/87 (20 %) faecal samples [110]. In light of the similarity with data we reported on another nanoarchaeon named *Nanopusillus massiliense,* observed by electron microscopy to be tightly associated with *Methanobrevibacter oralis* (*M. oralis*), we inferred that the same situation may exist for *M. smithii/Ca.* N. phoceensis. We therefore determined two *M. smithii* cellular forms that we named *M. smithii* small cell variant (SCV) (with no association with *Ca.* N. phoceensis), and *M. smithii* large cell variant (LCV) (*M. smithii* associated with *Ca.* N. phoceensis) [110,111].

7.1. Genomic diversity

M. smithii genome comprises one chromosome (no plasmid detected to date), the genomic characteristics of which (genome length, number of coding sequences (CDS), number of CRISPR, coding ratio, and GC%) are displayed in Table 1 for 95 *M. smithii* genome sequences available at the time of this review. No obvious difference between the different strains with the respect of source, human and pig *M. smithii* genomes yielded approximately same genome size measuring in average 1.6–1.7 Mb with 31 % GC content and 83–85 % coding ratio. The characteristics of four *M. smithii* complete genomes (ATCC 35061, KB11, CE91-St67, CE91-St68), including GC%, GC skew, as well as their restriction maps, are shown in Fig. 6. As an example, *M. smithii* ATCC35061 type strain derived from a primary sewage digester has a 31 % GC content, a 1 853 160 base pair (bp) genome sequence with a 90.2 % coding ratio of 1795 coding genes, 34 tRNAs, two rRNA clusters, and two CRISPR arrays [72] (Table 1). Interestingly, the genomic sequences of digestive tract isolates exhibit a higher number of genes coding for cell wall structure, defence, and metabolism of bacteria-end products than environmental *M. smithii* strains (binomial test; $P < .01$). Nevertheless, the genome sequences of the human feces *M. smithii* strain KB11

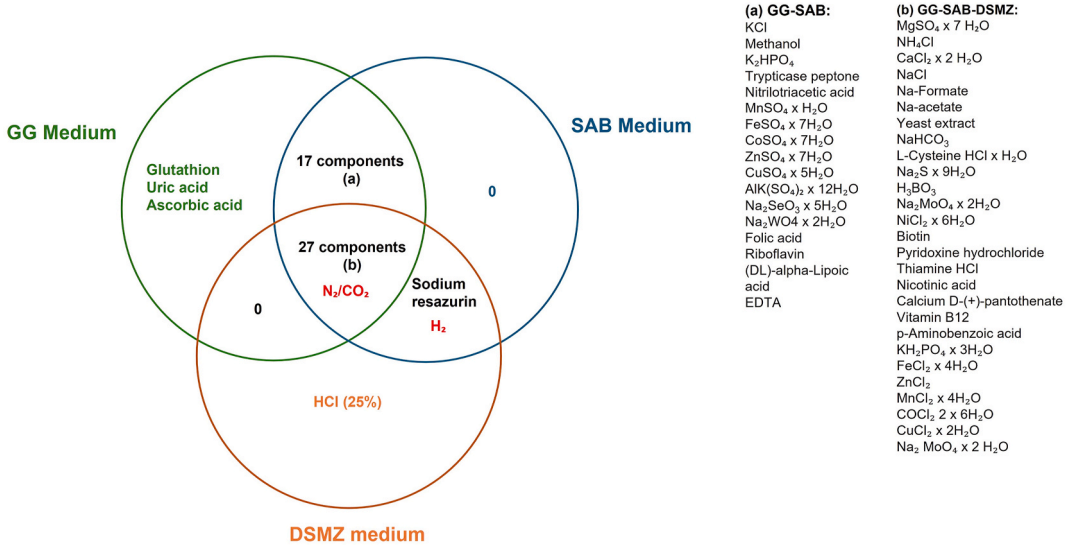

Fig. 4. Venn diagram comparing three culture media of *M. smithii* (DSMZ, SAB, and GG media). These media share between them 27 components including N_2/CO_2. SAB and GG media have 17 common components, SAB et DSMZ have sodium resazurin and H_2 gas as common components and no shared components between GG and DSMZ media. Specific components were present in GG medium (Glutathion, uric acid, and ascorbic acid) and DSMZ medium (Hydrochloric acid).

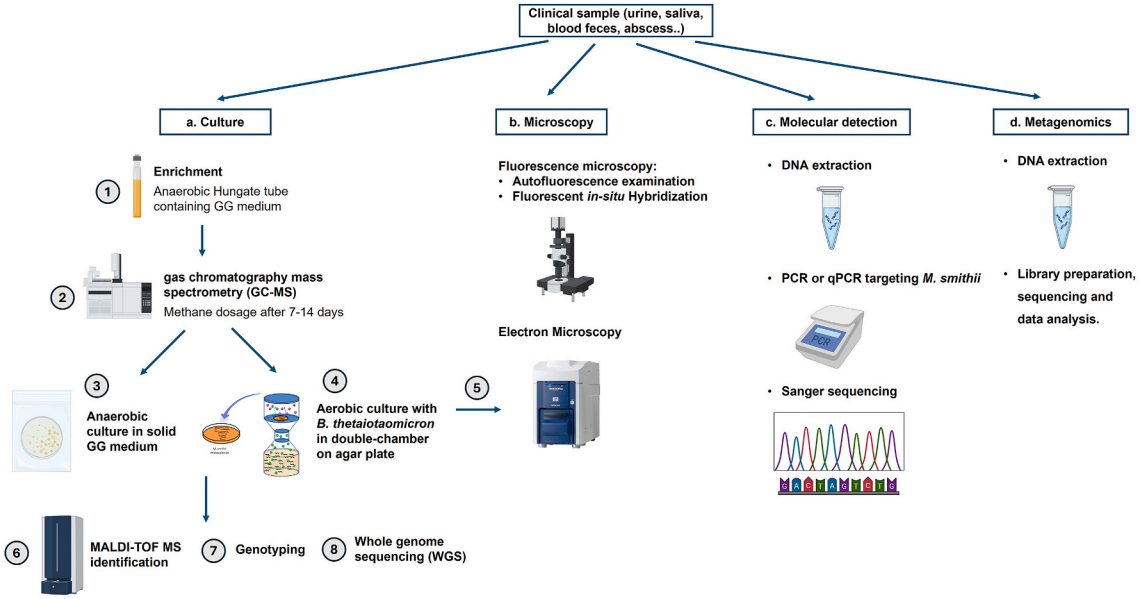

Fig. 5. *M. smithii* detection methods from clinical samples. Four protocols were shown for *M. smithii* investigation: a. Culture methods followed by methane measurement by GC, MADI –TOF identification or WGS b. Microscopy methods by autofluorescence, FISH observations or electron microscopy c. Molecular detection by qPCR and PCR followed by sequencing d. Metagenomic screening of clinical samples.

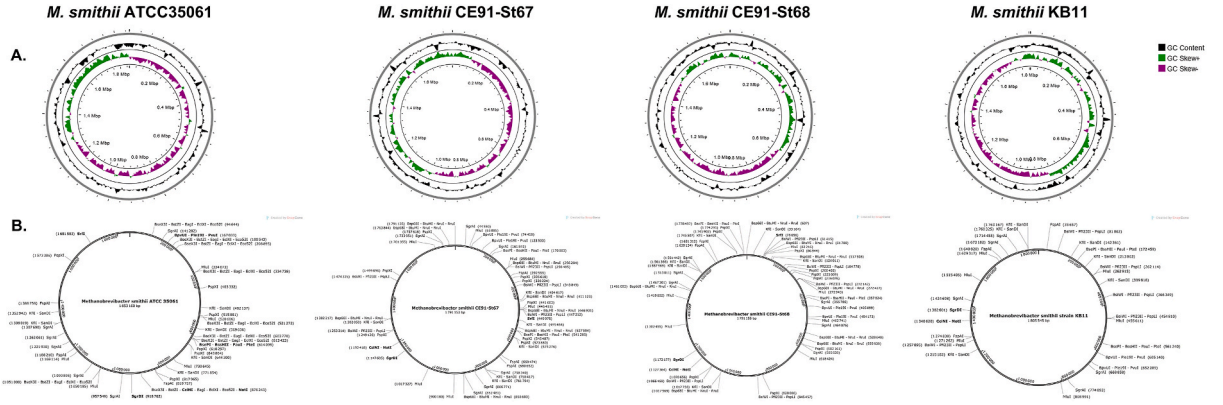

Fig. 6. Illustration showing genomic diversity between four complete genomes of *M. smithii* (ATCC 35061, CE91-St67, CE91-St68 and KB11). A. GC % (black graph) and GC skew (Green and purple graphs) (C-G/C + G), GC skew negative represents more G bases than C bases in the position, GC skew positive represents more C bases than G bases in the position. B. Restriction maps relative to *M. smithii* genomes using 678 restriction enzymes, 18 enzymes do not cut inside these four genomes while *Srf*I does not cut KB11 (Supplementary information, Table 1). Figures obtained using Proksee (https://proksee.ca) and SnapGene (https://snapgene.com) programs.

(CP017803.1) is 47 615-bp shorter than that of *M. smithii* ATCC35061 (CP000678.1). A C-terminal deletion includes DNA helicase UvrD (Msm_0731) and gaps in the adhesin-like protein (BK798_03360), while an additional 23 180-bp region (500216–523396) encodes for hypothetical proteins, type I restriction-modification system, and transposases [72,112]. *M. smithii* genomic diversity was also assessed by multispacer sequence typing (MST) through developing PCR systems targeting four intergenic spacers in reference *M. smithii* ATCC35061 [113]. Applying these systems to four *M. smithii* genome sequences and 22 *M. smithii* digestive tract isolates yielded 15 different genotypes: three distinct genotypes corresponding to each of the F1, ALI, and B181 reference genomes; five genotypes for five oral isolates and nine genotypes for seven gut isolates with two genotypes shared between the oral cavity and gut isolates [113]. Finally, comparing clusters of 29 gene ontologies in intestinal *M. smithii* and *Methanosphaera stadmanae* (genome size: 1 767 403 bp) indicated that *M. smithii* presented more groups relative to the surface variation (48 vs 30), persistence (8 vs 4) and metabolic activity (480 vs 389) [72]. In *M. smithii* LCVs, our further genome analysis of *Ca.* N. phoceensis identified a SAM-dependent methyltransferase (QFW68505.1) and glycosyltransferase (QFW68504.1) most probably transferred as a whole from Nanoarchaeota virus NAV1 to *M. smithii* LCVs (I. MALAT, personal data), suggesting that LCVs may have an unique glucoside-decorated cell wall profoundly modifying LCV cell wall properties such as recognisability by residing immune cells such as dendritic cells, macrophages,

plasma cells in the mucosae and Peyer patches in the gut-associated lymphoid tissues (Fig. 7).

7.2. Structural morphology and cell wall composition

M. smithii SCVs look like 1.5–2 μm coccobacilli (electron microscopy) which are blue-green autofluorescent due to F_{420}-reducing hydrogenase, a cofactor implicated in hydrogen metabolism and methane secretion with a 420-nm emission peak (Fig. 3). *M. smithii* is not motile, exhibiting a gram-positive cell wall lacking bacterial peptidoglycan, instead being composed of pseudomurein proteins forming a mesh-like structure surrounding the cell and providing strength and rigidity [1,114,115]. Specific sugar units and glycosidic linkages differ between pseudomurein and peptidoglycan, with pseudomurein consisting of N-acetylglucosamine (NAG) and N-acetyltalosaminuronic acid (NAT) repeats connected by β-1,3-glycosidic bonds, differing from N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) which are connected by β-1,4-glycosidic bonds in bacterial peptidoglycan [114,116,117]. The pseudomurein cell wall serves as a protective layer, conferring resistance to *M. smithii* against lysozyme and a wide range of peptidoglycan-targeting antibiotics in addition to osmotic pressure and mechanical stress, and also helps to maintain cell shape, preventing cell collapse and providing overall cell stability [118] (Fig. 8). Internal to the cell wall, the cell membrane is composed of a lipid monolayer constituted by isoprenoid chains rather than the lipid bilayer constituted by unbranched fatty acids observed in bacterial and eukaryotic membranes [119,120]. These isoprenoid chains are linked to glycerol by ether bonds, yielding an hydrophobic core barrier regulating ions and molecule flux, maintaining cellular homeostasis and providing stability and adaptability of *M. smithii* in the anaerobic environment [121] (Fig. 8). Screening Nanoarchaeota in human stool samples using PCR targeting the 30S L12 gene yielded 17 positive results, which were also positive for *M. smithii* [110]. Analysing the 17 positive samples by FISH co-localised *M. smithii* and *Ca.* N. phoceensis and further electron microscopy observation demonstrated *M. smithii* LCVs enlarging *M. smithii* with one 100–400 nm coccobacilli [110].

7.3. Physiology

M. smithii is a strictly aero-intolerant archaeon and experimental deadly exposure to ambient air varied from 15 to 30 min [10], a timescale which could guide the sampling, transport and storage of specimens in the search for *M. smithii* by culture. Accordingly, *M. smithii* plays a critical role in the metabolic dynamics of anaerobic ecosystems such as mammals, including the human digestive tract via hydrogenotrophic methanogenesis, including the reduction of CO_2 by H_2 into gaseous methane following this reaction: $4H_2+CO_2 \rightarrow CH_4+ 2H_2O$ [72]. *M. smithii* possesses a repertoire of enzymes crucial for its distinctive metabolic pathways.

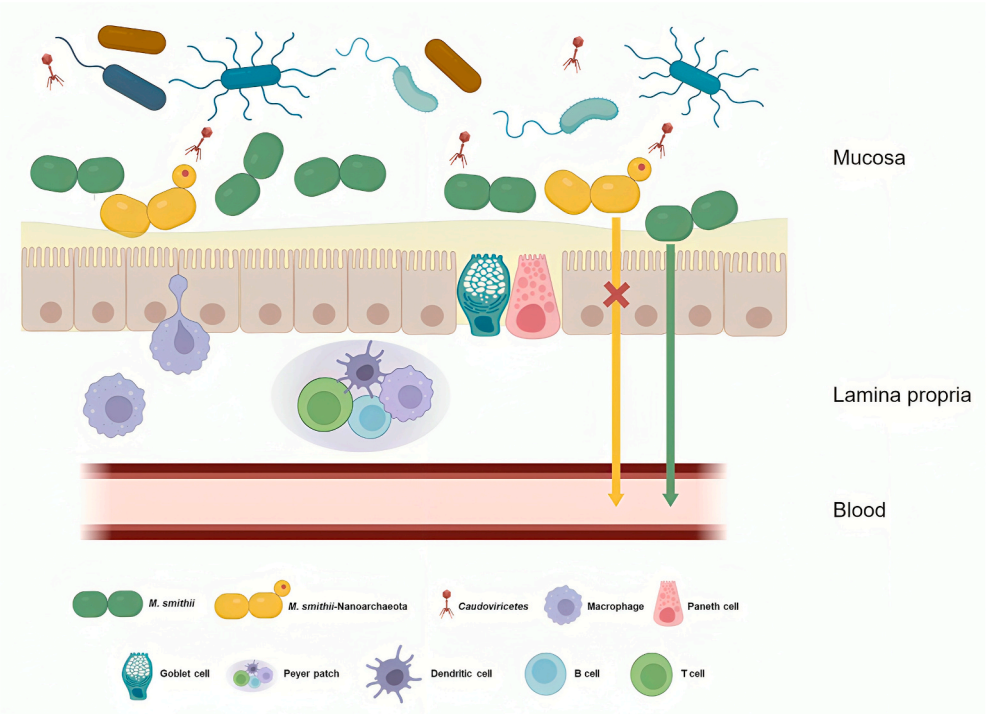

Fig. 7. Schematic illustration of *M. smithii* and *M. smithii*-Nanoarchaeota translocating and residing scenario from gut to blood. *M. smithii* alone can cross intestinal immune barrier and cells arriving to the blood. In contrast, *M. smithii*-Nanoarchaeota constitutes a novel microorganism with different cell size and different membrane profile (i.e., glycosylation caused by transferred glycosyltransferase gene from NAV1 to Nanoarchaeota) which inhibits intestinal barrier crossing. Scheme drawing using BioRender (https://www.biorender.com/).

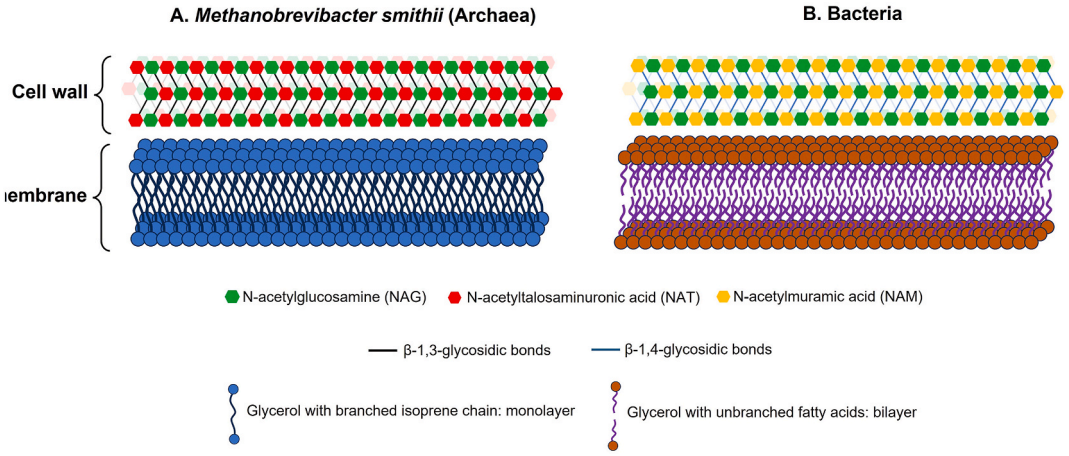

Fig. 8. Schematic illustration comparing *M. smithii* cell wall with bacterial cell membrane. *M. smithii* cell wall is composed of NAG and NAT connected by β-1,3-glycosidic bonds while bacterial cell wall is composed of NAM and NAG connected by β-1,4-glycosidic bonds. *M. smithii* cell membrane is constituted of glycerol with branched isoprene chain forming monolayer structure, while bacterial membrane is constituted of glycerol with unbranched fatty acids forming bilayer structure.

Methyl-Coenzyme M Reductase (MCR) is central in methanogenesis, catalysing the final step of methane synthesis by converting methyl-coenzyme M and coenzyme B to methane and coenzyme M [72]. In addition, the formylmethanofuran pathway relies on the activity of formylmethanofuran dehydrogenase (Fwd) for the reduction of CO_2 to formylmethanofuran, a pivotal intermediate in methanogenesis [72]. Furthermore, hydrogenases, including F_{420}-reducing hydrogenase, enable the hydrogenotrophic methanogenic capabilities of *M. smithii* [72]. All these methanogenesis reactions were illustrated in Fig. 9. The intricate interplay between these enzymes underscores the metabolic adaptability of *M. smithii* within the complex ecosystem of the gut microbiome.

7.4. Antiseptic and antibiotic susceptibility

The pattern of *M. smithii* antibiotic susceptibility was evaluated on 16 *M. smithii* isolates from freshly collected feces including the *M. smithii* ATCC35061 type strain [122]. All tested isolates exhibited high resistance to penicillin G, cephalothin, vancomycin, streptomycin, gentamicin, ciprofloxacin, and clindamycin (minimal inhibitory concentration (MIC) > 64 mg/L) [122]. In contrast, 10/15 isolates were susceptible (MIC <16 mg/L) to metronidazole, one was intermediately susceptible (MIC = 16 mg/L), and five were resistant (MIC >16 mg/L) [122]. Further studies highlighted resistance to chloramphenicol (MIC ≤25 mg/L) and susceptibility to bacitracin (MIC ≤4 mg/L), metronidazole, ornidazole, and fusidic acid [123,124]. Furthermore, *M. smithii* growth inhibition was observed after incubation with antimicrobial peptides (AMPs) human cathelicidin LL32 (MIC = 1 μM), porcine NK-lysin (NK2) derivatives (MIC = 3 μM), and synthetic antilipopolysaccharide Lpep 19-2.5 (MIC = 3 μM) while human cathelicidin LL20 and porcine

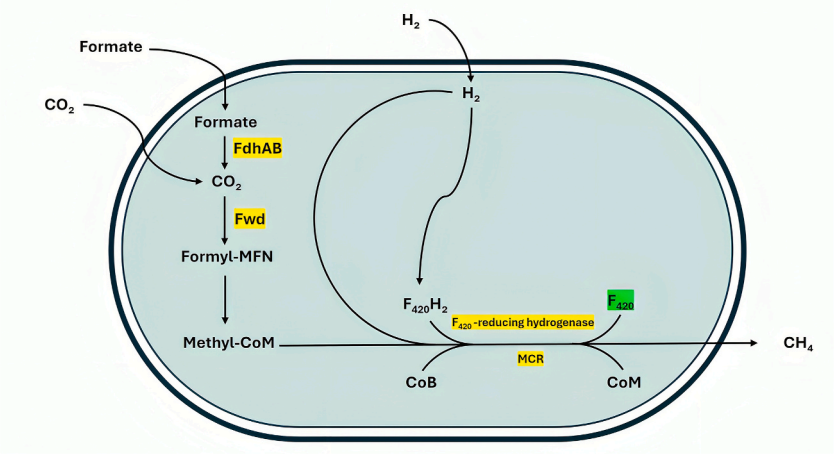

Fig. 9. *M. smithii* methanogenesis pathways from formate and H_2/CO_2. H_2, dihydrogen; CO_2, Carbone dioxide; CH_4, methane; FdhAB, formate dehydrogenase subunits; Fwd, formylmethanofuran dehydrogenase; MCR, methyl-coenzyme M reductase; CoB, coenzyme B; CoM, coenzyme M; MFN, methanofuran; CoM, coenzyme M.

NK-lysin C7S had MIC >10 μM [51]. In addition, transferring the *sat* gene to study methanogen resistance to nourseothricin led to using this gene as a selection marker for methanogens [125]. Among the species tested, *M. smithii* exhibited high susceptibility to nourseothricin, but colonies were still observed after one month of incubation with nourseothricin. WGS revealed four mutants in three hypothetical genes and a potassium transporter TrkA [125]. Lovastatin was also evaluated *in vitro* and no CH_4 was detected after five days of incubation of *M. smithii* with 4 μg/mL lovastatin compared to the control [126]. PCR amplifications indicated an increased expression of HMG genes due to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-Co-A) reductase (HMGR) inhibition by lovastatin leading to the inhibition of cell membrane synthesis [126]. No data issued regarding the susceptibility of *M. smithii* to decontaminants and antiseptics, except for squalamine exhibiting a MIC ≤1 mg/L [127].

8. *M. smithii* microbial interplays and networks

M. smithii was revealed to have relationships with bacteria, fungus and viruses, as well as with its host, constituting a wide network and shedding light on the importance of thoroughly characterising such interactions (Fig. 10).

8.1. Bacteria

The interactions between *M. smithii* and bacteria in the human gut is an intriguing aspect of the complex microbial ecosystem within the digestive system. Various gut bacteria, including genera like *Bacteroides*, *Prevotella*, *Ruminococcus* and *Faecalibacterium,* participate in the physiology of *M. smithii* through the production of hydrogen gas (H_2), an essential compound of methanogenesis [38, 66]. The metabolism of H_2 in the *in vitro* model designed to scrutinise the mutual interaction between *M. smithii* and *Anaerostipes rhamnosivorans* demonstrated a notable exchange of formate and hydrogen from the bacterium to methanogen [128]. This exchange coincided with a substantial increase in levels of bacterial butyrate production [128]. Moreover, examination of the interplays between *M. smithii* and *B. thetaiotaomicron*, a pivotal constituent of the gut microbiota, revealed a fascinating dynamic. The fermentation products and hydrogen produced by *B. thetaiotaomicron* served as vital sustenance for the thriving growth of *M. smithii* [73]. Paradoxically, the accrual of these fermentation byproducts triggers a disruption in the metabolic secretion pattern of *B. thetaiotaomicron*, ultimately impeding the growth of *M. smithii* [73]. Furthermore, the H_2 produced by *Christensenella minuta* enhances and supports the metabolic activities of *M. smithii* more efficiently than the presence of *Bacteroides thetaiotaomicron* [129]. Furthermore, to enhance our

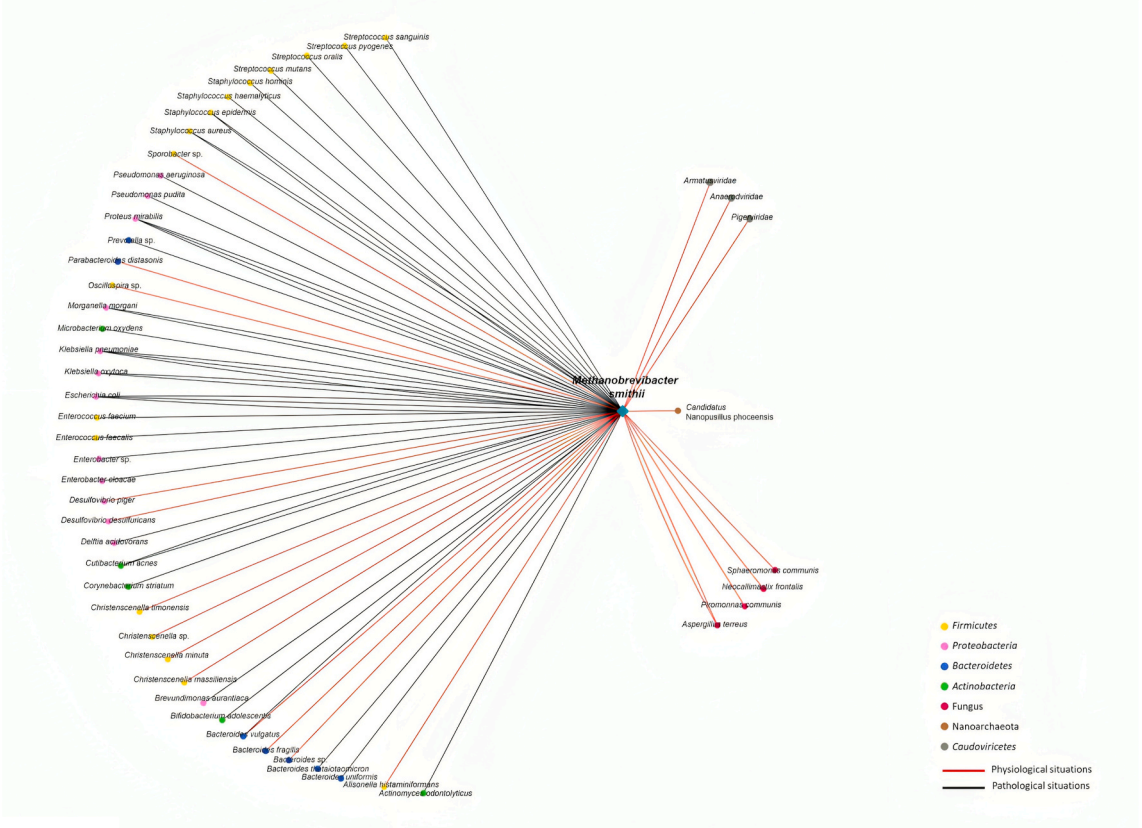

Fig. 10. Illustration showing *M. smithii* and its microbial networks. Four groups of microorganisms showed interactions with *M. smithii* including bacteria, fungi, nanoarchaeota and viruses. Figure drawing using Cytoscape (https://cytoscape.org/).

understanding of the role of *M. smithii* in the human gut microbiota and its interactions with other microbial families, an *in vitro* culture model was employed using *M. smithii* PCR-positive stool samples, initially devoid of methane post batch culture [130]. Intriguingly, the experiment revealed a depletion in *Desulfovibrionaceae* 200 h after *M. smithii* inoculation [130]. Conversely, inhibiting *M. smithii* using 2-bromoethanesulfonic acid resulted in an elevation in the *Desulfovibrionaceae* population ($r_s = -0.841$, P value < 0.05) [130]. These findings underscore the inhibitory impact of *M. smithii* on sulfate-reducing bacteria, suggesting a competitive relationship, particularly in terms of H_2 utilisation.

8.2. Viruses

The human virome encompasses a vast and diverse collection of viruses that inhabit human niches, influencing health, disease, and interactions with other microbial communities [131]. In the human digestive tract, the main families are found to belong to tailed double stranded DNA bacteriophages referred to as *Caudoviricetes* sp [132]. Recently, bioinformatic analyses have revealed a vast number of intestinal *Caudoviricetes* sp. nucleic sequences inserted in genomes of *M. smithii* derived from the human gut, suggesting infection of *M. smithii* by these bacteriophages [133–135].

8.3. Fungi

To date, no studies are available about *M. smithii*-fungi interactions inside humans. In contrast, the interaction between *M. smithii* and ruminal fungi has been better clarified. *M. smithii* co-culture with rumen anaerobic fungi *Neocallimastix frontalis* (*N. frontalis*) and *Piromonas communis* (*P. communis*) led to a decrease in fungal susceptibility to the ionophore antibiotics monensin and lasalocid, while in contrast no protective role of *M. smithii* was shown after its incubation with fungi in the presence of coumarin or p-coumaric acid [136,137]. Moreover, co-culture of *M. smithii* with *N. frontalis*, *P. communis* and *Sphaeromonas communis*, another anaerobic rumen fungus, increases their metabolic enzyme activity, thus xylan and cellulose fermentation, leading to the release of more acetate, xylose and hexose [138,139]. In addition, ryegrass and lignified secondary wall degradation was improved when *N. frontalis*, *Piromyces* and *Caecomyces* co-cultured with *M. smithii* [140]. In contrast, *Aspergillus terreus* in fermented rice straw extracts inhibits the growth of *M. smithii* and resulting methane production [141]. In greater depth, lovastatin secreted by fungus is able to inhibit 3-hydroxy-3methyl glutaryl CoA reductase, a crucial enzyme involved in the synthesis of the methanogenic cell membrane [141].

8.4. Host

In humans, the release of proinflammatory cytokines TNF-α and Il-1β was investigated using peripheral blood mononuclear cells (PBMC) and monocyte-derived dendritic cells (moDCs) collected from in healthy donors exposed to *M. stadtmanae*, *M. smithii* and *M. luminyensis* [142]. In addition, Bang et al. found that *M. stadtmanae* and *M. smithii* interfered with moDC expression of antimicrobial peptide genes [142]. The same experiments conducted with the Caco-2/BBe intestinal epithelial cells did not result in cytokine induction, suggesting that the recognition of *M. smithii* and *M. stadtmanae* might be restricted to immune cells [142]. Furthermore, in mice, the inhalation of *M. smithii* induced a tenfold accumulation of myeloid DCs compared to saline inhalation [143]. The term "archaeosome" was coined for spontaneous or synthetic liposomes composed of archaeal lipids and it has been showed that bovine serum albumin immunisation was more effective when *M. smithii*-derived archaeosomes were employed, as compared with conventional liposomes [144,145].

9. *M. smithii* in pathology

M. smithii, the predominant methanogen in the human gut, is found in higher concentrations in individuals with conditions such as constipation-predominant IBS, small intestinal bacterial overgrowth, and obesity [146,147]. Its production of methane is believed to slow intestinal transit time, exacerbating symptoms of constipation and contributing to bloating and discomfort [146]. Additionally, its presence has been linked to increased efficiency in extracting calories from food, which may play a role in weight gain and obesity [72,148]. Thus, *M. smithii* is implicated in both the manifestation and progression of these pathologies, highlighting its potential as a therapeutic target for related disorders.

9.1. Dysbiosis

9.1.1. Urinary tract infections

M. smithii was detected in 9 % of urine samples collected from patients with urinary infections. In addition, 53 %, 18 %, and 3 % of *M. smithii* in infected urine samples were associated with *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* sp., respectively [64].

9.1.2. Bacterial vaginosis

Methanogenic activity was detected in anaerobic vaginal fluid samples collected from patients with bacterial vaginosis (BV). After enrichment steps, methane monitoring by GC and indirect immunofluorescence assay, two isolates of *M. smithii* were identified close to strains *M. smithii* PS and *M. smithii* ALI [104]. Further study investigated *M. smithii* in bacterial vaginosis [47]. A group of 33 vaginal samples collected from BV patients showed 32 of them were positive by 16S rRNA RT-PCR for *M. smithii* [47]. Moreover, nine *M. smithii* strains were isolated in anaerobic SAB medium and two bacterial vaginosis samples showed the presence of *M. smithii* by FISH after

microscopic examination [47]. A patent on *M. smithii* as a biomarker for bacterial vaginosis has been deposited (WO 2019/122545 A1) [149].

9.1.3. Severe acute malnutrition

M. smithii has been defined as a roughly missed microorganism in the guts of African children with severe acute malnutrition due to disruptions in diet and lack of quality in consumed nutrients [150]. Moreover, a severe irreversible depletion in *M. smithii* was demonstrated by PCR screening of faecal samples from SAM children compared to controls, leading to the detection of *M. smithii* only in 4.2 % of cases compared to 40.9 % of controls [7]. Therefore, the role of *M. smithii* in malnutrition is linked to its involvement in the gut microbiome's energy-harvesting capabilities. Studies have shown that *M. smithii* is more abundant in the gut microbiota of obese individuals, suggesting it may contribute to more efficient caloric extraction from food, potentially exacerbating conditions related to overnutrition. Conversely, its role in malnutrition could be investigated in the context of its interactions with other gut microbes and its impact on nutrient absorption.

9.2. Abscesses

9.2.1. Muscular abscesses

M. smithii was isolated from a paravertebral muscular abscess sample collected from a 41-year-old patient [9]. Molecular detection of archaeal 16S rRNA and *mcr*A genes relative to *M. smithii* was verified by sequencing and *in vitro* resistance of the *M. smithii* isolate to amoxicillin-clavulanate and susceptibility to metronidazole was revealed after incubation of the strain with SAB medium supplemented with these antibiotics followed by methane production measurement using GC [9].

9.2.2. Cerebral abscesses

Only one study detected *M. smithii* from a brain abscess sample [151]. Of 18 brain abscess specimens, only one revealed *M. smithii* by RT-PCR [151].

9.2.3. Dental abscesses

M. smithii was detected and cultured from subgingival dental plaque samples [152]. After anaerobic incubation of collected samples in SAB medium, DNA extraction, followed by PCR targeting the *mcr*A gene of methanogens and sequencing, revealed the presence of *M. smithii* in 2/65 (3 %) of samples derived from moderate risk group patients. *M. smithii* was isolated in one sample after four months of anaerobic incubation at 37 °C [152].

9.2.4. Chronic rhinosinusitis

Methanogens were searched in 116 sinus surgical specimens collected surgically from patients diagnosed with refractory sinusitis [153]. Molecular detection by PCR yielded 12 methanogen-positive samples related to nine patients (10.3 %) [153]. Interestingly, *M. smithii* was identified in four patients after further analysis of methanogen positive samples by FISH and amplicon sequencing [153].

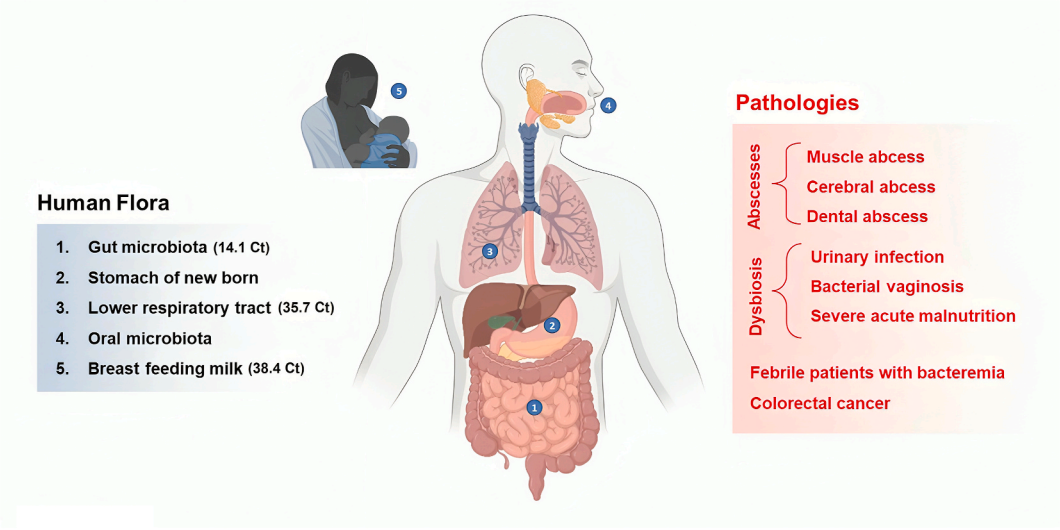

Fig. 11. *M. smithii* repertoire in human flora and pathologies. *M. smithii* was detected along the gastrointestinal tract including oral cavity, newborn stomach, and gut. Also in lower respiratory tract and breast feeding milk. In pathologies, *M. smithii* was detected in abscesses (muscle abscess, cerebral abscess, and dental abscess), dysbiosis (urinary infection and vaginosis) and febrile patients with bacteremia, noting its increase in feces of colorectal cancer patients and its absence in severe acute malnutrition patients. Scheme drawing using BioRender (https://www.biorender.com/).

9.3. Archaemia and endocarditis

M. smithii was detected and isolated from the blood of febrile patients, specifically those with infective endocarditis [10]. In detail, 27/5594 (0.5 %) anaerobic blood cultures collected from patients with fever showed the presence of bacterial species and were PCR-positive for *M. smithii* [10]. Further investigations were then applied to three samples relative to infectious endocarditis patients: *M. smithii* associated with *S. aureus* was detected by autofluorescence and electron microscopy in one blood culture, and two *M. smithii* strains, BC55 and BC84, associated with *S. mutans* and *S. epidermidis,* respectively, were isolated and sequenced from two blood culture of endocarditis patients [10]. A summary of the repertoire of *M. smithii* in human flora and pathological cases is shown in Fig. 11.

9.4. Others

9.4.1. Colon cancer

To investigate the archaeome in the human gut concerning colorectal cancer (CRC), a comprehensive analysis was conducted on 390 faecal metagenomic datasets, encompassing both apparently healthy individuals (n = 198) and CRC patients (n = 192) [154]. The data were sourced from diverse geographical regions, including Europe (Austria, Germany, and Italy) and Asia (Japan, China, and India), providing a broad perspective on archaeal species composition [154]. Within the pool of 217 identified archaeal species, the median density of *M. smithii* per faecal sample was significantly higher in samples collected from patients diagnosed with CRC than in apparently healthy individuals (Kraken abundance; mean: 36740,9844 VS 7693,05051) (P value < 0.001) [154], consistent with findings in an independent study that specifically emphasised Indian CRC cases [155]. Conversely, an investigation involving 73 Chinese CRC patients, using the MaAsLin approach (multivariate associate with linear models) ($Q < 0.1$), revealed an increase in the density of halophilic archaea and a decrease in methanogenic archaea, including *M. smithii*, compared to a cohort of 92 apparently healthy individuals [156]. Taken as a whole, these well-designed studies reported apparently contradictory results, suggesting that the faecal microbiota is not appropriate to resolving the question of the relationship between *M. smithii* and CRC. Further studies of cancerous tissues compared to apparently healthy tissue collected from paired individuals (including auto-controls) is required.

10. Conclusions and perspectives

M. smithii stands as an intricate and overlooked player in the microbiota physiology and associated pathologies. The culmination of several pieces of research has revealed the unique attributes of this methanogenic archaeon, painting a picture of its diverse roles and interactions within the complex ecosystems of the human body [157–159]. The taxonomic distinctiveness of *M. smithii*, confirmed through meticulous phenotypic, genetic, and genomic analyses, underscores its significance [160,161]. Its ability to detoxify hydrogen from bacterial fermentations and converting it into methane, positions *M. smithii* as a key contributor to metabolism-driven microbial networks [162]. The symbiotic association with *Ca.* Nanopusillus phoceensis [110] adds an additional layer of complexity, giving rise to distinct cell variants and influencing the dynamics of microbial communities. The model organism status of *M. smithii* in pathogenicity by association is a paradigm-shifting revelation. Its translocation with bacteria, the induction of detectable inflammatory responses, and co-culture from various bodily fluids open perspectives for exploring not only its physiological impact but also its potential implication in diseases. However, the limited availability of *M. smithii* strains in microbial collection databases represents a critical gap in our understanding. Only four strains are currently documented, prompting a call for a comprehensive effort to expand this repository. The need for in-depth investigations into the *in utero* acquisition, lifespan dynamics, diversity, and susceptibility of *M. smithii* is increasingly evident, holding promise for breakthroughs in microbiota-related studies.

Efforts should concentrate on expanding microbial collections to encompass a broader spectrum of *M. smithii* strains from various sources, including animals and derived products, facilitating a more nuanced exploration of its genotypic and phenotypic diversity. In parallel, investigations into the *in utero* acquisition of *M. smithii* could unravel critical aspects of early microbial colonisation and its potential implications for later health outcomes. Longitudinal studies tracking *M. smithii* throughout an individual's life will provide insights into its dynamic role within the microbiota, shedding light on its resilience and adaptability. Furthermore, advances in metagenomics, metatranscriptomics, metaproteomics, and metabolomics will enable a systems-level understanding of the host-microbe dialogue, offering unprecedented insights into the functional dynamics of these microbial communities.

The limited research works about *M. smithii* emphasize the importance of raising awareness about its roles and potential significance in health and diseases. Educational programmes and interdisciplinary collaborations could foster a collective effort to propel *M. smithii* into the forefront of microbiota research.

CRediT authorship contribution statement

Ihab Malat: Writing – original draft, Methodology, Conceptualization. **Michel Drancourt:** Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Ghiles Grine:** Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36742.

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