REVIEW

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Methanogens as hidden architects of intestinal ecology in monogastric animals: distribution and function

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Abstract

Methanogens, strictly anaerobic archaea within the gut microbiota of monogastric animals, play dualistic roles in host health through their unique molecular and metabolic characteristics. Distinguished by conserved 16S rRNA sequences, ether-linked membrane lipids, and archaea-specific cofactors (e.g., Coenzymes M and F_{420}), these microorganisms drive methanogenesis via hydrogenotrophic, aceticlastic, and methylotrophic pathways. Despite their low abundance (~1%–10% of gut anaerobes), methanogens critically regulate the host's metabolic homeostasis by scavenging hydrogen to enhance fibrolytic bacterial activity, improving dietary fiber degradation and nutrient absorption. However, their overgrowth correlates with metabolic disorders such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), obesity, and chronic constipation, underscoring a functional duality. Host-specific distribution patterns reveal the dominance of *Methanobrevibacter smithii* in humans and pigs, while *Methanomassiliicoccales* and *Methanosphaera* occupy niche roles in rabbits and companion animals. Their abundance is shaped by developmental stages (e.g., maternal transmission, post-weaning shifts), dietary fiber intake, physiological states (e.g., IBD-linked reduction, IBS/obesity-associated proliferation), and environmental stressors (e.g., ammonia tolerance). Current research limitations include bacterial-centric biases, undefined pathogenic thresholds, and scarce cross-species comparisons. Future directions emphasize multi-omics integration to elucidate methanogen–host interactions, develop 'archaebiotics' for targeted population modulation, and engineer ecological strategies (e.g., enhancing hydrogen sinks) to mitigate methane-related disorders. Advancing this knowledge will optimize therapeutic interventions for metabolic diseases, improve nutrient utilization, and reduce environmental methane emissions.

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Introduction

The intestinal tract, a central organ for nutrient digestion and absorption and a crucial immunological barrier in monogastric animals, harbors diverse microbial communities including bacteria, archaea, eukaryotes, viruses, and fungi. These microbes engage in intricate cross-kingdom signaling with the host. Through these interactions, they form complex networks that play essential roles in maintaining nutritional metabolic homeostasis and host health[1]. The development of the archaea domain's phylogenetic classification system by Carl Woese revolutionized microbiological research[2]. Among archaea, anaerobic methanogens have drawn significant attention because of their unique molecular features. These organisms possess conserved 16S rRNA sequences that distinguish them from eubacteria, lack peptidoglycan cell walls, and have membranes composed of isoprenoid-derived lipids linked to glycerol-1-phosphate via ether bonds[3,4]. In terms of energy metabolism, methanogens perform methanogenesis, an anaerobic respiratory process that reduces carbon dioxide, acetate, and methyl compounds to produce methane. This process relies on archaea-specific cofactors, such as Coenzymes M and F_{420}[5]. Although methanogens have a relatively low abundance in the gut microbiota, constituting approximately 1% of the porcine intestinal microbiome and approximately 10% of human gut anaerobes, they significantly influence the host's metabolic homeostasis[6,7] as hidden architects of intestinal ecology. They achieve this through mechanisms

such as modulating the partial pressure of microenvironmental hydrogen and remodeling the microbial interaction network[8,9].

Methanogens exhibit a dual nature in their ecological functions. On one hand, their hydrogenotrophic metabolism is beneficial, as it removes excess molecular hydrogen generated during fermentation. This process helps maintain transmembrane proton gradients, thereby promoting the growth of fibrolytic bacteria and enhancing the efficiency of dietary fiber degradation[10]. As a result, methanogens can improve nutrient absorption, particularly in malnourished hosts. On the other hand, methanogens have been associated with various metabolic disorders including irritable bowel syndrome (IBS), chronic constipation, and obesity[11]. Clinical studies show that individuals with more abundant methanogens often have elevated hepatic triglyceride levels. Moreover, obese individuals typically harbor more methanogens than those with a normal body weight, and bariatric surgery can reduce methanogen populations[10,12]. Given this metabolic complexity, the concept of "archaebiotics" has emerged. This strategy aims to dynamically regulate methanogen populations according to the host's metabolic phenotype, ultimately restoring the metabolic network's balance.

Despite significant progress in gut microbiome research, several principal limitations persist. The overwhelming dominance of bacteria in terms of abundance within the animal intestine has skewed research efforts predominantly towards functional analyses of the bacterial domain. Consequently, the contributions of methanogens to host physiological regulation have remained largely under the radar, leaving a critical knowledge gap in understanding their multifaceted roles. From a clinical perspective, the use of germ-free animal models has yet to establish a clear pathogenic threshold of methanogens[6,13]. This lack of definition complicates the diagnosis and management of potential methanogen-associated disorders. Moreover, the existing body of research is heavily concentrated on human and porcine intestinal ecosystems[7,14]. The scarcity of comparative metagenomic studies across other monogastric species hinders the development of a comprehensive understanding of methanogens' ecology and function across diverse hosts. This review endeavors to bridge these gaps by comprehensively examining methanogens' taxonomic characteristics, factors influencing their abundance, metabolic pathway intricacies, interactions with the host's metabolic and immune systems, and synergistic relationships with bacterial communities.

Classification

The methodologies employed in the taxonomy of methanogens have undergone substantial evolution, paralleling the rapid advancements in microbiome research technologies. In the early stages of investigation, 16S rRNA gene-based sequencing served as the cornerstone for delineating phylogenetic lineages among methanogens[15]. However, the subsequent advent of sophisticated techniques such as metagenomics, flow cytometry, quantitative real-time polymerase chain reaction (qPCR), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized the field, markedly enhancing both the detection efficiency and taxonomic resolution[16,17]. For instance, flow cytometry capitalizes on the autofluorescence property of Coenzyme F_{420}, a key cofactor in the methanogenesis pathway with a maximal absorption peak at 420 nm[18]. This characteristic allows for the rapid and accurate quantification of methanogens within complex microbial communities[16]. Similarly, qPCR offers highly sensitive detection capabilities by targeting specific functional genes associated with methanogenesis, such as *mcrA*, which encodes methyl-Coenzyme M reductase[19]. The Genome Taxonomy Database (GTDB) acts as a comprehensive resource, currently containing 2,339 archaeal genomes distributed across 19 phyla, over 70% of which represent uncultured organisms[20]. Notably, the establishment of a curated repository containing 1,167 archaeal genomes of the human gut has provided invaluable material for in-depth investigations into the structural organization and functional dynamics of methanogen communities[6].

From a phylogenetic perspective, methanogens are currently classified into nine distinct orders, namely *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanocellales*, *Methanopyrales*, *Methanomassiliicoccales* (formally recognized as the seventh methanogen order by the International Committee on Systematics of Prokaryotes in 2013[21])[22–24], and the more recently described *Methanonatronarchaeales*[25] and *Methanoliparales*[23]. Among these, the *Methanomassiliicoccales* order exhibits unique evolutionary divergence compared with the other methanogen groups. It is further divided into two families, the free-living clade *Methanomethylophilaceae*, which includes *Methanomassiliicoccus luminyensis* and *Candidatus Methanomassiliicoccus intestinalis*[8], and the host-associated clade *Methanomassiliicoccaceae*, encompassing organisms such as *Candidatus Methanomethylophilus alvus*[6,8]. Within the *Methanobacteriales* order, *Methanobrevibacter smithii* is classified into two species-level clades, '*smithii*' and '*smithii*_A', according to the GTDB classification system[20], highlighting the intricate taxonomic diversity within this group.

Morphologically, methanogens can be classified into six distinct cellular architectures. These include rod-shaped forms, exemplified by *Methanobacterium* and *Methanothermobacter*; coccoid shapes, such as *Methanococcus* and *Methanosphaera*; spiral structures, as seen in *Methanospirillum*; tetrad-forming cells, typified by *Methanosarcina*; plate-shaped organisms like *Methanoplanus*; and filamentous types, represented by *Methanosaeta*[18,26]. This morphological diversity provides a fundamental basis for initial taxonomic identification and offers insights into the physical adaptations of methanogens to various ecological niches.

In terms of metabolism, methanogens are commonly categorized according to their substrate utilization patterns into three major groups: hydrogenotrophic, aceticlastic, and methylotrophic. Among these, hydrogenotrophic lineages are the most prevalent (Table 1)[4,27]. Some methanogen taxa exhibit strict substrate specificity. For example, *Methanosphaera* spp., with *Methanosphaera stadtmanae* as a representative[28], exclusively reduce methanol, utilizing H_2 as an electron donor[29]. In sharp contrast, members of the *Methanosarcinales* order show remarkable metabolic versatility, being able to utilize multiple substrates, including acetate and methyl compounds[23]. This ability to employ diverse substrates defies simple classification into a single metabolic category. The functional diversity among methanogens not only underscores the evolutionary adaptation of these organisms to specific ecological niches but also uncovers potential molecular targets for modulating their ecological roles. Understanding these metabolic distinctions is crucial for predicting how methanogens interact with other members of the microbiota and for devising strategies to manipulate their activities in both natural and engineered ecosystems.

Distribution and influencing factors of methanogens' abundance in intestinal tracts of monogastric animals

Methanogens are widely distributed throughout the gut of monogastric animals, and their presence and abundance are influenced by a multitude of factors, including the growth stage of the animal, its dietary composition, the surrounding environment, and its physiological state.

Table 1. The substrates available to methanogens from different methanogenesis pathways

Order	Family	Genus	Species	Substrates	Ref.
Methanobacteriales	*Methanobacteriaceae*	*Methanobrevibacter*	*M. smithii*	H_2, methanol	[19]
			M. oralis	H_2	[59]
			M. arboriphilus	H_2, formate	[60]
		Methanosphaera	*M. stadtmanae*	H_2, methanol	[29]
Methanomicrobiales	*Methanomicrobiaceae*	*Methanogenium*		H_2, formate	[61]
Methanosarcinales	*Methanosarcinaceae*	*Methanosarcina*	*M. barkeri*	Acetate	[62]
		Methanimicrococcus		Methylamine	[58]
Methanomassiliicoccales	*Methanomethylophilaceae*	*Candidatus Methanomethylophilus*	*M. alvus*	Methanol	[63]
	Methanomassiliicoccaceae	*Methanomassiliicoccus*	*M. intestinalis*	Methylamines	[36]
			M. luminyensis	Methylamines	[64]

Distribution of methanogens in the intestines of monogastric animals

Multi-species studies aimed at exploring the distribution of methanogens within the intestinal ecosystems of animals have identified five key archaeal lineages. These lineages, namely *Methanobrevibacter* and *Methanosphaera* (both belonging to the *Methanobacteriales* order), *Methanomethylophilaceae* (from the *Methanomassiliicoccales* order), *Methanocorpusculum* (of the *Methanomicrobiales* order), and *Methanimicrococcus* (from the *Methanosarcinales* order), are recognized as the dominant archaeal components in the gut. Collectively, they account for more than 90% of the archaeal communities[15,30]. Among these, *Methanobrevibacter*[31] stands out as the most prevalent genus across the intestinal tracts of various animals, succeeded by the candidate taxon *Ca. Methanomethylophilaceae*[15]. Meanwhile, although *Methanosphaera* has a wide distribution across different animal species, its abundance is relatively lower[31].

In the context of the human gut microbiome, methanogens typically constitute approximately 10% of the anaerobic communities in healthy individuals[32]. Metagenomic analyses have shown that the *Methanobacteriales* (87.15%) and *Methanomassiliicoccales* (12.43%) are the predominant orders, with *Methanobrevibacter* being the dominat genus, accounting for 85% of the archaeal population[6]. At the species level, profiling has identified *Methanobrevibacter smithii*, *Methanobrevibacter oralis*, *Methanosphaera stadtmanae*, *Methanomassiliicoccus luminyensis*, *Candidatus Methanomassiliicoccus enteris*, and *Candidatus Methanomethylophilus alvus* as the core methanogens in the human gut[33]. *Methanobrevibacter smithii* exhibits an almost universal colonization rate (97.5% prevalence) and constitutes 84% of the adult archaeomes, with 16% belonging to the clade *Methanobrevibacter smithii*_A and 68% to the clade *Methanobrevibacter smithii*. On the other hand, *Methanosphaera stadtmanae* has a mean abundance of 13% and a prevalence of 29%[6,8,34]. Additionally, other taxa such as multiple species from the *Methanomassiliicoccales* order[35], *Ca. Methanomassiliicoccus intestinalis*[36], and *Methanosphaera cuniculi* (originally isolated from rabbit intestines)[37] often inhabit the human gut. When considering the strains *Mx02, Mx03,* and *Mx06*, the cumulative prevalence of these additional taxa reaches 80%[38].

The archaeal communities in pigs closely resemble those in humans in terms of composition. *Methanobacteriales* (57%–80%) and *Methanomassiliicoccales* (15.07%) are the dominant orders, with *Methanobrevibacter* (57%) and *Methanosphaera* (3%–14%) being the principal genera[7,39,40]. *Methanobrevibacter smithii* is detected in nearly all samples from the porcine colon (99.7%) and feces (99.9%)[7,41]. However, the dominance patterns of *Methanobrevibacter smithii* can vary, depending on the analytical methodology employed. qPCR often identifies it as the most prevalent archaeal species[30], whereas amplicon sequencing reveals that *Methanobrevibacter smithii* has a relatively minor prevalence, with *Methanobrevibacter millerae*, *Methanosphaera cuniculi*, and *Methanobrevibacter boviskoreanii* collectively accounting for 80%–90% of the total archaeal abundance[9]. Spatial analysis of the porcine gut shows a progressive increase in the relative abundance of *Methanobrevibacter* (comprising 44.2%–59% of archaeal communities) from the ileum to the colon. In contrast, methylotrophic *Methanomethylophilaceae* archaea remain scarce, accounting for less than 0.1% of the archaeal population throughout the porcine gastrointestinal tract[9].

In lagomorphs, archaeal communities are predominantly composed of *Methanobrevibacter* species[42,43]. In companion animals, such as dogs (where archaeal communities constitute 25% of the gut microbiota), cats (16.66%), and horses (4.16%), *Methanobrevibacter smithii* co-occurs with other archaeal species, including *Methanocorpusculum aggregans*, *Methanocorpusculum labreanum*, *Methanobrevibacter millerae*, *Methanobrevibacter thaueri,* and *Methanobrevibacter olleyae*[30]. These findings highlight the species-specific variations in the composition and abundance of methanogens across different monogastric animals, which may be associated with their unique dietary habits, digestive physiologies, and ecological niches.

Factors influencing the abundance of methanogens

The diversity and functionality of mammalian gut archaea are intricately regulated by a combination of the host's phylogeny, dietary habits, fiber content, and intestinal physiological characteristics[15]. These factors interact in complex ways to shape the composition and dynamics of methanogen communities within the gut ecosystem.

In addition to the previously mentioned differences in the prevalence of methanogens among different animal species, significant variations in the gut methanogen community structure are also observed among different breeds within the same species. For example, in pigs, the diversity of methanogens in the gut of the fat-type Erhualian breed is notably lower than that in the lean-type Landrace pigs[39].

The developmental stage of the host significantly impacts the structure of archaeal communities. In mammals, neonatal colonization by *Methanobrevibacter smithii* is likely facilitated by maternal transmission, primarily through breast milk[44]. As the host matures into adulthood, the abundance of *Methanobrevibacter smithii* increases substantially, while geriatric populations tend to exhibit an enrichment of *Methanomassiliicoccales*[45]. Similar trends are observed in pigs, where the dominant position of *Methanobacteriales* is gradually weakened by *Methanomassiliicoccales*[46]. Adult pigs generally display higher archaeal α-diversity compared with piglets. However, the weaning and growth phases in piglets are associated with a notable increase in archaeal richness. This change is largely attribute to dietary adjustments, such as an increased intake of fiber[7]. Weaning presents a pivotal transition point in the gut ecosystem, triggering a succession of archaeal communities. Suckling and nursery-stage piglets are predominantly colonized by *Methanobrevibacter smithii*, which is

gradually replaced by *Methanobrevibacter boviskoreanii* and members of the *Methanomassiliicoccales* after weaning[7,47]. These developmental changes underscore the dynamic and adaptive nature of host–microbe interactions, which aim to optimize nutritional metabolism while navigating niche competition.

Dietary fiber content plays a central role in determining the distribution and function of methanogens. The development of methanogen communities relies on the presence of anaerobic environments and diverse carbohydrate sources[45,48]. In pigs, high-fiber diets indirectly boost the abundance of certain methanogens, such as *Methanobrevibacter* sp900769095, by promoting the growth of hydrogen-producing bacteria. Methane production in this context is positively correlated with the fiber-degrading activities of the gut microbiota but negatively associated with starch metabolism[7]. A decrease in the fiber-to-starch ratio in the diet can lead to the accumulation of lactic acid and a subsequent drop in gut pH. This shift favors the conversion of lactate to propionate, a process that competes with methanogenesis for nicotinamide adenine dinucleotide (NADH)-reducing equivalents, ultimately suppressing methane production[49,50]. Moreover, high-fiber diets stimulate the production of bacterial methylamine, providing a substrate for methylotrophic methanogens, such as those belonging to the *Methanomassiliicoccales*[51]. Similarly, diets rich in protein have been shown to increase the overall abundance of methanogens[52], highlighting the multifaceted impact of dietary components on methanogens' ecology.

Disease states have a profound impact on the homeostasis of methanogen communities within the gut. While conditions such as colorectal cancer, polypectomy, and IBS have shown minimal effects on the abundance of human methanogens, inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are associated with reduced methanogen colonization[53,54]. This disruption suggests that chronic inflammation in the gut can alter the ecological niche, making it less hospitable for methanogens. In swine infected with influenza A, significant shifts occur in the archaeal community. The abundance of *Methanobrevibacter boviskoreanii* and *Methanosphaera cuniculi* decreases, while *Methanobrevibacter millerae* and *Methanomethylophilaceae* increase. Additionally, *Methanosphaera stadtmanae* is detected specifically in diseased pigs[8]. Notably, *Methanobrevibacter* species exhibit remarkable tolerance to antibiotics that target bacterial RNA and protein synthesis, and cell wall formation. This characteristic may influence the outcome of clinical interventions[55], as the persistence of methanogens during antibiotic treatment could potentially affect the recovery of the gut microbiota and host health.

Cross-regional studies of swine microbiota have revealed significant variations in archaeal diversity and community composition. Chinese swine populations exhibit lower archaeal diversity compared with their Danish and French counterparts, with distinct dominant taxa in each region. *Methanobrevibacter* is the predominant genus in Chinese (44.94%) and French (15.41%) swine, while *Candidatus Methanomethylophilus alvus* dominates in Danish herds (14.32%)[46]. Similarly, the marked enrichment of *Methanomassiliicoccales Mx06* in non-Westernized human populations highlights the role of lifestyle factors, including diet and environmental exposures, in shaping the biogeography of methanogens[6]. These findings underscore the complex interplay among environmental factors, host characteristics, and methanogen communities, which has important implications for understanding the ecological dynamics of the gut microbiota and its impact on host health.

Methanogenesis

Methanogenesis stands as one of the most ancient energy-conserving metabolic processes, exerting direct physiological effects on gastrointestinal systems[56]. Similar to the final workers in an industrial assembly line, methanogens occupy the terminal position in microbial trophic chains. They utilize the end-products of dietary substrate fermentation to produce methane[31,57] (Fig. 1). Acting as the ultimate electron acceptors, methanogens metabolize byproducts from bacteria and eukaryotes, such as H_2, CO_2, acetate, and methylamines, which are generated during the breakdown of dietary polymers, including short-chain fatty acids (SCFAs) and alcohols (Table 1). There are three primary methanogenic pathways: hydrogenotrophic (dependent on H_2 and CO_2), acetoclastic (involving acetate cleavage), and methylotrophic (utilizing methanol or methylamines)[58]. The metabolic flexibility of *Methanobrevibacter* is a key factor contributing to its dominance within the archaeal communities in the human gut[19].

Central to all methanogenic pathways is the terminal reaction catalyzed by methyl-Coenzyme M reductase (MCR). This reaction involves the reduction of CH_3–S-Methyl-Coenzyme M (CoM) using 7-mercaptoheptanoylthreonine phosphate (CoB-SH), resulting in the formation of methane and the heterodisulfide CoM-S-S-CoB (HDS)[65]. Encoded by the *mcrA* gene[66], MCR exists as an $(\alpha\beta\gamma)_2$ heterohexamer[67], featuring two Ni-centered (Ni I/Ni II) F_{430} active sites. These active sites are derived from 5-aminolevulinate[18] and are formed at the subunit interfaces ($\alpha/\alpha'/\beta/\gamma$ and $\alpha'/\alpha/\beta'/\gamma'$)[67].

The MCR active sites bind SH-CoM and SH-CoB in a sequential manner, triggering a conformational change that locks the enzyme into an inactive state (MCR_{silent}) with the formation of the CoM–CoB heterodisulfide[67]. The enzymatic activity of MCR is critically dependent on the redox state of Ni. The NiI-MCR form, with a midpoint potential of –650 mV, catalyzes the methanogenesis reaction. In contrast, the NiII-MCR form requires reductive activation, which is facilitated by dithiothreitol, adenosine triphosphate (ATP)-binding proteins (A2), and Fe-S complexes (A3a)[68,69]. This sensitivity of Ni to redox changes is fundamental to the strict anaerobic nature of methanogens and their vulnerability to inhibitors such as 3-nitrooxypropanol (3-NOP) and bromoethanesulfonate (BES). These inhibitors function by oxidizing the Ni center, thereby disrupting the methanogenic process[70,71].

The heterodisulfide reductase (HdrABC) and the methyl-viologen-reducing hydrogenase complex (MvhAGD) universally mediate the reduction of HDS, regenerating the coenzymes CoM-SH and CoB-SH[72,73]. HdrABC, a membrane-associated Fe-S protein complex, collaborates with $F_{420}H_2$ dehydrogenase (Fpo) under hydrogenotrophic conditions[58]. HdrA contains an electron-bifurcating flavin adenine dinucleotide (FAD) moiety, while HdrB forms the catalytic core responsible for the reduction of HDS[74,75]. Meanwhile, MvhA and MvhG constitute the conserved [NiFe] hydrogenase module, with MvhD facilitating the transfer of reducing equivalents to Hdr[73].

Methanogens possess two evolutionarily conserved Hdr systems, flavin-based electron bifurcation (FBeB), which is predominant in hydrogenotrophic methanogens, and cytochrome-dependent electron transfer (CDeT), which is characteristic of methylotrophic and acetotrophic methanogens[27]. FBeB enzyme complexes, such as HdrABC-MvhAGD[76], utilize flavin cofactors (FAD/FMN) for electron bifurcation[77]. These flavoproteins accept electron pairs from NAD(P)H, $F_{420}H_2$, H_2, or formate, generating low-potential electrons that are transferred via ferredoxin (Fd). The reaction can be represented as $2H_2 + F_{dox}$ + CoM-S-S-CoB → reduced ferredoxin (Fd_{red2}) + CoM-SH + CoB-SH + $2H^+$. This electron-bifurcating mechanism enables methane production with a minimal ATP requirement of ≤ 1 ATP per molecule by coupling Fd_{red} to CO_2 reduction, effectively closing the metabolic loop of the Wolfe cycle[78]. In CDeT systems, the reduction of the lipophilic carrier methanophenazine (Mp) is coupled with H^+ electrochemical potential ($\Delta\mu H^+$), driving both the generation

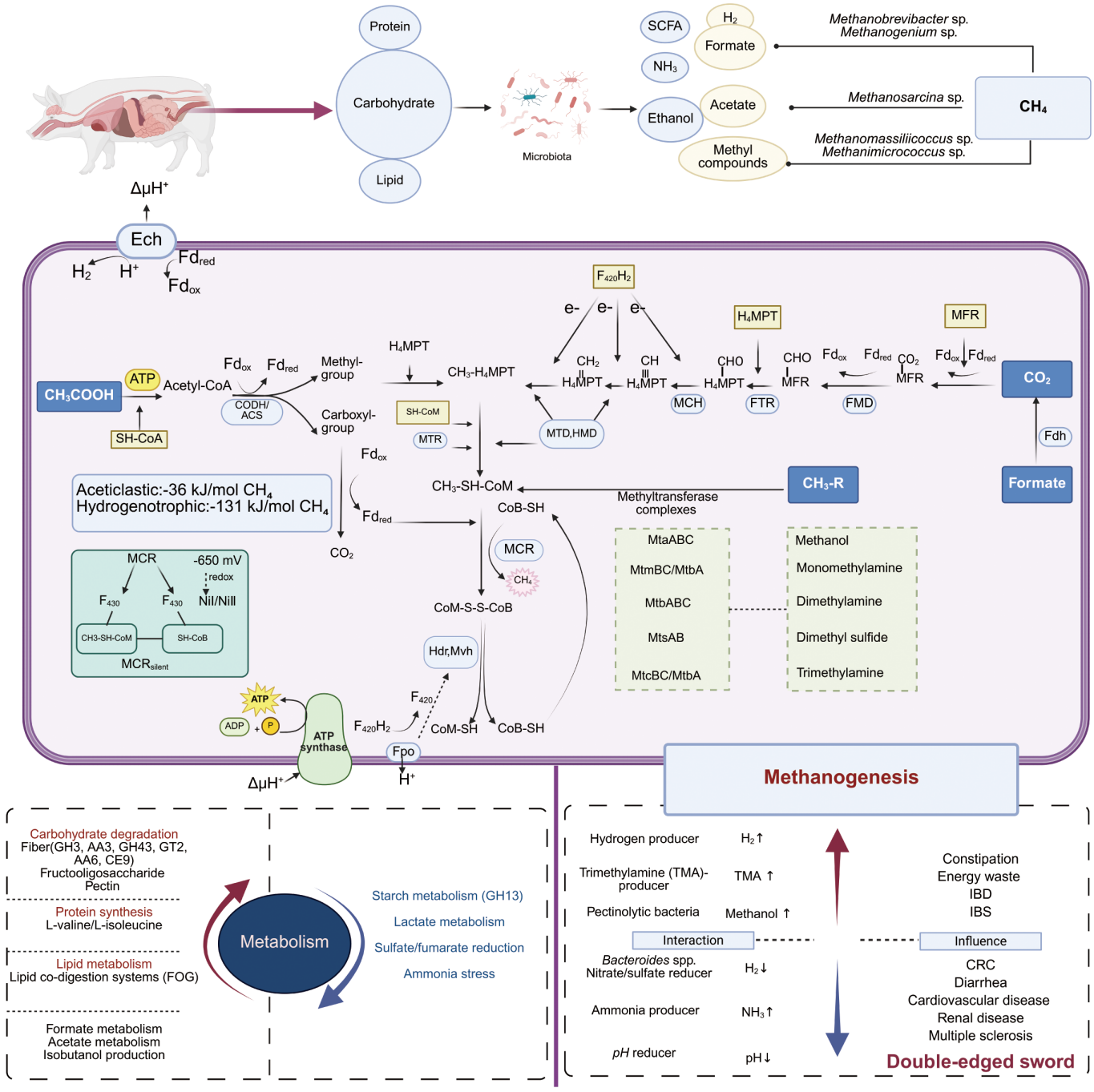

Fig. 1 Effects of methanogens on the host's metabolism and health and the mechanisms of methanogenesis.

of Fd_{red} and ATP synthesis[27].

In addition to these core modules, substrate-specific adaptations diversify the auxiliary pathways of methanogenesis. The methanogenic metabolic network integrates crucial methyl carriers, including methanofuran (MFR), tetrahydromethanopterin (H_4MPT) (derived via the Wood–Ljungdahl pathway), N^5-methyltetrahydromethanopterin, and enzyme complexes such as Coenzyme M methyltransferase (MTR), MCR, formate dehydrogenase (FMD), and methanophenazine-dependent enzymes (tetrahydromethanopterin formyltransferase [FTR], methenyltetrahydromethanopterin cyclohydrolase, methylene-tetrahydromethanopterin dehydrogenase/methenyltetrahydromethanopterin hydrogenase)[23,27,79], highlighting the evolutionary plasticity and functional versatility of these metabolic components within the archaeal domain.

Hydrogenotrophic methanogenesis

In methanogenic archaea, the vast majority of hydrogenotrophic lineages, including *Methanococcales*, *Methanopyrales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanocellales*, and *Methanosarcinales* (excluding *Methanomassiliicoccales*), use the Wood–Ljungdahl pathway for CO_2 fixation[80]. These organisms utilize H_2 as an electron donor to sequentially reduce CO_2 to CH_4[81]. For example, the metabolic activity of dominant methanogens in the porcine gut, such as *Methanobrevibacter* sp900769095), is intricately linked to hydrogenotrophic methanogenesis[7].

Notably, certain methanogens can utilize formate as an alternative electron carrier[4,82]. Formate dehydrogenase (FDH) oxidizes four formate molecules to CO_2[58], and subsequent hydrogenlyase (such as Hdr)-mediated reactions generate H_2[7]. Many members of the *Methanomicrobiales* lack genes encoding hydrogenase, rendering formate metabolism essential for providing reducing equivalents. In *Methanothermobacter* speices, for instance, Hdr and FDH exhibit activity only when formate serves as the electron donor, and the activity of formate hydrogenlyase is crucial for supporting Hdr reactions[82]. Additionally, some methanogens can utilize secondary alcohols, such as 2-propanol, 2-butanol, and cyclopentanol, or ethanol as electron donors[27].

Hydrogenotrophic methanogenesis commences with the reduction of CO_2 to formylmethanofuran (CHO-MFR), a reaction catalyzed by formylmethanofuran dehydrogenase (FMD)[83]. This initial step occurs under conditions where low-potential Fd_{red} is available, generated through either the FBeB or CDeT systems[58]. The endergonic nature of this reaction is energetically coupled with the generation of an ion gradient via the membrane-bound energy-conserving hydrogenase (Ech)[58]. Subsequently, formylmethanofuran: FTR transfers the formyl group from CHO-MFR to H_4MPT, forming CHO-H_4MPT. This cofactor-dependent reaction necessitates the formation of a ternary complex involving formyl-MFR, H_4MPT, and the apoenzyme[84]. The resulting CHO-H_4MPT undergoes a series of sequential transformations. First, methenyltetrahydromethanopterin cyclohydrolase catalyzes a dehydration reaction, yielding N^5,N^{10}-methenyl-H_4MPT (CH≡H_4MPT). This is followed by $F_{420}H_2$-dependent reduction steps mediated by methylenetetrahydromethanopterin dehydrogenase[85] and methenyltetrahydromethanopterin hydrogenase[86], which convert CH_2=H_4MPT into N^5-methyl-H_4MPT (CH_3-H_4MPT) and then N^5-methyl-H_4MPT (CH_3-HMPT). Throughout these dehydration and reduction processes, $F_{420}H_2$ provides the necessary electrons.

The methyl group from CH_3-H_4MPT is transferred to CoM-SH by MTR, resulting in the formation of CH_3-S-CoM[87]. MCR then catalyzes the terminal reductive demethylation of CH_3-S-CoM to produce CH_4, using CoB-SH as the electron donor. This reaction simultaneously generates the heterodisulfide CoM-S-S-CoB[67]. The metabolic cycle is completed through reduction of CoM-S-S-CoB back to SH-CoM and SH-CoB, a process mediated by Hdr complexes[72,73]. Electrons for this final reduction step are sourced from $F_{420}H_2$ or H_2[67], ensuring the continuous operation of the hydrogenotrophic methanogenesis pathway.

Aceticlastic methanogenesis

Acetotrophic methanogens employ a core mechanism to cleave acetate into methane and CO_2, a process involving intramolecular electron transfer from the methyl group to the carboxyl carbon[79]. Presently, only two genera, *Methanosarcina* (within the *Methanosarcinales* order)[88] and *Methanothrix* (previously known as *Methanosaeta*)[89], are recognized for their ability to perform this metabolic function. *Methanothrix* represents a strictly acetoclastic lineage that reduces acetate to CH_4 through direct interspecies electron transfer (DIET) with syntrophic bacteria, such as *Geobacter metallireducens*. This unique pathway is the only known means by which *Methanothrix* can carry out autotrophic respiration, as it allows for the direct uptake of extracellular electrons from organic donors[89,90]. In contrast, *Methanosarcina acetivorans* displays metabolic versatility. It can oxidize carbon monoxide to CO_2 while simultaneously reducing CO_2 to CH_4 via the Wood–Ljungdahl pathway[91]. The metabolism of CO in *M. acetivorans* generates auxiliary substrates, such as acetate and formate, which are integrated into the methanogenic metabolic network to conserve energy. Despite the occasional colonization of animal intestines by low-abundance *Methanosarcina* strains, the physiological roles of these organisms in acetoclastic methanogenesis within the gut remain largely uncharacterized[15].

In the process of acetoclastic methanogenesis, acetate is first activated through an ATP-dependent reaction to form acetyl-CoA[92]. Subsequently, acetyl-CoA is enzymatically cleaved into enzyme-bound methyl and carbonyl moieties[93]. The methyl group is transferred to H_4MPT, entering the final two steps of the pathway that are shared with hydrogenotrophic methanogens. Meanwhile, the carbonyl moiety is oxidized to CO_2, with ferredoxin (Fd_{ox}) serving as an the electron acceptor and generating reduced ferredoxin (Fd_{red})[93]. The Fd_{red} then participates in the reduction of the heterodisulfide (CoM-S-S-CoB) during the catalytic cycle of MCR[94]. The change in free energy associated with acetoclastic methanogenesis is –36 kJ/mol of CH_4[95], which is markedly lower than that of hydrogenotrophic pathways (–131 kJ/mol of CH_4[75]). This lower energy yield results in reduced energy capture efficiency, necessitating compensatory adaptations in acetotrophic methanogens. Such adaptation may include high substrate affinity or syntrophic metabolic coupling, enabling these organisms to persist within their ecological niches.

Methylotrophic methanogenesis

Methylotrophic methanogens catalyze the reductive demethylation of various methyl compounds, including methanol, monomethylamine, dimethylamine, trimethylamine, dimethyl sulfide, and methanethiol, through the methylotrophic methanogenesis pathway[94]. This metabolic process comprises two consecutive methyl transfer reactions. First, substrate-specific methyltransferase complexes, such as MtsA/MtsB for dimethyl sulfide, MtaABC for methanol, and MtmBC/MtbA for monomethylamine, transfer the methyl group from CH_3-R donors to the corresponding corrinoid proteins, forming CH_3-corrinoid intermediates. Second, the subsequent transfer of the methyl group to Coenzyme M (CoM-SH) results in the formation of methyl-Coenzyme M (CH_3-S-CoM)[94]. When H_2 is abundant, MCR reduces CH_3-S-CoM to CH_4. However, under conditions of electron donor limitation, the reverse Wood–Ljungdahl pathway oxidizes CH_3-S-CoM to CO_2, releasing electrons that support the subsequent reduction of methyl groups. This establishes an autocatalytic electron cycling mechanism, ensuring the metabolic pathway's continuous operation[96–98].

Methanogens that utilize methyl compounds in the presence of H_2 represent a substantial proportion of archaeal populations within animal microbiomes[8,64]. For example, members of the *Methanomassiliicoccales* order employ methylated amines, such as trimethylamine, as methanogenic substrates[99,100]. The model species *Methanomassiliicoccus luminyensis* couples H_2 oxidation with heterodisulfide reduction via the membrane-bound Fpo-HdrD electron transport chain. This coupling generates a proton motive force ($\Delta\mu H^+$) that is essential for ATP synthesis, while simultaneously eliminating the organism's dependence on sodium ions, thereby enabling efficient energy conservation[64].

Methanosphaera stadtmanae, which lacks carbon monoxide dehydrogenase/acetyl-CoA synthase, relies exclusively on methanol and H_2 for methanogenesis. This process is mediated by the methanol: coenzyme M methyltransferase encoded by the *mtaABC* genes[28,101]. In porcine gut, *M. smithii* may engage in methanogenesis through methyl metabolic bypass pathways[7]. These methanogenic strains employ specialized enzymatic systems tailored to specific substrates, energy-coupling strategies, and reverse reaction electron cycling mechanisms. These adaptations allow them to maintain metabolic activity under variable conditions of methyl compound availability and energy constraints, highlighting their resilience and versatility within the gut

ecosystem.

Influence of methanogens on nutrient metabolism and intestinal health in monogastric hosts

Methanognes and host metabolism

In porcine models, developmental stages and dietary regimes exert profound regulatory effects on methanogenic activity. Suckling piglets display low gene abundance related to the acetoclastic pathway, which rapidly increases to parental levels during the nursery phase. In contrast, genes associated with the methylotrophic pathway decline with age[7]. Metatranscriptomic analyses have revealed that *Methanobrevibacter* dominates in hydrogenotrophic metabolism, whereas *Methanosphaera* relies on methyl reduction pathways[9]. Notably, the transcriptional activity of these taxa significantly surpasses their genomic abundance. For instance, the transcript levels of *Methanosphaera cuniculi* and *Methanosphaera stadtmanae* exceed their genomic abundance by 27- and 30-fold, respectively[7,102], underscoring their central role in H_2/methyl metabolism.

The metabolic networks of methanogens intricately interact with the host's nutritional metabolism. A high-fiber diet can enhance the activities of fibrolytic enzymes (GH3) and the metabolic pathway of formate in the porcine gut[7]. Furthermore, in vitro studies shows that *Methanobrevibacter* can maintain a high abundance under fecal microbiota co-culture conditions with high concentrations of oligofructose and pectin[103]. Conversely, hydrogenotrophic methanogenesis is negatively correlated with starchase (GH13) and lactate metabolism[7]. Methanogenic activity is positively associated with the intestinal concentrations of formate and acetate[57]. In the microbiomes of piglets, the activation of the sulfate/fumarate reduction pathway reduces the acetate/propionate ratios and suppresses methanogenesis, indicating ecological competition for hydrogen sinks[7]. Additionally, ammonia inhibition disrupts acetoclastic methanogenesis and syntrophic chains by binding to coenzymes (such as Coenzyme M) or blocking the active sites of MCR[104].

Specific methanogen strains have been shown to correlate with host phenotypes. The abundances of *Methanobrevibacter smithii* and *Methanobrevibacter sp900769095* are positively associated with porcine body weight[105]. The symbiotic strain *Candidatus Methanomethylophilus alvus Mx1201* potentially modulates the host's protein synthesis and lipid metabolism through the regulation of the shikimate pathway and bile resistance genes[100]. The metabolic repertoires of methanogens include L-valine/L-isoleucine biosynthesis, isobutanol production, and carbohydrate-active enzyme (CAZyme) families (AA3, GH43, GT2, AA6, CE9), indicating their potential in amino acid and carbohydrate metabolism[106]. The dominance of acetotrophic *Methanosarcina* in FOG (Fats, Oil, and Grease) co-digestion systems highlights its role in lipid metabolism[107]. In rat models, the depletion of methanogens induced by bromochloromethane increases daily weight gain and adiposity, suggesting that methanogen-targeted interventions could be useful for weight management. The diversity of methanogens is positively correlated with the intensity of fiber fermentation in the porcine hindgut[108], and their redox-balancing metabolism affects the host's energy allocation and adipogenesis[39]. These findings elucidate the profound metabolic plasticity of methanogens in the host's energy partitioning and lay the molecular foundations for the targeted modulation of intestinal methane emissions and nutrient utilization efficiency.

Methanogens and gut health

Methanogens have been found to be disproportionately abundant in patients suffering from IBD, periodontal disease, obesity, cancer and diverticulosis[109,110]. The ecological functions of gut methanogens and their associations with various diseases have become crucial areas of focus in clinical microbiological research.

Recent scientific progress has established intestinal methanogen overgrowth (IMO) as a distinct pathological condition that is independent of small intestinal bacterial overgrowth (SIBO). IMO is characterized by the excessive proliferation of methanogens and elevated levels of methane in the breath (≥ 10 parts per million [ppm])[111]. This condition has a strong correlation with constipation and an extended colonic transit time[109,112]. This phenomenon highlights the dual metabolic impacts of methanogenesis. On one hand, the scavenging of hydrogen by methanogens alleviates metabolic inhibition. On the other hand, the associated energy expenditure might exacerbate the metabolic burden on the host. Studies in horses have demonstrated a positive association between long-term colonization by *Methanobrevibacter* and mortality[113], while syntrophic interactions between *Christensenella* and methanogens have been linked to weight loss in humans[114].

In the context of specific disease, patients with constipation-predominant irritable bowel syndrome (IBS-C) show increased fecal microbial α-diversity and a higher abundance of *Methanobrevibacter*, especially *Methanobrevibacter smithii*[115,116]. Breath testing has revealed that in individuals who are high methane emitters (with CH_4 levels ranging from 5 to 75 ppm), there is a 1,000-fold enrichment of *Methanobrevibacter smithii*[57]. Mechanistically, mevalonate pathway inhibitors, such as lovastatin, can alleviate constipation by suppressing the methanogenic activity[117]. Paradoxically, in patients with IBD, there is a dysbiosis in the methanogen community. The total abundance of methanogens in IBD patients exceeds that in healthy controls[110], yet the core species *Methanobrevibacter smithii* is depleted[54], while *Methanosphaera stadtmanae* experiences proliferation[118]. This pathogen activates the TLR8-dependent NLRP3 inflammasome pathways in monocyte-derived dendritic cells (moDCs), which, in turn, triggers the release of pro-inflammatory cytokines and leads to hyperactivation of the innate immune system[119,120]. In colorectal cancer (CRC) patients, the abundance of *Methanobacterium* and *Methanosarcina* is reduced, and *Methanocaldococcus* and *Methanotorris* are depleted in the advanced stages of CRC. This suggests that the exhaustion of methanogens may accelerate the process of tumorigenesis[121]. These findings emphasize the functional heterogeneity among methanogens within inflammatory microenvironments.

The influence of methanogens on the host's metabolic health exhibits bidirectional regulation (Fig. 1). Zhou et al. reported that an increase in fumarate reductase activity leads to the accumulation of succinate in the intestines of piglets, which can contribute to post-weaning diarrhea[122]. In contrast, Chen et al. observed a sharp decline in fumarate reductase expression in healthy piglets after weaning[7]. These findings suggest that methanogens may reshape their intestinal H_2 consumption patterns through hydrogenotrophic methanogenesis, thereby competing with fumarate reductase. Consequently, targeting this interaction may represent a potential therapeutic strategy for alleviating post-weaning diarrhea in piglets. The enrichment of *Methanobrevibacter* associated with anorexia may adapt to hypocaloric states through H_2 oxidation-induced thermogenesis, contributing to the maintenance of metabolic homeostasis[114,123]. Members of the *Methanomassiliicoccales* order, such as *Methanomassiliicoccus luminyensis*, metabolize trimethylamine (TMA) through pyrrolysine-dependent methyltransferase systems[38,124] by methylotrophic methanogen-

esis. In this way, they inhibit the conversion of TMA into the pro-atherogenic trimethylamine-N-oxide (TMAO)[125], thereby presenting potential therapeutic applications for cardiovascular and renal diseases[100,126]. In patients with multiple sclerosis (MS), there is a negative correlation between the abundances of *Methanobrevibacter smithii* and *Methanobrevibacter* sp900766745 and disease severity. Additionally, treatment with dimethyl fumarate increases the colonization levels of these methanogens, accompanied by weight reduction[127]. Conversely, long-term colonization by methanogens has been inversely correlated with host longevity, potentially accelerating the aging process via the depletion of redox potential[113].

From an immunological perspective, ether lipid vesicles (archaeosomes) derived from *Methanobrevibacter smithii* can induce influenza hemagglutinin-specific $CD8^+$ T cell responses and facilitate the vertical transfer of maternal antibodies[128,129]. In MS patients, *Methanobrevibacter smithii* activates the TLR8-NLRP3 inflammasome pathway, leading to the upregulation of genes such as *CASP1*, *TRAF5*, and *STAT5B*, which are associated with interferon (IFN) signaling, IL-2 pathways, and *PPAR*/*RXR* regulation[130]. It also significantly alters the expression of antimicrobial peptide genes in moDCs[120]. Similarly, *Methanobrevibacter stadtmanae* can induce the robust release of pro-inflammatory cytokines in moDCs[120]. Antimicrobial peptides (AMPs) are a crucial component of intestinal immunity. They exert immune functions not only against bacteria and fungi but also against methanogens. Bang et al.[131,132] compared the sensitivity of three methanogenic archaea—*Methanobrevibacter smithii*, *Methanomassiliicoccus luminyensis*, and *Methanosphaera stadtmanae*—with human cathelicidin-derived peptides LL32 and LL20, as well as the antimicrobial peptide NK-lysin. The tested methanogens exhibited different levels of sensitivity, with *M. smithii* being the most susceptible. These findings clearly demonstrate that the antimicrobial peptides released by human innate immune cells target not only bacteria and fungi but also archaea.

Developmental studies have shown that intestinal methanogenesis in piglets, as indicated by the abundance of the *mcrA* gene, is lower than that in adult pigs. Elevated activities of sulfate reductase (encoded by *asrA* and *aprA*) and fumarate reductase (encoded by *frdA*) suggest that H_2 is preferentially diverted towards sulfate and fumarate reduction processes[7]. Notably, the accumulation of succinate mediated by fumarate reductase can trigger weaning-associated diarrhea in piglets, indicating the potential of modulating H_2 sinks as a therapeutic strategy[7,122]. These findings systematically elucidate the involvement of methanogens in the host's pathophysiology through mechanisms such as metabolic network remodeling, immunophenotypic regulation, and energy homeostasis modulation. As a result, they provide the molecular basis for targeted microbiome engineering and the development of novel therapeutic approaches.

Interaction between methanogens and bacteria in the gut of monogastric animals

Metabolic interactions between methanogens and bacteria significantly influence methanogenic efficiency through intricate hydrogen metabolism and electron transfer mechanisms. Methanogens play a pivotal role in sustaining syntrophic bacterial activity by maintaining extremely low hydrogen partial pressures (H_2 < 0.1 Pa), thereby establishing cross-domain metabolic coupling[133]. The hydrogen generated by carbohydrate-fermenting bacteria, including *Mogibacterium*, *Pyramidobacter*, *Christensenella*, *Anaerostipes*, *Ruminococcus,* and *Aminipila*, serves as a substrate for methanogens (such as *Methanobrevibacter* species) to reduce CO_2 to CH_4[57,113,134]. Conversely, *Bacteroides* species can alter hydrogen's availability by recycling mucin glycans, thus fueling nitrate/sulfate-reducing bacteria and subsequently suppressing methanogenesis[135]. Sulfate-reducing bacteria (e.g., *Desulfovibrio*) and *Fibrobacter succinogenes* (through phosphotransacetylase-driven succinate/propionate synthesis) further limit the accessibility of H_2 through substrate competition[45,113,135]. Methylotrophic methanogens, like *Methanosphaera stadtmanae*, can inhibit hydrogenotrophic methanogens by reducing the H_2 concentration below 0.1 Pa, inducing interspecific metabolic suppression[136]. Additionally, the ammonium produced from bacterial protein degradation is assimilated by methanogens (e.g., *Methanobrevibacter smithii*) via ammonium transporters (AmtB, encoded by MSM0234)[10].

Associations between health or disease states and the gut microbiota reveal diverse correlations between methanogen abundance and specific bacterial taxa. In healthy individuals, *Akkermansia*, *Phascolarctobacterium*, and *Eubacterium* exhibit positive associations with methanogens, whereas *Bacteroidetes* and *Veillonellaceae* show negative correlations[137]. In patients with IBS, the positive associations between methanogens' abundance and bacterial diversity/richness are more pronounced. Co-occurring taxa, such as *Christensenella* and members of the *Ruminococcaceae* family, synergistically contribute to metabolic dysregulation[57,138]. Notably, the abundance of *Methanomassiliicoccales* is correlated with TMA-producing bacteria[100], and *Bacteroides fragilis* may modulate methanogens' distribution by regulating the colonic tumor microenvironment[121]. Mathematical modeling has demonstrated that sulfate-reducing bacteria compete more strongly with methanogens for H_2 than reductive acetogens in the human intestine[139]. Methylotrophic archaea (e.g., *Methanosphaera stadtmanae*) engage in metabolic coupling with pectinolytic bacteria (e.g., *Bacteroides*) by utilizing the methanol released by the latter[28]. Overgrowth of *Lachnospiraceae*, *Lactobacillaceae*, and *Streptococcus* can suppress methanogens' activity by reducing the pH, thereby decreasing CH_4 production[140]. These complex interaction networks shed light on the dynamic equilibria of carbon, hydrogen, and electron fluxes within gut microbiomes, offering valuable ecological insights for the targeted modulation of methanogenic modulation.

Conclusions

Methanogens, as integral archaeal constituents of the gut microbiota in monogastric animals, display distinct host-specific distribution patterns. *Methanobrevibacter smithii* is predominant in the intestines of both humans and pigs, whereas *Methanomassiliicoccales* and *Methanosphaera* assume specialized ecological roles in rabbits and companion animals. The abundance of methanogens is intricately influenced by multiple factors. The host's developmental stages play a crucial role, as evidenced by maternal transmission in neonates and significant shifts post-weaning. Dietary components, such as high-fiber diets that promote the growth of hydrogenotrophic methanogens, also profoundly impact their population dynamics. Disease states have differential effects. For instance, IBD leads to reduced methanogen colonization, while IBS and obesity are associated with methanogen overgrowth.

The three primary methanogenic pathways (hydrogenotrophic, acetoclastic, and methylotrophic) exemplify the metabolic duality of methanogens in modulating host health. The hydrogenotrophic pathway, which enhances fiber degradation, has been linked to constipation in certain contexts. The acetoclastic pathway is mainly involved in syntrophic lipid digestion and is restricted to specific methanogen lineages. The methylotrophic pathway can reduce the toxicity of trimethylamine but may also trigger inflammatory responses. Methanogens engage in syntrophic interactions with fibrolytic bacteria, such

as *Christensenella*, by efficiently scavenging H_2. However, they also compete with sulfate-reducing bacteria and acetogens for substrates, influencing the overall metabolic balance within the gut microbiota.

Notwithstanding the significant progress in the field, several research gaps remain. Current studies often exhibit a bacterial-centric bias, overlooking the unique contributions and functions of methanogens. The pathogenic thresholds of methanogens in various host conditions are yet to be precisely defined, and cross-species comparisons are relatively limited. To fully elucidate the roles of methanogens in the microbiota–host axis, future research endeavors should focus on integrating multi-omics approaches to comprehensively map methanogens' metabolic networks. Developing 'archaebiotics' for targeted modulation of methanogen communities and engineering ecological strategies, such as enhancing hydrogen sinks, hold promise for mitigating methane-related disorders. Unraveling these dynamics will not only advance the development of novel therapies for metabolic diseases but also optimize their utilization in animal production and contribute to reducing environmental methane emissions, thereby addressing both health and environmental challenges.

Ethical statements

Not applicable.

Author contributions

The authors confirm contributions to the paper as follows: study conception, manuscript revison and funding acquisition: Luo Y; writing – original draft: He J, Dai Z; writing – review and editing: Deng X, Li H, Huang S, Wu A, Mao X. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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