Chapter 16
Methanogenesis and Its Role in Climate-Change Alleviation

Shahnaz Anjum, Asma Nazir, Bisma Farooq, Madeha Farooq, and Shayista Yousuf

Abstract Methanogenesis is the biological generation of methane (CH_4) by anaerobic microbes belonging to the Archaea domain, also known as methanogens. Understanding how microbial methanogenesis reacts to temperature is crucial for anticipating how this powerful greenhouse gas will interact with climate change. Microorganisms in the environment play a significant role in both global and terrestrial methane emissions and sinks. Climate change mitigation efforts strive to reduce and prevent the emission of harmful greenhouse gases. Researchers have expanded on the importance of methylotrophic communities in global carbon cycle and reducing the influence of greenhouse gases such as methane, carbon dioxide, water vapours, and indirectly carbon derivatives in the environment because of their function in climate change mitigation. The positive response of the methylotrophic community is therefore changing the warm ground surface to cooler temperatures, resulting in a more adaptable habitat for species to survive. The reaction of respiratory carbon (C) emission to temperature change can be reduced over time by a compensatory thermal response in microbial activity. The mass-specific CH_4 respiration rates of the methanogens drop with warming and rise with cooling, implying that microbial methanogenesis has temperature-dependent compensatory responses. However, a complete mechanistic understanding of the reaction of methane cycle to global

S. Anjum (✉)
Department of Botany, Lovely Professional University, Phagwara, Punjab 144411, India
e-mail: shinaz.anjum@gmail.com

A. Nazir
Department of Clinical Biochemistry, University of Kashmir, Hazratbal, Srinagar, J&K 190006, India

B. Farooq
Department of Biomedical Engineering, Satyabhama Institute of Science and Technology, Chennai 600119, India

M. Farooq
Departement of Microbiology, Asian International University, Imphal, Manipur 795113, India

S. Yousuf
Department of Zoology, University of Kashmir, Hazratbal, Srinagar, J&K 190006, India

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J. A. Parray (ed.), *Climate Change and Microbiome Dynamics*, Climate Change Management, https://doi.org/10.1007/978-3-031-21079-2_16

warming is still deficient. This chapter discusses the role of the methylotrophic community in reducing greenhouse gas emissions that cause climate change.

Introduction

For more than 12,000 years, the global climate has been steady, and this stability is essential to human survival [1]. However, throughout the past century, the average global temperature surged up by 1.5 °F, and within the next 100 years, it is predicted to rise by an additional 0.5–8.6 °F. This is a critical problem since even little changes in the average global temperature can lead to significant changes in the climate and weather [2]. According to the IPCC's most recent Fifth Assessment Report, it is very likely that human activity is to blame for the phenomena of climate change that have been seen over the past few decades. Without a doubt, since the 1950s, the atmosphere and the seas have warmed, the amount of snow and ice has decreased, the sea level has risen, and greenhouse gas concentrations have pitched in a way that hasn't happened in centuries or millennia [3]. Emitted greenhouse gases are the primary determinants of anthropogenic radiative forcing. Together, CO_2, CH_4, and N_2O account for more than 80% of the total radiative forcing (the cause of the greenhouse effect), and their present concentrations and rates of growth are higher than those seen in the previous 800,000 and 20,000 years, respectively [4]. While CH_4 (1.804 ppm) and N_2O (0.324 ppm) have far higher warming potential than CO_2, which is by far the most prevalent greenhouse gas (GHG) in the atmosphere (390 ppm; without accounting for H_2O), this has moved research focus and potential mitigation techniques towards these non-CO_2 GHGs [5]. At the moment, one of the most complicated challenges in the world is climate change, which has implications for the scientific, economic, social, political, moral, and ethical realms [49]. It is primarily brought about by the impacts of four greenhouse gases—carbon dioxide, methane, nitrous oxide, and chlorofluorocarbons—having greater atmospheric concentrations [6]. The first three gases that are released as a result of microbial activity have a 1, 12, and 298 year atmospheric lifespan and a 100, 25, and 114 year global warming potential, respectively (Center for Climate and Energy Solutions, USA Web site). Natural ecosystems are seen to be carbon sinks, like the ocean and forest, and protecting them through silviculture and green technology is seen as another strategy to mitigate the problem. Through its efforts to mitigate climate change, United Nations Environment Protection (UNEP) plays a significant role in maintaining a low-carbon society on a global scale. To reduce greenhouse gas emissions, a variety of innovative technologies are used, including solar power, tidal power, hydrogen fuel cells, wind power, and geothermal power [7]. Processes like the flow of greenhouse gases are impacted by climate change, particularly changes in temperature and moisture content, in one of two ways: by altering the physiology of already existing microbial populations, or by altering the makeup of the microbial community. It is commonly acknowledged that microbes influence the concentration of GHGs such as CO_2, CH_4, and N_2O [8]. The microbial world is extremely significant in this context because it

plays a crucial role in the carbon and nitrogen cycles and is engaged in the emission and removal of gases that contribute to climate change, such as carbon dioxide and methane [9–11]. In 2005, the average global CO_2 concentration was roughly 380 ppm, which was nearly 80 ppm higher than the previous record high over the previous 650,000 years [12]. Numerous changes in the global environment brought about by microorganisms have also impacted them [13–15]. In reality, a number of microbes may be impacted by climate change, which might have an adverse effect on the environment, the economy, and society [16, 17]. While heterotrophic microorganisms break down organic substances to release greenhouse gases, photosynthetic microbes consume atmospheric carbon dioxide. The net carbon flow is primarily determined by the balance between the two processes, and it varies across different ecosystems based on climatic factors like temperature. As a result, microbial reactions play a critical role in the earth's carbon cycle since they not only lock up large amounts of carbon but also release it [18–20]. It is important to emphasise that most greenhouse gases, including CO_2, CH_4, and N_2O, are produced by bacteria [21]. Methane (CH_4) is a GHG that is released into the environment by some microbial communities, including those found in termite guts, rumens, marshes, and seas. As a carbon source, methane may be used by microorganisms like methanotrophs, which helps to lower the amount of GHGs in the environment. There is a knowledge deficit about the major reactions of soil bacterial and fungal populations to climate change, despite their active participation in terrestrial ecosystem function. Microbes that use reduced carbon substrates without a carbon–carbon bond are known as methylotrophs. Methanotrophic bacteria include both methylotrophs, which do not consume methane, and methanotrophs (which consume reduced carbon substrates other than methane). Apart from methane, this functional group may use substances like methanol, methylamine, dimethylamine, formate, and formaldehyde as its only sources of carbon and energy, and it frequently participates in the global carbon cycle [22, 23]. Only 5% of the world's atmospheric CH_4 sink is accounted for by methanotrophs' biological oxidation of CH_4 [20]. Prior to being released into the atmosphere, up to 90% of the CH_4 generated in the soil is additionally oxidised by methanotrophs [24]. Since there is less microbial variety and less evaluation of bacterial and fungal communities, there is a vacuum in our understanding of dryland environments in particular. By discussing and describing the impact of aridity change (a sort of climate change) to soil bacterial and fungal diversity, this gap is partly narrowed [25]. They examined the composition and abundance of distinct dryland ecosystems across all continents, with the exception of Antarctica, and came to the conclusion that as aridity increased, bacterial and fungal populations shrank. The composition and number of Chloroflexi and Proteobacteria increased as a result of this sort of climatic change, while Verrucomicrobia and Acidobacteria dropped. A potentially effective method of reducing the effects of global climate change is the management of the microbial ecosystem. The ecology and function of beneficial microbial communities must be understood in order to be managed. Due to the simplified CH_4 pathway and the involvement of specialist bacteria, the CH_4 biocycle is easier to understand than other GHG cycles.

Methane

Methane (CH_4) is one of the three primary greenhouse gases, along with carbon dioxide (CO_2) and nitrous oxide (N_2O), and it has a 25-fold greater potential to cause global warming than CO_2. The ozone layer's deterioration is also impacted by CH_4 [26, 27]. About two thirds of the worldwide CH_4 emissions, or total anthropogenic methane, are caused by men [25]. According to a study, agriculture is responsible for 47–56% of all anthropogenic CH_4 emissions, of which 12–37% may be of enteric origin [6, 28–30]. After stabilising for a while, methane concentrations have been rising again since 2007, which is now ascribed to changes in climate-induced methane releases from natural wetlands. Methane contributes 17% of radiative forcing [9]. The primary sources of human-related methane emissions include domestic ruminants, rice fields, carbon mines, landfills, and the use of fossil fuels [25]. On the other hand, methane is also released naturally from sources including termites, wetlands, and seas [31]. Ruminants are the main producers of CH_4 among animals. Their huge fore stomach, or rumen, features an ongoing fermentation mechanism. More than 70% of the stomach's capacity is taken up by the rumen, which has a volume of 15 L in sheep and 100–150 L in cattle [32]. The primary source of methane synthesis is microbial fermentation of hydrolyzed carbohydrates, which is seen as an energy loss for the animal [33–35]. Ruminant CH_4 generation is influenced by a variety of variables, including ruminant intake, feed quality and type, energy intake, animal size, growth rate, output level, genetics, and ambient temperature [36]. Ruminant methane emission lowers the effectiveness of nutrient uptake. Therefore, one of the most significant objectives for animal nutritionists is to manipulate the rumen microbial environment to reduce methane emission by ruminants and to increase their performance. Reducing ruminant methane emissions improves production, increases nutrient use efficiency, and lessens the impact of methane on global warming [8].

Carbon Cycling and Climate Change

The global carbon cycle of different ecosystems on earth provides the best explanation for the fluxes of carbon in the environment. As a component of life and one of the most plentiful substances on earth, carbon is also a key factor in determining the world's climate, its unpredictability, and the availability of energy for humanity. In the end, CO_2 is used by plants during the process of photosynthesis after being removed from the atmosphere by the bacterial and fungal breakdown of dead tissues and organic components. A crucial class of bacteria known as methylotrophs uses greenhouse gases like CO_2 and CH_4 to reduce the effects of global warming [37]. Along with the many other autotrophs, including plants and bacteria that can make photosynthetic material, methanogens are among the organisms that use CO_2 as a source of energy. Heterotrophs use organic substances for growth as well, converting them to CO_2. Through a variety of chemical processes, including methanogenesis,

methanotrophism, carbon dioxide fixation, anaerobic respiration, and fermentation, the equilibrium in carbon cycling is maintained. Methylotrophic bacteria oxidise methane, the second most prevalent and strong greenhouse gas, together with its derivatives (methanol, formaldehyde, methylamine, dimethylamine, trimethylamine, and formic acids) [24, 36, 37]. Methane is the second most important gas after CO_2 in terms of its contribution to global warming and the destruction of the ozone layer. Methanogenesis in animals and the decomposition of organic matter are significant contributors to global warming since it is a powerful greenhouse gas with a global warming potential 25 times greater than carbon dioxide [38]. Although it may not be a net contributor since it uses ambient carbon dioxide to form organic material, its ultimate impact is to turn that carbon dioxide into methane, a considerably more powerful greenhouse gas. Degradation and decomposition are processes that methylotrophic bacteria use to keep the environment's carbon cycle in check. Organic molecules undergo biodegradation, which releases CO_2 into the atmosphere [1]. Prokaryotes, such as Actinomycetes, Arthrobacters, Pseudomonads, and Fermicutes, in addition to methylotrophic bacteria, play a critical role in the biodegradation of hazardous carbon and carbon derivatives. These microbial communities react to environmental change sensitively by looking at the various microbial populations of soil, which are markers of climate change. Numerous anthropogenic activities and interferences, such as, deforestation, construction of industries, combustion of fossil fuels by vehicles, air and water pollution have an impact on climate change or unanticipated environmental variation [39]. Changes in the cycle of carbon and nitrogen across the globe have been impacted by these interferences. Climate change is caused by both the rise in greenhouse gases and the sum of all these atmospheric changes. Microbes have long had an impact on humanity, and we play a part in changing the energy balance and atmospheric composition. Methane, carbon dioxide, and nitrous oxide have been brought into the atmosphere as a result of human meddling and activity, and this induction predominates over greenhouse gas fluxes brought about by microorganisms [40]. Researchers also looked at the idea that bacterial and fungal communities expand more quickly in response to global warming. As they expand quickly, their respiration increases the amount of CO_2 in the atmosphere, which warms the climate [41, 42]. In this way, microbial organisms contribute to and have an impact on climate change. Additionally, complicated metabolic processes in the carbon and nitrogen cycles are impacted by inorganic nutrients [43]. In the past, several methylotrophic strains have been described as actively contributing to climate change and lowering greenhouse gas emissions [2, 29–31]. On an individual level, action is required to combat global climate change across all nations. By using other fuels and adopting low-carbon lifestyles, GHG emissions may be minimised. Mitigation studies show that the amount of GHGs in the atmosphere is decreasing which slows down climate change. This reduction in GHGs is made possible by using less energy. Numerous bacteria are also contributing to the lowering and decrease of these hazardous gases.

Methanogenesis

Methanogenesis, also known as biomethanation, is a multi-step process involving several microorganisms, including those that are hydrolytic, fermentative, acetogenic, and most importantly, methanogenic. The term "methanogens" refers to anaerobic bacteria from the domain Archaea that are involved in the biological synthesis of methane. The sole metabolic process carried out by methanogens is methanogenesis. Methanogens are only able to employ a few number of substrates that are derived from the anaerobic basement of the organic matter by hydrolytic and fermentative bacteria for this metabolism [18]. That suggests that methanogens accept a terminal place in the trophic chains of microbes. These methanogens vary from bacteria and eukarya because they lack the peptidoglycan that bacteria and eukarya have in their cell walls [39]. Based on the substrate used for methane generation, there are three main routes for producing the gas: hydrogenotropic, acetoclastic, and methylotropic. The most common route among them is hydrogenotropic and acetoclastic. The majority of rumen methanogenesis is produced by hydrogenotrophic methanogens, which turn CO_2 into CH_4 [16]. Methanogenesis, or the process of producing methane, depends on alkyl radical-containing substances such formate, acetate, methanol, methyl sulphides, and methylamines. Substrate-specific methyltransferases convert the alkyl radical in these substances into CH_4. Other bacteria and fungi found in the local microbial communities largely create these substrates by decomposing organic materials. Aerobic methanotrophic bacteria can utilise methane that escapes from anaerobic environments as a source of carbon and energy, or it can escape into the atmosphere, where it participates heavily in atmospheric chemical processes and is a significant greenhouse gas [44]. Methane generation is a significant and common kind of microbial metabolism. It is the last stage of the breakdown of biomass in anoxic settings. The majority of natural gas accumulations are due to thermogenesis, with methanogenesis accounting for a sizeable portion of them [10, 32, 33]. The methyl-oxidation route, similar to the first, is used to further oxidise an alkyl radical into CO_2, which causes the hydrogenotrophic pathway to operate in the opposite direction. This results in the abbreviation equivalents for this methanogenesis. Without oxygen and other electron acceptors like nitrate, sulphate, and iron, methanogenesis takes place. The release of ATP for numerous cellular functions results from the synthesis of methane. The methyl-coenzyme M reductase (Mcr) complex, which catalyses the last step of reducing methyl-coenzyme M to methane, is the essential enzyme in methanogenesis. As an alternative to the reducing equivalents produced by the methyl-oxidation route, this mechanism makes absolute use of the H_2 that is already available in the environment and is associated with an electron donor. It appears that the methanogens limited to this other pathway start to bond with the surroundings found in the gut. Acetate is a smart substrate for methanogenesis used by a few archaea that are connected to the Methanosarcinales [45]. Methanogens produce methane from $H_2 + CO_2$ (hydrogenotrophic), acetate (acetotrophic), or methanol and methylamines to provide energy (methylotrophic). These substrates are a byproduct of the decomposition of organic matter in anoxic

habitats (such as wetlands, sediments, permafrost, and landfills), which is facilitated by a network of bacteria hydrolyzing polymers into monomers that may then be fermented. Temperature, quantity, and type of organic matter are all regulated by physical variables (such as water table/flooding in wetlands) or other microorganisms or plants, which in turn govern concentrations of oxygen and alternative electron acceptors (e.g., NO_3, NO_2, Fe_3+, SO_4) [7, 44]. In general, nitrogen is thought to hinder the production of methane, either directly or indirectly, through hazardous denitrifying intermediates (NO_2, N_2O, and NO) or as an oxidant for denitrifiers (NO_3, NO_2 that can compete with methanogens for substrate [3, 4]. Methanogens also require nitrogen as a nutrient, which they can obtain either by fixing N_2 or by absorbing NH_4+ or NO_3. For the latter two, they must contend with plants and other bacteria (such denitrifiers), a relationship that has received little research.

Methylotrophs Mitigating Methane

Methane is the second most significant greenhouse gas after carbon dioxide in terms of its impact on short-term climate change. Future climatic harmony may be threatened by the ongoing release of methane from many sources, whether from immediate anthropogenic sources or perhaps quickly from the Arctic. As a result, there is a considerable worry about using different ways to reduce methane emission. Numerous anthropogenic and Arctic-related causes have given rise to the development of a wide range of mitigating methods, but they still need to be improved upon before being used more widely. However, there are still a lot of unknowns regarding the precise processes, scope, and techniques of the Arctic's fast methane emission. Being a significant GHG, methane has a variety of paths and mechanisms for release into the environment, including wetlands, lakes, and oceans. It may also be distributed equally across wide regions or concentrated in tiny patches [46]. However, one of the most important processes for methane emission into the atmosphere is bubbles that are produced from the sediments of Arctic sources. A few sources in the Arctic, where methane is concentrated in pockets, may be used with the methane release mitigation technologies, even though most of them are based on restricted gas streams of 0.1% methane or greater. In addition to other methods, a few mitigating techniques designed specifically for rice fields and agricultural soils have also demonstrated promise for Arctic wetlands and thawing permafrost. However, a number of additional Arctic-specific mitigation techniques have been proposed; they need more research. In order to address current methane sources and prospective Arctic sources, experts have so far identified four relevant research and development areas: (1) Methane emission detection and measurement; (2) Small and distant methane stream mitigation; (3) Dilute (1000 ppm) methane stream mitigation; and (4) Methanotroph and methanogen ecology understanding. Additionally, the use of methylotrophs and a thorough explanation of soil methanotrophy might be a useful tool to address methane emissions naturally released from closed landfills

and a significant drop in waste-related GHG emissions after methanotrophic reactions [22]. Methanotrophs have developed and gained the ability to use CH_4 as their only source of carbon and energy to grow aerobically. These bacteria are crucial in converting CH_4 into organic compounds and releasing CO_2 for use by autotrophs [40]. Additionally, the major component breakdown that results from a number of photochemical processes is the oxidation of methane in the atmosphere in the presence of hydroxyl (OH) radicals. The primary reactive species in the trophosphere is the hydroxyl radical, which is created photochemically in the atmosphere and interacts with many types of organic molecules [20]. A study on the biodegradation of methane and the buildup of polyhydroxybutyrate (PHB) utilising an isolated strain and a methanotrophic consortia has produced encouraging findings for the reduction of methane. It went on to explain that the isolate and the consortium had specific methane consumption rates of 100 and 17 mg CH_4 g h^{-1}, respectively. Additionally, the two-phase partitioning bioreactor (TPPB) was tested for its ability to remove methane from an air stream while containing 10% volume-to-volume silicon oil. The TPPB encouraged PHB production at rates of 34 and 38% w/w and advocated a 33–45% rise in methane removal. Under these circumstances, the consortium's particular methane degradation rate reduced to that of the isolated strain while remaining unchanged for the collaboration. According to the study, strain CZ2 of the bacterium *Methylobacterium organophilum* is able to use methane and accumulate up to 57% (w/w) of PHB when nitrogen is scarce. Additionally, it was shown that *Methylobacterium organophilum* CZ2 and *Methylosinus trichosporium* OB3b had similar specific CH_4 (methane) consumption rates and capacities for accumulating PHB. So, methylotrophs contribute to reducing GHS emission into the environment and have enormous potential for producing PHB industrially from waste gases [47]. Since it is known that methylotrophic bacteria may use C1 chemicals, such as methane, there is a persistent effort to identify and describe new species of methane-degrading bacteria. Therefore, by effectively using methane, such new methylotrophic bacteria may contribute to lessening the effects of global warming. Additionally, identifying and assessing specific plant growth-promoting (PGPR) strains for their capacity to decompose methane would undoubtedly open new doors for many uses of such cultures, including the promotion of plant growth, the tolerance of abiotic stress, and methane mitigation [30, 48]. The simplest spectrophotometric assay for methane screening using microbial strains was recently studied and compared to other methods available, including the traditional gas liquid chromatographic technique, assay of specific enzymes, and molecular analysis of the genes encoding methane monooxygenase and methanol dehydrogenase (mmo and mxaF) respectively. Jhala and associates were able to effectively restore bacterial cultures that degrade methane by enriching soil with water and using methane as the only carbon source [29]. Additionally, colorimetric plates assay identified the existence of soluble methane monooxygenase (sMMO) enzyme and measured their survival in evacuated tubes containing methane. By finding the genes encoding the enzymes (methane monooxygenase and ethanol dehydrogenase) and qualitatively estimating the enzyme activity in the isolates, it was possible to further confirm the ability of the isolates to degrade methane. Research on the slurry material taken

from the Herman Pit, a former mercury mine, showed the importance of methanotropic bacteria in the aerobic removal of CH_4 from sediments. Furthermore, the existence of acidophilic or acid-tolerant methanotrophs was shown by the methanogenic activity that was carried out under artificially acidic circumstances. Thus, maximal activity at pH 4.5 with incubated slurries was used to validate acid-tolerant methanotrophs. Such methanotrophs also had their sterol and hopanoid lipids extracted, which is a feature of methanotrophs, and their abundance was augmented by a rise in sediment methane consumption. Additionally, the genomic DNA isolated from methane-oxidizing enrichment cultures revealed an amplified sequence for the pmo A gene that matched methanotrophic Gammaproteobacteria. An enrichment culture was created under acidic conditions (pH 4.5) using methane oxidation [2]. Another important worry of the scientific community is the environment's rising CO_2 concentration, and much focus is currently being placed on determining how methylotrophs contribute to CO_2 mitigation. Since it is anticipated that waste-related biomass will be harvested sustainably and there would not be any net CO_2 emissions because it is believed that CO_2 produced by food waste decomposition can be absorbed by the following year's crop, most biomass or biomass-based waste degradation is typically not included in domestic or international greenhouse gas inventory totals. GHG inventories, however, also include methane emissions from waste caused by anaerobic decomposition [22]. Formaldehyde (HCOH) and CO_2 are typically two C1 oxidation products involved in methanotrophic activities. Additionally, there are two mechanisms for assimilating carbon during methanotrophic metabolism: the serine pathway and the RuMP system. During methanotrophic metabolism, the serine route uses two moles of HCOH and one mole of CO_2 to create a three-carbon intermediate. In the RuMP route, three moles of HCOH are used up, resulting in the generation of three major metabolic carbon intermediates. The RuMP route is therefore more effective than the serine pathway. Additionally, the RuMP route is superior than the serine pathway for both ATP consumption and molar yield values (g of cell dry weight/mol of substrate consumed), where bacteria utilise C1 compounds [23]. Because all methanogens are capable of removing CO_2 from the air, they do so by converting it to cell material and CH_4. Methanotrophs have little effect on the carbon cycle, but they do have an impact on the amount of plentiful greenhouse gases in the atmosphere due to their metabolism.

Methylotrophs Mitigating Methane in Paddy Fields

One effect of the methane imbalance throughout the atmosphere is the global shift in the physiochemical characteristics of the climate. The finest illustration of significant methane sources is a rice field [12, 49, 50]. Since methane is produced in large quantities in rice fields, methanotrophic bacteria play a significant role in reducing methane through biodegradation. In the paddy field, there is a cycle of microbial activity wherein flooding circumstances encourage the methanogens, which produce methane gas. The methanotrophic bacteria there then trap the methane gas, converting

it to methanol and biomass in the process. Methane monooxygenase (mmo) enzyme is a necessary component for methanotroph activity, and oxygen is needed to make it reactive. This methane oxidation enzyme system is stimulated by aerobic methanotrophs. The green algae that cover the surface of the flooded rice field typically cause this aerobic situation [51]. Methylotrophic isolates with functioning enzyme systems were collected from Gujarati wetland paddy fields, and upon biochemical and molecular analysis, they were identified as several species of *Bacillus* and *Penibacillus*. The existence of the particulate methane monooxygenase (pmoA) genes that encode the subunits in gene cluster is demonstrated by the working enzyme system. While the mmoX gene encodes (part of the hydroxylase component) in *Methylobacterium extrorquens*, the presence of the pmoA gene implies methane use by bacteria like *P. illinoisensis*, *B. aerius*, *B. subtilis*, and *Rhizobium* sp. In a research, communities that are effective at using methane, such as *P. illinoisensis* and *Rhizobium* sp., were shown to have the mxaF gene, which codes for the subunit of the methanol dehydrogenase enzyme. A recognised bacterial group that promotes plant development was found to have methane breakdown enzymes and genes in the methane reducing communities isolated from wetland rice fields [30]. These particular methylotrophic communities are systematically arranged over the soil surface in paddy fields, with the capacity and power to digest the greenhouse gas methane, resulting in aerobic soil surface conditions. This well-organized film is related to the algal populations that are mostly seen in rice fields. By driving the activity of methane oxidation, the algal communities play a significant part in reducing greenhouse gas emissions in the environment. A thin coating of algae reduced methane emission in a microcosm experiment without rice plants. In addition, the presence of algae on the surface of submerged rice fields encouraged methanotrophs and constrained the number of methanogens. According to a study, in the presence of rice, CH_4 emission occurs mostly through aerechyma [52, 53]. Studies confirm the involvement of methylotrophs in the reduction of greenhouse gas emissions in the environment.

Enzymes Involved in Methane Production

The complexity and uniqueness of methanogenesis as a type of anaerobic respiration lies in the need for six exceptional coenzymes, including methanofuran, ferredoxin, methanopterin, coenzyme M, coenzyme B and coenzyme F420: a pathway and several specific membrane-bound enzyme complexes coupled to the creation of a proton gradient driving ATP synthesis [15]. CO_2, acetate, and substances containing methyl groups, such as methanol, methylated amines, and methylated sulphides, are the three main substrates for the production of methane. Due to this, there are three separate routes for the formation of CH_4: hydrogenotrophic, acetoclastic, and methylotrophic [11, 14]. Although the three routes have different intermediates and enzyme processes, they nonetheless have common characteristics in the ultimate stages of CH_4 synthesis. The yield of a carrier-bound methyl intermediate is influenced by both the hydrogenotrophic and acetoclastic processes. Methanopterin, a product of

the hydrogenotrophic route, and sarcinapterin, a product of the acetoclastic pathway, are the carrier proteins. All three processes include the addition of the methyl group to coenzyme M via a particular, membrane-bound methyltransferase and the consequent decrease of methyl coenzyme M to CH_4 via the crucial enzyme methyl coenzyme M reductase [54]. The three methanogenic processes are further explained in the supporting information in small print. Methyl coenzyme M reductase is made up of a dimer of the three subunits (McrA), (McrB), and (McrG), and it has a special active site termed coenzyme F430 that includes porphinoid nickel [19]. About 300 kDa is the apparent molecular mass of the enzyme. Methyl coenzyme M reductase has two specific isoenzymes that have been found [66]. The second enzyme has a different substrate affinity and is known as methyltransferase for methyl reductase two [5]. The mcrBDCGA operon codes for methyl coenzyme M reductase activity, whereas the MRT is encoded by the mrtBDGA operon [55, 56]. The mrt operon lacks the identical counterpart of gene mcrC [55]. The byproducts of the genes mcrC (McrC), mcrD (McrD), and mrtD (MrtD) are under 20 kDa are the. Their purpose is yet unknown and it is still unclear how primary sensors and signal transduction cascades work [57]. However, evidence for regulation was found in the availability of trace elements [58]. This is because many methanogenesis-related enzymes have trace metals (such as molybdenum, tungsten, selenium, and nickel) in their active sites. It was discovered that the abundance of the substrate H_2 regulates the synthesis of various important methanogenesis-related enzymes together with MRC. The two isoenzymes of methyl coenzyme M reductase are differently expressed in Methanothermobacter species with the help of H_2 availability, with isoenzyme I (methyl coenzyme M reductase) being predominately expressed in H_2 limiting environments [47–56]. Control of gene expression of the methanogens is still poorly understood, necessitating more research.

Current Status and Future Perspective

The use of DNA extraction, PCR, sequencing, and probe biases, and a lack of bioinformatics support for next-generation sequencing and metaproteomics, continue to limit innovative technologies. The development of bioinformatics tools, however, has led to a noteworthy advancement in this sector in recent years. The current dispute will create quantitative information for bacteria involved in the CH_4 cycle and to parameterize this data for substantial use in climate and ecological models. Because their metabolic capacities are not well known, many methanogens and methanotrophs are not cultivable. This is a crucial need for the accurate integration of microbiological data in the prediction forms. Stable isotope probing and methods like DNA and RNA analysis can help determine the physiological capacities of different animals. Due to information gaps about DNA and RNA, stable isotope probing methods with a relatively high substrate concentration are required to label DNA sufficiently [57, 58]. PLFA-SIP, which combines stable isotope probing with PLFA, may detect active bacteria at ecologically relevant concentrations. This method, however, is

unable to precisely identify microorganisms at the species level due to a lack of phylogenetic precision. Environmentally substantial amounts of substrate may be used for metagenomic and metaproteomic investigations thanks to technological advancements in SIP and associated apparatus [59–65]. Additionally, it is necessary to classify the habitats used by populations of methanogens and methanotrophs. Therefore, a demonstration of niche adaptation in methanogens and methanotrophs was provided before [38, 66–72]. However, in the next three millimetres of water-saturated soils, Reim and colleagues discovered vertical niche divergence in gamma proteobacterial methanotrophs [73]. Given the local commerce that may be identified on a small scale, this is very significant and indicates the necessity for specific niche identification.

Conclusion

Methanogenesis is the anaerobic production of methane by methanogenic Archaea. Methanogenesis can come from a variety of anthropogenic and natural sources (human sources). Methylotrophic bacteria use and break down reduced carbon molecules like methane, contributing significantly and significantly to climate change. This particular bacterial group is unusual in that it helps to maintain the climate by lowering greenhouse gas emissions. The rice field is the most prevalent environment for methanotrophs, where enzymatic activities are aided by other species including methanogens and algae. Although methane (CH_4) emissions are projected to vary due to climate change, the dynamics of methanogens and methanotrophs under this transition have not yet been thoroughly studied. Agriculture, particularly the rearing of cattle, is the largest anthropogenic source of methanogenesis. Methanogenesis from the production of animals and organic matter decomposition contributes significantly to global warming. The inclusion of microbial knowledge into the development of prediction models will be greatly aided if we can identify the niche separation for certain microbial groups with specified physiological capabilities and their control. Furthermore, such information may be used to investigate extensive data on the generation of methane and the use of particular unidentified genes as a molecular pathway.

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