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Research Progress on the Isolation, Purification and Adaptation Mechanism of Methanogens from Extreme Environments

Zhang Mengqi

School of Life Sciences, Yantai University, 264006, China.

Corresponding to: Zhang Mengqi

Abstract: Anaerobic fermentation is one of the ways to treat organic waste, among which methanogens are one of the main strains for anaerobic fermentation, which exist in most terrestrial and aquatic environments. The study of the isolation and purification of methanogens in extreme environments and the elucidation of their adaptation mechanisms are conducive to anaerobic fermentation in a variety of environments, such as reducing energy consumption and increasing the fermentation rate in the anaerobic fermentation process in cold regions. Ordinary methanogens do not have enzymes adapted to the thermal environment or the lack of expression of genes related to heat adaptation may lead to the loss of activity during high temperature and anaerobic fermentation in summer in low latitudes, and thermophilic or heat-tolerant methanogens are one of the good choices for anaerobic fermentation in this area. At the same time, the ocean occupies most of the earth, and salt-tolerant or halophilic methanogens are a wise choice for fermentation of anaerobic fermentation substrates derived from seawater, as the substrate does not need to be desalted, which helps to reduce the number of steps and improve efficiency. Therefore, this paper mainly reviews the isolation, purification and adaptation mechanism of methanogens from extreme environments, and puts forward the prospect of future research content.

Keywords: methanogens, extreme environment, separation and purification, adaptation mechanism

1 INTRODUCTION

The International Energy Agency (IEA) predicts that by 2050, global demand for bioenergy will include solid bioenergy/biomass (60%), liquid biofuels (30%) and biogas (10%)[1]. Under pressure to mitigate climate change, there is an urgent need to develop methods and practices to enhance methane capture, pursuing biogas upgrades to improve biogas quality and expand its use[2]. Methane is a major component in natural gas and biogas, and as global crude oil resource consumption increases, methane is gaining traction as an alternative and promising resource for chemical commodity production[3, 4].

Methanogens are one of the main producers of biomethane, a near-pure source of methane found in most terrestrial and aquatic environments, and one of the essential biological components in environments with extreme temperatures and salinity[5]. For methanogens, most organic substances, such as carbohydrates, are not direct substrates for methane production, they must be processed by intermediate microorganisms to produce hydrogen and carbon dioxide, acetate, or methylated compounds, substrates actually used by these methanogens[6]. To date, four pathways for anaerobic methanogenesis have been identified: hydrotrophic, acetic acid, methyltrophic and methylreduction[5].Methanogens are strictly anaerobic bacteria, their isolation and purification and culture are difficult, for methanogens growing in various extreme environments, its isolation and purification process will be more difficult, the existing research is more focused on methanogens in the conventional environment or only a single study or summary of one or a class of methanogens, for a variety of extreme environments of methanogens There are few general articles, so this paper mainly comprehensively summarizes the progress of the isolation and purification of thermophilic, cold-philic, halophilic methanogens and the research status of their adaptation mechanisms. In order to provide a certain reference for more people to understand methanogens in extreme environments.

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2 Methods For Separating And Purifying Methanogens

Isolation and purification of strictly anaerobic bacteria using solid media is the most challenging part of anaerobic microbiology, mainly due to the slow growth rate of methanogens and the need to maintain an anaerobic environment during operation[7]. At present, the isolation and purification of methanogens generally adopts the Hengate roller tube method, anaerobic glove box, anaerobic tank method, etc., at present, the most commonly used culture vessels are: Hungate or Balch tubes and serum bottles, sealed by butyl rubber plug and screw cap or aluminum cap to ensure an oxygen-free environment, for strict anaerobic bacteria sometimes use anaerobic glove box to operate to ensure strict anaerobic throughout the operation process[7]. In 1969, the Hungate Roller Pipe Act[8]The invention of the method has promoted the process of anaerobic bacteria isolation and purification, and improved the success rate of anaerobic bacteria isolation and purification, which is still one of the most important and widely used methods for isolating and purifying anaerobic bacteria, and its derivatives are also being widely used. In 1978,D. J. COX et al. invented a simple anaerobic glove box that can remove trace amounts of oxygen by combining hydrogen and palladium catalysts[9]. Since then, researchers have used various methods to isolate and purify methane-producing strains. Kohei Nakamura et al. developed a simple six-well plate method to culture and isolate obligate anaerobic microorganisms, and after experimenting with 21 representative specialized anaerobic bacteria, all detected anaerobic bacteria could form visible colonies on six-well plates, and these colonies could be successfully subcultured in liquid medium. This method is comparable to the roller pipe method, or even better than the roller pipe method, and using this method, a sulfate-reducing bacteria was successfully isolated from environmental samples[10].Peter H. Janssen successfully separated the electron microscope grid in a tube using the method of Friedrich Widdel Methanosaeta spp.[11]. Marisa Hohnadel et al. manually removed individual cells from the sample using a capillary tube and saline using a microsyringe and injected into the medium using an improved single-cell micromanipulation technique, and tested to have a high growth recovery rate for multiple strains[12].

3 Species Of Methanogens In Extreme Environments And Their Adaptation Mechanisms

3.1 *Thermophilic Methanogens And Their Adaptation Mechanisms*

Thermophilic methanogenic bacteria Topt 35℃ and 55℃, respectively, and the temperature range of its growth is 25~80℃, and extreme thermophilic bacteria Topt is above 80℃ and can survive in high temperature conditions, and low temperature will have an inhibitory effect on its growth, and even make it unable to survive in a low temperature environment[13].In 1972, Zeikus et al[14] isolated the first thermoautotrophic methanogens Methanobacterium thermoautotrophicus sp. n. using carbon dioxide as the sole carbon source. The only carbon source can be continuously cultivated on a large scale in completely inorganic medium. The lower and upper limits of the growth temperature of the bacterium were 40℃ and 75℃, respectively, the optimal growth temperature range was 65~70℃, and the optimal growth pH range was 7.2~7.6, and it could not grow when the pH value was lower than 6.0 or higher than 8.8. Subsequently, researchers successively collected sediments from the seabed, hot springs, craters, anaerobic digester, Mine sewage, several thermophilic methanogens were isolated in anaerobic environments such as high-temperature oil reservoirs[15], among them, hot springs are one of the most isolated and purified sources of thermophilic methanogens. For example, Zhenbo Lv, etc[16] isolated and purified a thermophilic hydrogenic methanogen DL9LZB001 from Tengchong Hot Springs, China, a class I methanobacteria belonging to the class Methanomycetes, its optimal growth temperature is 65℃, was named Methanothermobacter tengchongensis, length 2.80~6.43 μm, elongated rod-shaped cells with a diameter of 0.436 ± 0.052 μm, and the cells mostly appear in pairs, using CO_2 and H_2 to produce methane. Mackenzie M Lynes et al[17]. Candidatus is enriched from one of the hot springs in Yellowstone National Park Methanoglobus hypatiae, a methanogen belonging to the archaeaceae family, grows at temperatures of 64~70℃ and pH 7.8, and uses monomethylamine, dimethylamine and trimethylamine to produce methane. Calendula M, etc.[18]use M.voltae with M. maripaludis Archaeae detergent OP-10 separates "whole" archaea (i.e. with a knob-like anchor structure at one end), and then by briefly shearing the cells, archaea in the form of clusters or clumps are isolated from the thermophiles.Takao Iino et al[19]A thermophilic and hydrotrophic methanogen was isolated from the puddle soil in Kisarazu, Chiba Prefecture, Japan, as Ki8-1T, is a gram-negative bacterium, curved or wavy rod-like,11-25 μm long, The optimal growth temperature and pH were 30℃ and 7.5, respectively. This strain grows best in basal medium without the addition of NaCl.

Because both methanogen growth and methanogenic processes are highly dependent on temperature. High temperature will denature and inactivate enzymes, while thermophilic methanogens can grow and produce methane under high temperature conditions. How thermophilic methanogens respond to temperature changes in ecosystems above 60℃ and how this response differs from the response of well-studied temperate ecosystems, It is key to understanding the co-evolution of methane cycle ecosystems and the Earth's climate[20].Zhenbo Lv, etc[16]Thermomethanogens (50~70℃) and mesophilic methanogens (28~40℃) were compared, found that mesophilic methanogens lost genes encoding enzymes that may be involved in thermal adaptation, such as intact membrane proteins that help methanogens adapt to thermal environments under thermal conditions to help stabilize membranes, and enzymes that repair DNA, prevent DNA damage, or promote DNA transcription. At the same time, thermophiles make

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various modifications in protein structure and sequence and possess heat shock proteins to prevent denaturation under high temperature conditions[21]. Studies have shown that GC content affects hyperthermophile. The main factors of bacterial tRNA stability[22], rather than the traditional inclusion Archaea of MCR encoding reverse rotase (topG).is a key determinant of a hyperthermophilic lifestyle above 85°C[23].

3.2 Methanogens Psychrophilus And Their Adaptation Mechanisms

Psychrophilic microorganisms refer to microorganisms that can grow in cold (0~10°C) conditions and have an optimal growth temperature in the low temperature range (below 25°C)., according to the optimal growth temperature range. It can be divided into two groups: obligate psychrophilic microorganisms (Stenopsychrophiles) and facultative psychrophilic microorganisms (Eurypsychrophiles) [24]. Obligate psychrophilic microorganisms. It refers to a microorganism with a limited growth temperature range. The optimal growth temperature is low, cannot tolerate higher temperatures; And both Cold-loving microorganisms refers to a microorganism that "likes" a permanently cold environment, its optimal growth temperature is high and can be tolerated in the temperature range compare specific cryophilic microorganisms wide, can still grow under medium temperature conditions[13].Because the isolation and purification of methanogenic methanogens were difficult until 1992, P. D. Franzmann et al. isolated a methylated methanogen from Lake Ace in Antarctica Methanococcoides burtonii, It is irregular spherical, with a diameter of about 0.8~1.8 μm. The optimal temperature for growth is 23~24°C, and methanol can be used as a substrate for growth. Since then, researchers have isolated psychrophilic methanogens in various cold environments, and most of the psychrophilic methanogens are generally isolated from frozen soil or areas affected by frozen soil, such as, Simankova M V etc[25]Five strains of methanogenic archaea (MT, MS, MM, MSP, ZB) were isolated from permanently and periodically cold land. Strain MT is: Methanosarcina mazeiand the strains MM and MS are cold-tolerant species Methanosarcina lacustris. Hydrotrophic strains MSP are: Methanocorpusculum. A new ecotype of the genus. Obligate methyltrophic strain ZB is Methanomethylovorans hollandicanew ecological type, The minimum growth temperature of all isolates was 1~5°C, but the best growth was achieved The temperature is all moderate(25~35°C). Dirk Wagner et al[26]Strain SMA-21T was isolated from permafrost-affected soil at the northern tip of Samoilov Island (72°22′N, 126°28′E) in the Lena Delta of Siberia by serial dilution in liquid medium, is a gram-negative bacterium, which is an irregular coccus with a diameter of 1.3~2.5 μm. The optimal culture condition is a temperature of 28°C,pH 7.8 and salinity 0.02 M NaCl, this strain grows on H_2/CO_2, methanol, and acetate. Based on the above content, it can be seen that most of the melanogenic methanogens that have been isolated and purified are methyl methanogens, and there are also H_2/CO_2 methanogens, and there are fewer acid-type methanogens.

Proteins from cold-adapted archaea have a high content of uncharged amino acids, particularly glutamine(Gln) and threonine (Thr), as well as lower hydrophobic amino acid content, especially leucine (Leu). The main factor that may affect the composition of amino acids is the change in genomic GC content(31%-61%), and at the same time in M. A cold shock domain (CSD) protein (CspA homologue) was identified in frigidum, which was identified in M. Two hypothetical proteins with CSD folding were identified in burtonii, in M. A unique wing spiral was identified in burtonii DNA-binding domain proteins, indicates that these types of nucleic acid-binding proteins are in Cryophilic methanogensmay play a key role[22]. The fluidity of the cell membrane is the basis of cell structure and function, and low temperature will lead to blocked cell membrane mobility and even lead to cell death. Second, studies have shown that psychrophiles may have evolved the ability to adapt to cold through genomic plasticity, including nucleotide skew, horizontal gene transfer, and transposase activity[27]. At the same time, compatible solutes in the cytoplasm have important biomolecular protection in vitro, which can stabilize protein and nucleic acid structures at low temperatures[28, 29].

3.3 Halophilic Methanogens And Their Adaptation Mechanisms

In addition to the class of salt bacteria, methanogenic archaea living at NaCl concentrations of >0.2 M have been identified as halophiles[30].In high-salinity habitats, methyltrophic methanogenesis is often considered the main pathway, generally is Methanosarcinals. At neutral pH, Methanohalophilus is moderately salt-tolerant, Methanohalobium is extremely salt loving. The genus can grow at concentrations up to 5 M NaCl, and a single moderately halophilic genus has been found. Methanosalsum can grow in highly saline water. 1985 Miguel Pérez-Filloletc[31] isolated the first obligate halophilic methanogenic bacteria from solar salt pool sediments, with a minimum and maximum salt concentration of 17% and 30% at grow thand. The best salt concentration is 30%, and H_2/CO_2 (80/20) is used as the energy and carbon source. Methanonatronarchaeum thermophilum sp. nov. It was isolated from the sediments of a highly salty soda lake in the Altai Kurunda steppe of Russia,Irregular cocci with a size of 0.4~0.5 μm, in the salinity sum of 2 M Na or greater than 0.2% SDS is lysed, Strictly anaerobic methanogens, using MeOH, methylamine and dimethyl sulfide as electron acceptors and formate or H_2 as electron donors.Obligate basophil, growth pH range of 8.2~10.2 (optimal pH value is 9.5~9.7), extremely halophilic, growing between 3~4.8 M total sodium (optimal 4M), Moderately thermophilic with a growth temperature range between 30~34 and 55~60°C (optimally 50°C)[32].Candidatus Methanohalarchaeum thermophilum was enriched in the sediments of the high salt lakes of the Kurunda steppeirregularity, Amotor coccus, 0.4~0.5 μm, Lysed in the presence of salinity below 2 M NaCl and greater than 0.2% SDS strictly anaerobic methanogens, using MeOH and trimethylamine as electron acceptors and H_2 or formate as electron donors. It is extremely salt-philic and grows best at 4~5 M NaCl. The optimal pH for growth is 7~7.5. Moderate thermophilic and best grown at 50°C, death occurs above 60°C[32].

In a high-salt environment, halophilic methanogens are divided into two osmoregulation strategies, one is the "salt in" strategy

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commonly used by halophilic methanogenic archaea, that is, increasing the concentration of cytoplasmic salts, usually potassium chloride, The second is the "low salt intake" strategy or "salt out" strategy, That is, compatible solutes are produced to maintain osmotic balance in the cytoplasm without increasing cytoplasmic salt concentrations[33, 34]. Methyl-reduced methanogenesis is thought to be the main mode of methanogenesis in oxygen-deficient oceans, freshwater, and high-salt sediments[35]. There are two reasons: first, sulfate reducing agents do not compete for methylated compounds like hydrogen or acetate; Second, methanogens can utilize methyl-containing compounds due to the breakdown of compatible solutes produced by prokaryotes and algae. Methanohalophilus and Methanohalobium are generated Organically compatible solutes, but also accumulating intracellular potassium in high concentrations in their cytoplasm, hence a hybrid osmosis strategy, similar to that documented in extremely salt-tolerant bacteria and some salt archaea[32]. Methanogens typically synthesize nitrogen ε-acetyl-β-lysine and β-glutamic acid as compatible solutes, which is a particularly effective osmoregulatory strategy because β amino acids are not incorporated into proteins or other essential macromolecules and are easily synthesized with α amino acids as precursors, and these molecules can be produced rapidly under salt stress and accumulate in high concentrations within cells[36, 37].

4 APPLICATION

4.1 ANAEROBIC DIGESTION OF FOOD WASTE TO PRODUCE METHANE

Anaerobic digestion mainly includes monodigestion and anaerobic co-digestion, although the monodigestion technology has matured, but the disadvantages of the monodigestion technology are slow and unstable due to its poor biodegradability and high toxicity[38, 39]. People are more inclined to use anaerobic co-digestion, which can make up for the limitations of the degradation of a single raw material, improve the stability of fermentation characteristics and reaction system, and effectively avoid pollution caused by the accumulation of agricultural and animal husbandry waste[40]. Anaerobic co-digestion of food waste and other substances is gaining traction as an effective way to achieve more balanced nutrient levels, mitigate ammonia inhibition, dilute toxic pollutants, and increase biogas production[41]. As one of the main components of organic solid waste, food waste plays a key role in the recycling of biomass energy[42], it is often considered an ideal anaerobic digestion substrate due to its high organic matter content[43]. The quality of the inoculum is the key factor affecting the stable operation of the anaerobic digestion reactor[44]. Inoculants refer to cultures containing functional microorganisms used for fermentation, which can be divided into mixed inoculum and pure inoculum. It is feasible to use specific pure microorganisms as inoculants for simple substrates, and kitchen waste collected from kitchens usually contains some local microorganisms, because it is inevitable to be contaminated by microorganisms during collection, transportation, storage and treatment, and the inactivation of natural microorganisms and the maintenance of sterile conditions are expensive on an industrial scale, so pure inoculation is difficult for complex kitchen waste treatment. Therefore, most food waste treatment plants usually use mixed inoculants, including sludge, animal manure, some soil, etc[45].Xuedong Zhang and so on[46] proposed a new strategy was developed to effectively produce hydrolases and improve the anaerobic digestion of waste activated sludge for methanogenesis by fermenting food waste, which was effectively enriched by pre-fermentation of food waste and directly used to enhance sludge hydrolysis, adding 15 g of fermented food waste per 50 g of sludge, the improvement of hydrolase activity increased the sludge hydrolysis rate by more than 200%, and by adding 7187 g of fermented food waste to 2140 g of sludge, the amount of methane produced by sludge digestion was significantly improved and increased from 30 mL to 200 mL, which about 24.3% was contributed by the addition of enriched hydrolases. Na Wang et al[47] evaluated garden waste by multicomponent synergistic anaerobic fermentation (GW) or food waste (KW) as a co-substrate for hydrogen production by thermophilic dry anaerobic co-fermentation (FW). The results show that when the food waste (FW) with garden waste (GW) ratio was 60:40, the maximum cumulative hydrogen yield and organic matter removal rate reached 85.28 NmL g^{-1} VS and 63.29%. When the food waste (FW) and kitchen waste (KW) ratio was 80:20, the maximum cumulative hydrogen yield and organic matter removal rate reached 81.31 NmL g^{-1} VS and 61.91%. Anaerobic fermentation treatment of kitchen waste can not only reuse waste resources, but also generate clean energy similar to methane, alleviate the energy crisis and world environmental protection problems, give full play to the maximum utilization value of resources, and realize the harmonious coexistence of people, resources and the environment.

4.2 ANAEROBIC DIGESTION OF ALGAE TO PRODUCE METHANE

Algae growth generally does not involve the use of fertilizers, pesticides or other chemicals, only natural nutrients from seawater and solar energy and carbon dioxide, and algae contain easily hydrolyzable sugars and proteins, low-fraction lignin and high-fraction hemicellulose, and good hydrolysis yields, making this biomass suitable for anaerobic fermentation[48]. The algae that have been used for anaerobic fermentation are: Chlorella[49], spirulina[50], Microcyst[51], Shi Ying[52]etc. Some researchers used whole cells of S. obliquus and extracted algae residue for anaerobic fermentation treatment in a batch reactor filled with calcium alginate encapsulated digestion sludge, and the results showed that the encapsulated digestion sludge formed by 4% sodium alginate could maximize the yield of biomethane, and the biomethane produced increased by 58%[53]. Ana Eusébio et al. utilized both autotrophic and heterotrophic microalgae materials as anaerobic digestion substrates while producing biogas/methane and pigments, in which fully autotrophic microalgae are better suited for anaerobic fermentation than heterotrophic microalgae because it is more efficiently converted to methane (279 vs 180 L CH_4/kg VS)[54]. In addition to producing biomethane, the nutrients in

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the anaerobically digested sewage can be further recycled back for algae cultivation, which is conducive to sustainable energy and economic development[55].

4.3 OTHERS

In addition to anaerobic digestion of kitchen waste and algae, methanogens can also be used to degrade petroleum, coalbed methane, etc. in situ. Ding Mingshan et al[56]aiming at the problem of a post-chemical flooding limit water-bearing reservoir in Shengli Oilfield, a biogasification technology based on co-production of oil and gas oil extraction was established to achieve the effective use of residual oil by in-situ degradation of the top remaining oil with high water content to produce methane gas. Microbial enhancement of coalbed methane has become an important research topic in recent years. Bioconversion of coal to methane is a feasible and environmentally friendly way to increase coalbed methane production. In yield stimulation strategies, the addition of microbial consortium or biofortification can be considered as a promising alternative[45]. Tian Jie Ao et al [57] developed a single-chamber anaerobic digestion integrated microbial electrolytic cell system (AD-MEC) to increase methane yield by 55% and in situ methane content to 82% by converting CES to biogas using voltages from 0 to 2.5 V, while mitigating negative effects on the original microorganisms. Cameron Hepburn et al. consider methane as one of the potential energy carriers for CO_2 transportation and CO_2 capture for methane production[58], which not only solves the problem of excessive carbon dioxide emissions, but also provides a solution to the problem of energy shortage. Mads Ujarak Sieborg et al[59] proposed a low-temperature alternative based on biointegrated carbon capture and utilization, which can capture and utilize biocatalysts at low temperatures, replace the traditional heat-based CO_2 stripping device with a lower-cost biomethanation step, and use it for diamine regeneration and coupled conversion into renewable synthetic natural gas, which has a strong adsorption capacity for impurities in flue gas. Methanogens can also be used as adsorbents or reducing agents to adsorb or reduce metal ions from the environment, and methanogenic sludge particles can reduce platinum group metals, such as platinum (Pt)[60]and Palladium (Pd) [61], pure cultures of methanogens can chelate copper (Cu^{2+}) [62], thermophilic methanogens Methanothermo-bacter thermautotrophicus can restore chromium (Cr^{3+})[63]. Both metrapophiles and thermophilic methanogens are able to convert vanadium (V^{5+}) restored [64], halophilic or salt-tolerant methanogens can recover lithium from brine[30].

5 CONCLUSION

Due to the low temperature in high latitudes and high altitudes, the energy required for the anaerobic fermentation process will be higher than the energy in the process of room temperature, resulting in energy loss, and low temperature will lead to the deterioration of methanogenic membrane fluidity and the decrease of enzyme activity, affecting the activity of methanogenic bacteria, greatly reducing the anaerobic fermentation rate, isolating and purifying cold-loving or cold-tolerant methanogens and applying them to actual production will help reduce the energy consumption in the process of anaerobic fermentation in cold areas and improve the fermentation rate. Ordinary methanogens do not have enzymes adapted to the thermal environment or the lack of expression of genes related to heat adaptation may lead to the loss of activity during high temperature and anaerobic fermentation in summer in low latitudes, and thermophilic or heat-tolerant methanogens are one of the good choices for anaerobic fermentation in this area. At the same time, the ocean occupies most of the earth, and salt-tolerant or halophilic methanogens are a wise choice for fermentation of anaerobic fermentation substrates derived from seawater, as the substrate does not need to be desalted, which helps to reduce the number of steps and improve efficiency. This review only summarizes the progress and adaptation mechanism of methanogen isolation and purification in some extreme environments, and there may also be methanogens in extreme environments that are compatible with the environment. Their findings may provide a deeper understanding of the evolutionary genetic choices made by methanogens in response to various environments.

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