

## Review Article

# Methanogenic archaea: A Multipotent Biological Candidate Focusing Toward Realizing Future Global Energy

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## ABSTRACT

Methanogenic archaea are obligate anaerobes belong to the domain archaea. Those are a multi-potent candidate for their different physiological and metabolic activities. It offers a great innovative thrust among researchers in place of its challenging laboratory analysis as well as its effective microbiological practical applications. In economic and environmental point of view, the Methanogenic archaea covers a major area like biofuel production and neutralization of global carbon cycle. Biological sequestration process in the form of methanogenesis and formation of methane from carbon dioxide is one of the processes which produce a valuable fuel (the methane) that can be used for the generation of electricity and for other applications. This process is eco-friendly and sustainable one. Thus, this is a novel approach to enlighten our knowledge about the benefits of Methanogens. Furthermore, it involves anaerobic digestion, climate controller, model organisms for extreme environments and nitrogen fixation. Owing to these distinct features, the study of Methanogenic archaea creates a touch of excitement in our environmental research world. This review covers all the applied aspects of methanogens.

### Keywords

Methanogenic archaea,  
Biofuel,  
Methanogenesis,  
Carbon cycle,  
Methanogens

## Introduction

Methanogens are unicellular, slow growing, strictly anaerobic (Liu and Whitman, 2008) and obligate methane-producing unicarbonotrophs (Woese et al., 1990). These are the largest, most diverse, phylogenetically distinct, and the best-studied group within the domain Archaea (Balch et al., 1979; Ferry and Kstead, 2007). Those are widely distributed in various anoxic environments. The methanogens itself has captured the curiosity

of many environmental issues for decades. Methanogenic archaeal biodiversity represents a considerable fraction of the Archaeal world in different ecosystems, indicating that organisms from this domain might have a large impact on global energy cycles. They have increasingly attracted research interests because of their special metabolic characteristics and challenging laboratory analysis.

The nature of methanogen is of methane makers, which is one of the peculiar metabolisms for this group. Their unique properties make them a potentially valuable resource in the development of novel biotechnological processes, industrial as well as environmental applications such as new alternate green energy production (biofuel), carbon recycling, industrial processes, cellular products from novel methanogenic communities, and identification of new compounds with potential biotechnological and other applications. In the present review, we show and discuss some exclusive beneficial characteristics of Methanogenic archaea and the current knowledge about the biotechnological and environmental potential of the methanogens (Alqueres et al., 2007). The application of Methanogenic archaea for our environment is more likely in the near future.

### **Methanogenic archaea**

Methanogens are one of the potent biological candidates for resolving many environmental issues by means of its effective biosequestration system. Methanogens are different scientifically notable bacteria in archaeal kingdom. Today, Methanogens have received much attention because they catalyze the terminal step in the anaerobic breakdown of organic matter under sulfate-limiting conditions and are essential for both the recycling of carbon compounds and the maintenance of the global carbon flux on the earth.

### **Commercial applications of Methanogenic archaea**

***Biofuel: a fascinating feature of Methanogenic archaea:*** Awareness of rising energy demands and the notable environmental impacts of current energy

sources have strongly stimulated the search for sustainable, readily available, carbon-neutral (environmental-neutral), or carbon-free alternative energy resources and innovative fuel technologies. So, currently there is a huge global interest in finding new sources of renewable energy. One of the potential ways in which we can fill this energy gap is through methanogenesis, which leads to the formation of methane, a natural gas produced via the breakdown of organic materials.

Methane gas is actually produced by a group of specialized microorganisms known as methanogens, which are known to be extremely sensitive to the types, to availability of substrates, and to the conditions surrounding them. Methanogenic archaea is the only organism that produces significant amounts of methane in the atmosphere. They are responsible for approximately 75%–85% of all methane emissions, and 1 billion tons of biological methane is produced by them annually, which is paving the way to strong interest in studying the environmental aspects of methanogenesis (Kumar et al., 2011). In anaerobic microorganisms, the Calvin cycle for CO<sub>2</sub> fixation does not operate, and acetyl-coenzyme A is the central intermediate in carbon dioxide metabolism. Nonphotosynthetic pathways for carbon dioxide fixation are those used by the bacterial methanogens. Methanogenesis also has an important function in the global carbon cycle as the terminal step by degrading organic matter as well as by supporting many aerobic methanotrophic communities. Economically, methane is a major component of natural gas and is an important source of heat and energy (Hungate, 1966).

Sustainable production of methane would serve the purpose of producing a biofuel

while also helping to slow and possibly cap the net accumulation of carbon dioxide in the atmosphere. Capturing carbon dioxide from the atmosphere (Lackner, 2009) would allow us to remove it and recycle the greenhouse gas to use as a plentiful carbon source. On combustion of the methane biofuel, the carbon dioxide released back to the atmosphere will not cause a net increase in the amount of carbon dioxide. This carbon-neutral process would help to reduce the rapid accumulation of atmospheric carbon dioxide and greatly improve the chances of meeting carbon emission reduction. Methanogenic biogas is an authentic and eco-friendly energy source that can be used for heating and running power plants as it requires minimal treatment (Figure 1 shows the conceptual pictorial graph for our future biofuel production).

***Methanogenesis (biomethanation or biogenic methane formation):*** The formation of methane by Methanogenic archaea is a well-known natural process (Jones et al., 1987; Zeikus, 1977) through methanogenesis. Methanogenesis is used for conversion of carbon dioxide into methane fuel. Biomethanation is the biological production of methane mediated by anaerobic microorganisms commonly called methanogens. The production of methane is due to the energy-yielding metabolism of methanogens and it is a major catabolite unique of these organisms. Methanogenesis is thought to be one of the most ancient forms of cellular metabolism and may have been the original method of energy acquisition in the evolution of life. On the basis of the substrates, three different catabolic pathways are found to be involved in the conversion of methane. Three major pathways are the following: Hydrogenotrophic or carbon dioxide-reducing pathway (reduction of carbon

dioxide), Aceticlastic pathway (fermentation of acetate), and Methylophilic pathway (dismutation of methanol or methylamines/ C1 compound conversion pathway).

Many coenzymes and cofactors, such as C1 unit carriers, methanofuran (MF/MFR), coenzyme F<sub>420</sub> (methanophenazine, MP), and coenzyme F<sub>430</sub>, are involved in the methanogenesis pathway. All three pathways have the common feature of the demethylation of methyl-coenzyme M to methane and the reduction of the heterodisulfide of coenzyme M as well as coenzyme-Me B catalyzed by methyl-coenzyme M and heterodisulfide reductases. These vary in the carbon compound used as the substrate, as well as the source of reducing potential.

About two-thirds of the methane produced in the nature derives from the reduction of the methyl group of acetate, and about one-third from reduction of carbon dioxide with electrons from hydrogen or formate. Lesser amounts of methane are produced by the oxidative and reductive dismutation of methanol or methylamines. Recently, it has been described that methanogens produce methane from dimethyl sulfide or reduce carbon dioxide to primary, secondary, and cyclic alcohols as electron donors (Hedderich and Whitman, 2006). (Figure 2 shows overview of methanogenesis pathway)

***Methanogenic archaea: all-rounder for anaerobic digestion:*** Anaerobic digestion (AD) is widely used in wastewater treatment plants for stabilization of primary and waste-activated sludge. Increasingly energy prices as well as stringent environmental and public health regulations ensure the increasing popularity of anaerobic digestion. Anaerobic digestion is a multistep biological and chemical process that is beneficial not

only in waste management but also energy creation. Methanogens have a pivotal role in the degradation of organic matter. They form the basis of the method of waste treatment aimed at reducing their hazardous effects on the biosphere. The mutualistic behaviour of various anaerobic microorganisms results in the decomposition of complex organic substances into simple, chemically stabilized compounds, mainly methane and carbon dioxide. The conversion of complex organic compounds to methane and carbon dioxide is possible due to cooperation of four groups of microorganisms, that is, fermentative, syntrophic, acetogenic, and methanogenic bacteria (Shah et al., 2014).

Anaerobic treatment of organic wastes has been used in sewage treatment plants for nearly a century. Now, there is an increased interest in treating various industrial and agricultural wastes using methanogenic mixed culture, because methanogenic waste treatment systems can be energy efficient and even energy-producing. Methanogenic mixed cultures are being seriously considered for the treatment of certain toxic wastes, including halogenated and aromatic organic compounds (Figure 3 shows schematic diagram showing anaerobic degradation of organic matter).

***Methanogens holds key for fifth pathway for carbon dioxide fixation, neutralization, and maintenance of global warming:***

Methanogens are ancient organisms that are key players in the carbon-neutral process in our environment. They are important actors in the biogeochemical cycling of carbon on the earth. They act as a carbon dioxide scrubber in our environment. They perform the final stage of anaerobic degradation of organic matter with their unique ability to produce methane (Helijoutonen et al., 2006). Biological methanogenesis is crucial in the global carbon cycle, with a significant

impact on global warming (Claus and Konig, 2010; Ferry, 1993). The biological formation of methane (methanogenesis in our environment) is one of the direct essential links and important actors in the neutralization and maintenance of global carbon cycle (biogeochemical cycling of carbon) because if organic matters are not reduced to gaseous methane, they will accumulate in our environment, directly leading to the global warming (Figure 4 shows A novel pathway of CO<sub>2</sub> fixation found by Berg et al., (2007) in Archaea. Four other pathways are known by which autotrophic representatives of bacteria, archaea, and eukarya fix carbon).

***Nitrogen fixation:*** The Methanogenic archaea bring an excellent perspective in the field of nitrogen fixation. The biochemical mechanism of nitrogen fixation in the domain Archaea is evidently similar to that of the well-studied mechanism and operates on the same fundamental mechanism. In the year 1984, nitrogen fixation was discovered in the archaea, in both *Methanosarcina barkeri* strain 227 (Murray and Zinder, 1984) and *Methanococcus thermolithotrophicus* (Belay et al., 1984). Six *nif* genes (*nifH*, D, K, E, N, and X), present in bacteria and also found in the diazotrophic methanogens, are probably of the molybdenum type. However, differences exist in gene organization and regulation (Leigh, 2000). The first report of nitrogen fixation on methanogen was published by Pine and Barker (1954) for *Methanobacterium omelianskii*. However, since this organism was later shown to be a mixture of two species of bacteria, the report of nitrogen fixation was discounted. After that, Belay et al. (1988) showed that the methanogenic component of *Methanobacterium omelianskii*, *Methanobacterium bryantii* strain M.O.H., does indeed carry out nitrogen fixation.



Figure.1 The conceptual pictorial graph for future biofuel production

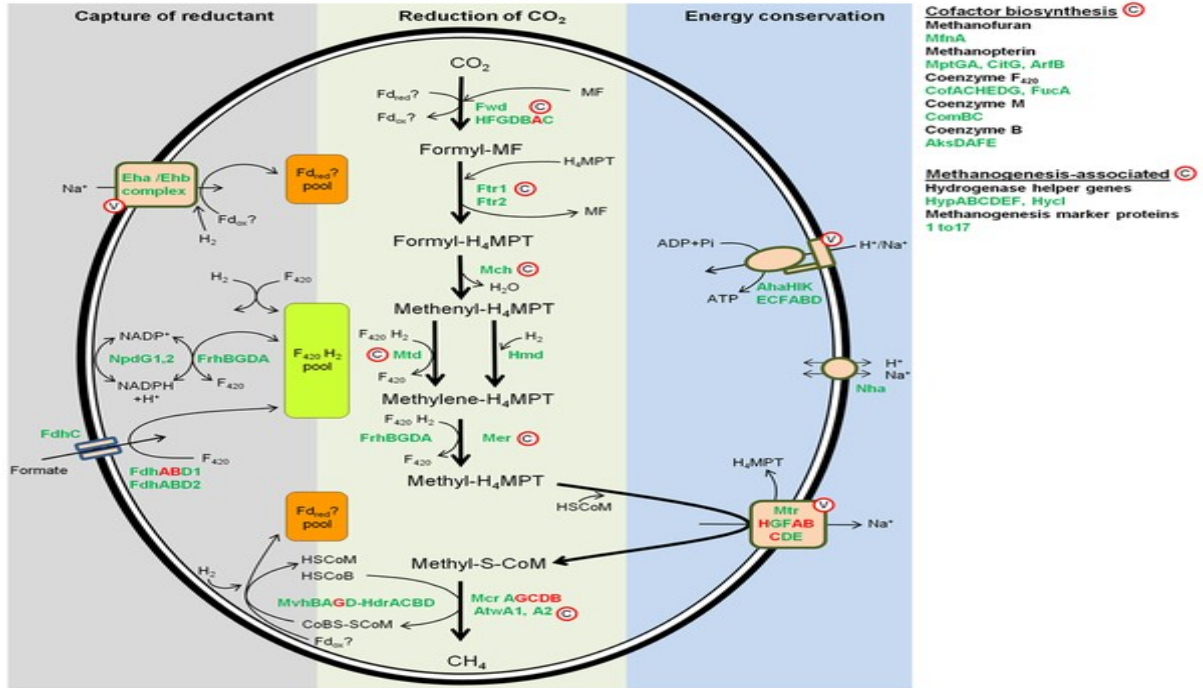
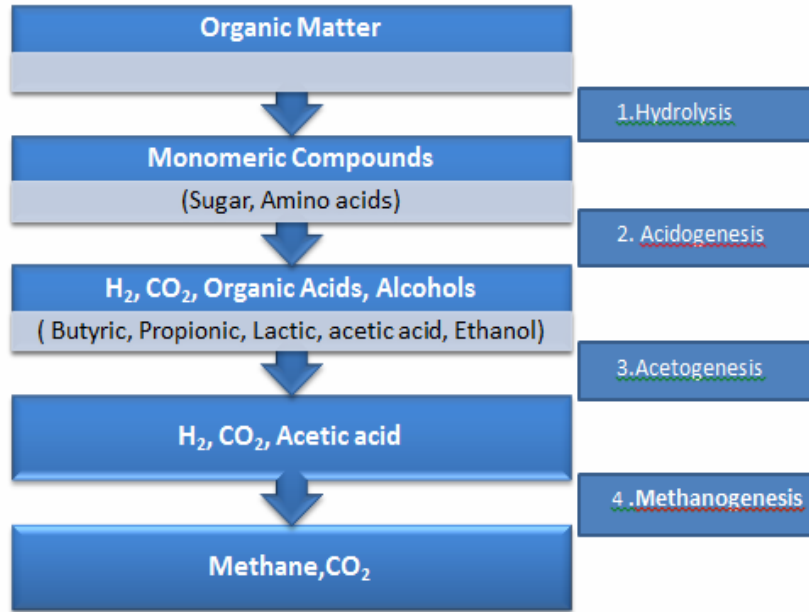


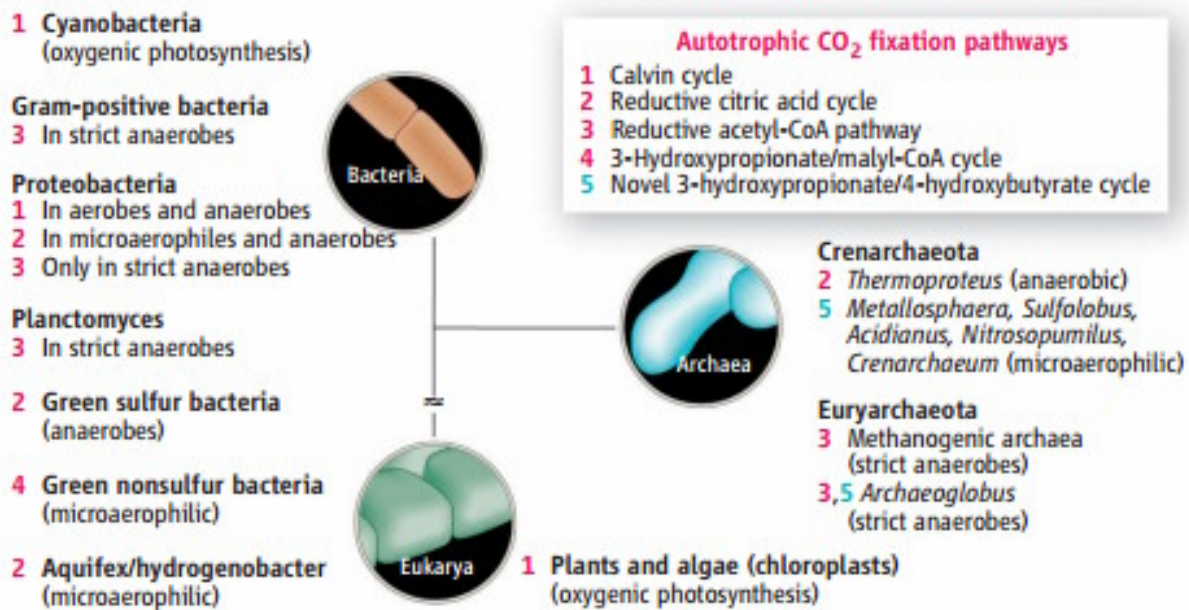
Figure.2 Overview of methanogenesis pathway (Leahy et al. 2010)



**Figure.3** Schematic diagram showing anaerobic degradation of organic matter



**Figure.4** A novel pathway of CO<sub>2</sub> fixation found by Berg et al. (2007) in Archaea. Four other pathways are known by which autotrophic representatives of bacteria, archaea, and eukarya fix carbon



***Methanogenic archaea acts as model organism for life in extreme habitats:***

Methanogenic archaea is one of the simplest and oldest organisms on our earth. They do not require organic nutrients and they are non-photosynthetic, which indicates they could exist in subsurface environments. Methanogenic archaea, derived from Siberian permafrost in addition to known species, are also used as model organisms to investigate their potential to survive in adverse living conditions.

Current research suggests that it is one of the ideal and survival organisms on Mars. These organisms have been postulated as a model candidate for life on Mars because of their ability to act as the primary producers of a subterranean ecosystem using only CO<sub>2</sub> and H<sub>2</sub>. It has become increasingly clear that life thrives in the most challenging environmental conditions found on the planet. Methanogens also act as model organisms for genetics (Leigh et al., 2011; Science Daily- Science News, 2014).

***Other activities of methanogens:***

*Methanoflorens stordalenmirensis* manages surprisingly well in the acid peat lands for annual cycles of freezing, melting, flooding, and drought. It seems to be an indicator species for melting permafrost (Koffmar, 2014). The presence of Methanogenic archaea in ecosystem with very different salinities indicates that they have a mechanism to cope with salt or osmotic stress.

They accumulate compatible solute, commonly glycine betaine, from the environment but only *Methanohalophilus* sp. synthesizes on its own (Spanheimer et al., 2008). The genetic materials namely Ota and Otb (for glycine betaine transports) could be used for creating transgenic plants that may overcome viable salinity stress problem.

**Current issue about methanogens**

Methanogenic biofuel is the hottest, attractive, as well as specially emerging research study topic globally. It has attracted greater attention to date because of fast growing concern that both increasing global energy demand and environmental pollution are central issues in developed as well as developing countries, particularly in India. So, it is considered as one of the major scientific and environmental problems and is the biggest challenge to scientists. That is the reason why we were extremely interested in working in such an area. It is also one of the interesting scientific oceanic areas. Our present efforts have been mainly concentrate to improve and optimize the potent as well as sustainable production of biofuel. Today methanogens have improved standards of living. This novel innovative green technology, which is a gift for our world, provides an effective carbon-neutral fuel source. It also maintains the global equilibrium between oxidative and reductive pathways of single carbon sources. This would likely require a paradigm shift in future energy technologies. It is a promising method not only for recycling the gas but also for producing an alternative fuel source. So it has high public perception toward our clean, green environment. Compared to all biofuels, methanogenic biofuel is the most effective alternative method for realizing our global energy requirements.

**Future directions**

The recent research mainly focuses on biochemistry and pathways related to carbon dioxide fixation and biofuel (methane) formation in methanogens. Upcoming studies also cover optimizing the parameters and its kinetics for effective methane fuel production. Molecular biology and genetics studies for improved production of biofuel

have been carried out in many laboratories. The overall research study mainly provides a scientific basis to evaluate the possible technical solutions to environmental and energy problems. If it is proved that the above-discussed methanogenic methodology for quantitative assessment of total conversion capacity is practically successful, it will be applicable on worldwide basis.

greatest when complemented by other techniques. Methanogenic research will serve our future society. It will reduce pollution in our environment and provide a sustainable, affordable energy source to our nation.

In conclusion, for this review shows methanogens are dominant candidate that is potentially used to resolve many environmental as well as economic issues. It is a worthwhile research topic for enhancing our understanding about the role of the biofuel production in a green, clean environment. The significance of methanogens on the energy research will further increase in the coming years. It also acts as a bio-climate controller because of the presence of its natural carbon dioxide sequestration system. So it will fix, neutralize, and maintain the global carbon cycle effectively.

### Acknowledgments

We acknowledge the financial support provided by the Department of Science and Technology (DST), New Delhi. We also acknowledge the academic and team support by the K.S. Rangasamy College of Arts and Science.

### Reference

Alquerque, SMC., Clementino, MM., Vieira, RRP., Almeida, WL., Cardoso, AM., Martins, OB. 2007. Exploring the

biotechnological applications in the archaeal domain. *Braz J Microbiol* 38: 398–405

Balch, WE., Fox, GE., Magnum, LJ., Woese, CR., Wolfe, RS. 1979. Methanogens: re-evaluation of a unique biological group. *Microbiol Rev* 43: 260–296

Belay, N., Sparling, R., Daniels, L. 1984. Methanogen fixation powerfully effect on the environment. *Microbiol Rev* 48: 101–105

Belay, N., Sparling, R., Choi, BS., Roberts, M., Roberts, JE., Daniels, L. 1988. Physiological and <sup>15</sup>N-NMR analysis of molecular nitrogen fixation by *Methanococcus thermolithotrophicus*, *Methanobacterium bryantii* and *Methanospirillum hungatei*. *Biochim Biophys Acta*. 971: 233–245

Berg, IA., Kockelkorn, D., Buckel, W., Fuchs, G. 2007. A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. *Science* 318: 1782–1786

Claus, H., König, H. 2010. Cell envelopes of methanogen. In: König H, Claus H, Varma A (Eds) *Prokaryotic Cell Wall Compounds*. Springer-Verlag, Berlin pp 231–251

Ferry, J G. 1993. *Methanogenesis*. Chapman and Hall, New York

Ferry, JG., Kestead, KA. 2007. *Methanogenesis*. In: Cavicchioli R (Ed) *Archaea: Molecular and Cellular Biology*. ASM Press, Washington DC pp 288–314

Hedderich, R., Whitman, WB. 2006. Physiology and biochemistry of the methane-producing Archaea. In: Falkow, S., Rosenberg, E., Dworkin, M. (Eds) *The Prokaryotes*. Springer-Verlag, New York 3(2): 1050–1079

Helijoutonen, H., Galand, PE., Yrjala, K. 2006. Detection of Methanogenic



- archaea in peat: comparison of PCR primers targeting the *mcrA* gene. *Res Microbiol* 157: 914–921
- Hungate, RE. 1966. *The Rumen and Its Microbes*. Academic Press, New York
- Jones, WJ., Nagle, DP Jr., Whitman, WB. 1987. Methanogens and the diversity of archaeobacteria. *Microbiol Rev* 51(1): 135–177
- Koffmar, L. 2014. Methane-producing microbe blooms in permafrost thaw. Available at: <http://phys.org/news/2014-03-methane-producing-microbe-blooms-permafrost.html>
- Kumar, VS., Ferry, JG., Maranas, C D. 2011. Metabolic reconstruction of the archaeon methanogen *Methanosarcina acetivorans*. *BMC Syst Biol* 15, 28
- Lackner, KS. 2009. Capture of carbon dioxide from ambient air. *Eur. Phys. J. Special Topics*, 176: 93-106
- Leahy, SC., Kelly, WJ., Altermann, E., Ronimus, RS., Yeoman, CJ., Pacheco, DM., Li, D., Kong, Z., McTavish, S., Sang, C., Lambie, SC., Janssen, PH., Dey, D., Attwood, GT. 2010. The Genome Sequence of the Rumen Methanogen *Methanobrevibacter ruminantium* Reveals New Possibilities for Controlling Ruminant Methane Emissions. *PLoS ONE* 5:e8926.
- Leigh, JA. 2000. Nitrogen fixation in methanogens: the archaeal perspective. *Curr Issues Mol Biol* 2: 125–131
- Leigh, JA., Albers, SV., Atomi, H., Allers, T. 2011. Model organisms for genetics in the domain Archaea: methanogens halophiles *Thermococcales* and *Sulfolobales*. *FEMS Microbiol Rev* 35: 577–608
- Liu, Y., Whitman, WB. 2008. Metabolic phylogenetic and ecological diversity of the Methanogenic archaea. *Ann NY Acad Sci* 1125: 171–189
- Murray, PA., Zinder, SH. 1984. Nitrogen fixation by a methanogenic bacterium. *Nature* 312: 284–286
- Pine, MT., Barker, HA. 1954. Studies in the methane bacteria XI Fixation of atmospheric nitrogen by *Methanobacterium omelianskii*. *J Bacteriol* 68: 589–591
- Science Daily Science News. 2014. Earth organisms survive under martin conditions: methanogens stay alive in extreme heat and cold University of Arkansas Fayetteville Available at: <http://www.sciencedaily.com/releases/2014/05/140519114248.htm>
- Shah, FA., Mahmood, Q., Shah, MM., Pervez, A., Asad, SA. 2014. Microbial ecology of anaerobic digestors: the key players of anaerobiosis. *Sci World J* 2014:183752
- Spanheimer, R., Hoffmann, M., Kogl, S., Schmidt, S., Pfluger, K., Müller, V. 2008. Differential regulation of Ota and Otb Two primary glycine betaine transporters in the Methanogenic Archaeon *Methanosarcina mazei*. *Göl J Mol Microbiol Biotechnol* 15: 255–263
- Woese, CR., Kandler, O., Wheelis, ML. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. *Proc Natl Acad Sci, USA*, 87: 4576–4579
- Zeikus, JG. 1977. The biology of methanogenic bacteria. *Bacteriol Rev* 41 (2): 514–541