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Are Archaea Periopathogens? Truth or Falsehood

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Abstract

An exclusive association of *Archaea* has been observed in oral infections like periodontitis, apical periodontitis and even in certain types of GIT diseases. Owing to their unique physiology and energy metabolism, it is highly plausible that their role in infected areas is beyond that of just as secondary colonizers. They have been reported to be actively involved in the overall poly-microbial infection process. However, it is highly challenging to clearly demonstrate their possible active participation mostly due to the difficulty faced in growing them in routine microbiology laboratories. These organisms are considered to make up the third domain of cellular life and are also a part of human oral microflora. They are strikingly less diverse in comparison to oral bacteria and appear to be relatively rare with respect to their numerical abundance. This current review highlights the importance of understanding the medical-dental impact of methanogens and also aims to devise strategies for elucidating the true function of archaea in the human ecosystem.

Keywords: Methanogenic Archaea; Human Microbial Ecosystems; Oral Infections; Interspecies Hydrogen Transfer, Periodontitis

Introduction

Periodontal disease is a polymicrobial infection where sequence of microbial colonization is essential for the onset and progression of the disease.[1] The microbial etiology of periodontitis has been studied extensively and evidences have shown more than 100 noncultivable bacterial species in subgingival microflora by16S ribosomal RNA ribotyping to be associated. Thus, it is understood that dental biofilm is composed of nonspecific opportunistic pathogens that induce the disease.[2] Molecular evolutionary perspective has unexpectedly invigorated in the field of microbial ecology with the discovery of a major new evolutionary lineage known as "The Archaea" (1977).[3]

Archaea are single-celled organisms lacking a nucleus or other membrane-bound organelles, which are usually found residing in high numbers in both humans and animals.[1] They have been shown to effect other resident microbes and host directly or indirectly. In particular, the methanogens, which respire hydrogen to produce methane, have attracted considerable attention, being contributors to both host disease and health.[4] Unlike bacteria, the diversity of Archaea in human body varies substantially, representing only one phylum. In recent research, main focus of interest is laid on the effects and composition of human intestinal microbiota especially eubacteria and, to a lesser extent, single-celled eukaryotes.[4] In humans, oral microorganisms primarily belong to the bacterial domain. Surprisingly, the members of Archaeal domain have been underrepresented in the oral microbiome, even though they are widespread in nature and capable of occupying almost any ecological niche, including extreme environmental conditions.[5]

Considering their dominance and diversity in human microbiota, one can question that whether Archaea holds any importance in terms of being a part of oral health and infection? Archaea has shown unique physiology and energy metabolism. Their occurrence have been recorded at sites of infection with reasonably high proportions and prevalence.[5] The present review is therefore an attempt to elucidate the questions regarding role of Archaea as a possible pathogenic oral microbiota, highlighting its basic biology and classification. This literature review also attempts to develop a realistic perspective for assessing the impact of Archaea on the human ecosystem.

Classification and Diversity

Prokaryotes have been thriving as microbial communities on Earth for the past 3.5 billion years. They display morphological simplicity and high number that has resulted in a rich and complex diversity like Archaea. This diversity also encourages the search for new groups that are still undetected. The observation reported by Carl Woese and George Fox in 1977, had shown that even though methanogens looked like bacteria, they still had different cell wall structures, unique methanogenesis related coenzymes and 16S rRNAs. Their oligonucleotides also differ from bacterial 16S rRNAs and from eukaryotic 18S rRNAs owing to which they have been placed in a third phylogenetic kingdom, the *Archaebacteria*. In 1990, Carl Woese, Otto Kandler, and Mark Wheelis replaced the name of *Archaebacteria* with *Archaea*, and kingdoms were termed as domains.[7]

The phylogenetic tree of life reveals two very important facts related to evolution: (1) All prokaryotes are not phylogenetically closely related, and (2) Archaea are more closely related to Eukarya than to Bacteria (Table 1). The domain Archaea has two phyla namely, the Euryarchaeota and the Crenarchaeota, each contributing to a major branch on the Archaeal tree. Most of the Archaea that have been cultured in laboratories are extremophiles with potential to grow at the highest temperatures (upto 122^0C), salinities, and extremes of pH known for any microorganism. These organisms are known to utilize hydrogen gas (H_2) in their energy metabolism to a great extent when compared to other microorganisms which thrive mostly on organic and inorganic compounds. [8]

Euryarchaeota is known to particularly inhabit humans. It is further divided into four groups of organisms namely, the methanogens, the extreme halophiles, the thermoacidophiles, and some hyperthermophiles. Some of them require oxygen (O_2) for survival while it is lethal to other groups. Few of them are also capable of surviving in the extremes of pH. Recent molecular research has also unveiled the presence of another branch of archaea called Crenarchaeota in the human gastrointestinal tract. [9]

Archaeal Biology

Prokaryotes are extremely small cells with defined shapes which differentiates them from cells of bacteria and archaea. They share similarity with bacterial cells in lacking muramic acid in the extracellular polysaccharides and possessing lipopolysaccharide-containing outer membrane. It has a unique N-glycosylated protein surface layer (S-layer) which cannot be subjected to Gram stain with few exceptions (*Thermoplasma*, *Halococcus*, *Methanobrevibacter*, *Methanosphaera* and *Ignicoccus*). The outer surface of the S-layer is smooth while the inner surface is corrugated amounting to total thickness of 5 and 25 nm respectively. [10]

The methanogens are rod shaped cells, enclosed in a paracrystalline proteinaceous sheath. This sheath varies from the S-layer in formation of an unusual stable layer of fine p2 lattice structure with several cross links. The lattice network involves cysteine which not only resists dissociation but also allows molecules like H_2, carbon dioxide (CO_2), methane (CH_4) and water (H_2O) to pass through the layer.[10] Some archaeal species like Methanobacteriaceae also consist of a polymer called pseudomurein. The pseudomurein is structurally similar to eubacterial murein except for the replacement of murein with talosaminuronic acid and glucosamine by galactosamine. It also lacks D-amino acids and contains ε- and γ-peptide bonds that makes them resistance to most bacterial antibiotics like β-lactams, lysozymes and proteases.[10]

Archaea consist of many distinct cell surface structures that enable them to move across, sense and adhere to surfaces. Cannulae, hamus, and flagella (pili) are some of the surface appendages. The archaeal flagellum has demonstrated similarities with bacterial type IV pilins. Flagella and pili are used by archaeal to attach to host cells. Cell division in archaea takes place by either a putative system that relies on archaeal actin-like proteins, the endosomal sorting complex, required for transport III(ESCRT-III) based system[11] or by a cytokinetic ring, that acts as its bacterial homolog FtsZ, and establishes constriction during cell division and tubulin.[12]

Archaea display a process called Methanogenesis where materials like polysaccharides, proteins, nucleic acids and lipids are degraded into CO_2 and methane as shown in Table 2. It involves a complex microbial community interaction where all the species function in harmony and help in each other's activity.[9] While most of the knowledge on methanogens comes from investigations in both natural and artificial methanogenic environments, studies have shown existence of these microorganism in the gastrointestinal tracts of humans and animals as well as in the human oral cavity.[9] Research has revealed that methanogens closely interact with a variety of bacterial species to degrade organic matter under anaerobic conditions. This cross-feeding behavior or syntrophic growth on substrates is obligatory for these partners without which none of them would be able to utilize the required substrates under thermo-dynamical constraints.[6, 5]

For the oxidation process, a low partial pressure of H_2 is required which is provided by methanogens by using simple molecules like H_2 and CO_2 as substrates and converting them to CH_4.[13] This conversion results in free energy which is sufficient for the growth of methanogens and bacterial populations which in turn convert volatile fatty acids (VFA) into more H_2. This relationship of growth is termed as 'interspecies hydrogen transfer'.[13] In order to avoid inhibition of VFA fermentation by excess accumulation of H_2 (end-product inhibition), a non-enzymatic removal of H_2 out of the ecological system takes place which keeps running the reaction autonomously. The energy which becomes available through the anaerobic oxidation of H_2 is used by

anaerobes to utilize VFAs. For an efficient interspecies hydrogen transfer, methanogens should be physically and closely connected to syntrophic bacteria.[6,5] Thus, methanogens favour the growth of fermenting anaerobic bacteria by removing the end products,[9] which is in turn a key factor for pathogenesis of many human diseases.

Archaea in Humans

Methanogenic archaea have been shown to be involved in a metabolic cascade with a complex biosystem[9] observed exclusively at sites of poly-microbial anaerobic biofilms in the oral cavity[14] and other mucosa of the body,[5,15] supporting the growth of fermenting bacteria that are opportunistic pathogens.[6,5] Hence, methanogens, when active, are involved in the infectious process with interspecies hydrogen transfer as an indirect mechanism of virulence.[6, 5] They also directly harm the host through their capability to transform heavy metals into volatile methylated derivatives that are toxic[16], and such toxins have been identified in the human gut.[10] It has been reported that when H_2 is being used as a substrate, a support is extended to the fermenting pathogens by methanogens through interspecies hydrogen transfer.[6, 5]

Early researchers have made efforts to identify methanogens from human fecal samples and have successfully isolated *Methanobrevibacter smithii* that accounts for upto 10% of human gut microbial flora.[17] In 1985, first research associated with Archaea in human gastrointestinal disease was published.[18] Researchers have found *Methanobrevibacter* at low concentration in GIT associated with diverticulosis [18,17] and *Methanosphera* in inflammatory bowel disease and chronic constipation.[17] Detection of high concentration of methane in breath is one of the indirect ways to determine a possible pathophysiology of active methanogenic archaea in diseases like precancerous conditions (e.g. ulcerative colitis, colonic polyposis) and cancer of the colon.[2] On the contrary, chemotherapy-induced diarrhea in cancer patients has resulted in the decrease of methanogenic archaea along with a parallel loss of beneficial bacteria.[18]

Recent studies have confirmed the presence of abundant Archaea microorganisms in Type-2 Diabetes(T2D).[18] A possible important contribution of the intestinal microbiome in the development of T2D has also been pointed out that's results from increase in the membrane transport of sugars, branched-chain amino acids and methane metabolism, which in turn influences the hormone balance contributing to the disease.[19] Evidence from various human studies strongly supports the association of *M. smithii* with leanness. Based on studies conducted in mice, it has been suggested that gut methanogens contribute to human obesity.[17] Methanogenic archaea has also been found among the microbial flora of bacterial vaginosis where *Methanobrevibacter* species play a contributing role.[15]

Archaea in the oral cavity and clinical relevance

With the recent advances in PCR-based techniques targeting 16SrRNA genes, it has become easier to find evidence of Methanogenic archaea in human oral ecosystem. A functional gene encoding for the Methyl-Coenzym-M reductase, a key enzyme involved in methanogenesis, has been identified[20] in one of the representatives of methanogenic archaea, namely *Methanobrevibacter oralis,* a predominant species in the oral cavity.[6,5] Newer *Methanobrevibacter* phylotypes (*Methanomassiliicoccus luminyensis* and *Candidatus Methanomethylophilus alvus*) have been recently reported in the oral cavity.[17] These methanogens have been identified in samples from periodontal plaque[6,5] and in some cases even in association with apical periodontitis,[21] suggesting clinical relevance.

An increased proportion of *M. oralis* has been noted with increased severity of disease. Yamabe *et al.* had

investigated its distribution in Japanese patients with periodontitis.[22] As *M.oralis* has never been detected from healthy sites, it can serve as a positive predictive value for periodontitis. Although the mean relative proportions of these archaeal species varies, no other periodonto-pathogenic species has shown an equivalent proportional level of as high as 18% that has been reported with archaea.[6] Methanogenic archaea are more detectable in diseased sites when compared to healthier sites in patients with aggressive periodontitis.[23] *M. oralis* has been identified in 2 of the 34 pulp tissue samples collected from patients with and without endodontic infections.[24] These findings suggest a significant role of methanogens which is more than being just secondary colonizers of infected areas.

It is well understood that periodontal damage is caused by initial periodontal inflammation followed by the pathogen-host response that results in connective tissue breakdown and bone loss in periodontitis[25] In a study by Yamabe *et al*., researchers have detected IgG antibodies against *M. oralis* in 72% of patients with severe periodontitis, supporting the potential role of *M. oralis* in pathogenesis of periodontitis.[22] Krishnan *et al*., suggested from their study that archaeosomes (archaeal liposomes) induce humoral T-helper response, long-term cytotoxic T cell response and enhance the antigen presenting cell recruitment and activation *in vivo*.[26,27] Furthermore, they have identified one of the antigenic molecules as a subunit of the group II chaperonins (also known as thermosomes in Archaea and chaperonin-containing T-complex polypeptide - CCT in Eukarya) and have demonstrated its cross-reactivity with the human chaperonin CCT (highly antigenic molecule).[28] This data is suggestive of the potential role of antigenic molecules of *M. oralis* as modifiers or even initiators of an inflammatory process such as periodontitis.[6, 5]

It has been observed that *M. oralis* is resistant to many antibiotics including tetracyclines, even at minimum inhibitory concentration (MIC) <100 mg/L, but is susceptible to metronidazole with MIC < 1 mg/L. An interesting fact that surfaced is that the most common combination (metronidazole and amoxicillin) used in periodontal disease is also effective against *M. oralis* species.[29] Therefore, all antibiotics are not effective against methanogenic archaea mainly due to metabolic process and cell wall structure of archaea that differs from bacterial species.

M. oralis serves as keystone species in H_2-consumption of oral ecosystems

Archaeal species play an important role in pathogenicity in humans which is in accordance with a concept known as 'key-stone species'. According to this concept, keystone species are rare members of a complex community and are usually noticed only when they are removed or disappear from an ecosystem, resulting in dramatic changes to the rest of the community.[30]

Microbial communities are usually composed of members which carry out similar biochemical reactions contributing to functional redundancy as well as members which exhibit unique physiological traits forming the keystone species. Such complex microbial communities display a functional diversity that is much lower than their phylogenetic diversity. A symbiotic activity among the species is observed during anaerobic degradation of organic matter. In case of extinction of any species, another species with same functional activity replaces it thereby maintaining the microbial homeostasis. By contrast, this is not true for keystone species, which are considered as niche specialists with unique physiologies and cannot be replaced in most of the cases. In case of functional failure of keystone species in macro-organisms, the effect may be beneficial or harmful to the host

depending on the ecological role of the keystone species in the physiological or pathogenic microbial community.[6,5]

In case of oral infection, methanogen at the site of infection may theoretically be replaced by two different functional groups, dissimilatory sulfate reducers (SRB) and reductive acetogens, both of which can grow on H_2 and produce hydrogen sulfide (H_2S) and acetate as end-products, respectively. All these hydrogenotrophic groups have been identified in sub-gingival plaque samples. But the association of these compounds with periodontal disease could be established only for methanogens and SRB. Furthermore, methanogens and SRB were observed to exclude each other in 46% of multiple plaque samples of patients that were pooled for analysis. The results indicated an apparent lack of one particular group in the oral cavity that may be attributed to host-specific factors. In the absence of an appropriate alternative syntrophic partner, functional replacement of keystone species is not possible. Hence, it was suggested that H_2-consumption in a substantial number of periodontitis patients is a process performed by only one type of species i.e. the keystone species.[6, 5]

Future Strategies

Latest technologies like PCR are being used now-a-days to identify newer prokaryotic species. A major limitation that occurs in most of the cases is the cross-reaction with human DNA that is rarely observed with bacteria-specific primers. This may be due to unfavorable ratio between archaeal-to-human DNA and a higher degree of sequence similarity between archaea-specific primers and human gene sequences. Careful design of novel primers as well as the use of multiple molecular targets (16S rDNA and mcrA) is highly important in order to recover a wider range of human methanogens.

Methanogenic archaea has the potential to cause periodontal diseases in oral cavity apart from its pathogenic potential in human gut but its identification still remains a challenge. Two new strategies have been designed for identification of phenotype of organism that are based on monitoring of interspecies hydrogen transfer. One of the strategies is “via stable isotope probing” and other one is “via inhibition of methanogenesis”.[6,5]

Stable isotope probing (SIP) of different organic compounds such as butyrate or propionate, has become a popular method to investigate syntrophic methanogen-bacterial interactions in various habitats. A major challenge arises in achieving appropriate *in vivo* conditions for incubation of sub-gingival plaque samples in the laboratory. However, such techniques have been launched for studying the impact of the gut microflora on inflammatory bowel disease. Also, metabolic activity of methanogens in sub-gingival plaque takes place through its biochemical inhibition. The complex biochemical cascade of methanogens is linked in such a way that once the final step of methane (CH_4) formation is inhibited, the entire process remains blocked. A compound named 2-bromoethane sulfonate (BES), which is a structural analogue of coenzyme M, is often used to specifically inhibit methanogenesis. BES is known to stop CH_4 formation and as a consequence also impedes interspecies hydrogen transfer. The DNA can then be incubated and subjected to analysis for *mcr*A gene which is indicative of presence of methanogens in the periodontal disease.[6, 5]

It is possible that the interspecies hydrogen transfer based on methanogenesis may be a virulence mechanism for periodontal diseases. In order to confirm such a mechanism, it is crucial to identify the activity of the syntrophic bacterial partner from co-aggregation and interspecies hydrogen transfer in the liquid media (i.e. VFA).

Conclusion

Methanogenic archaea including *M. oralis* are significantly associated with human inflammatory diseases like periodontitis. Experiments in the past have provided sufficient evidence regarding the occurrence of interspecies hydrogen transfer in periodontal samples with *M. oralis* as an emerging periodontal pathogen. This also paves way for new vistas in the treatment of periodontal disease. Thus, our future investigations may be directed towards this novel channel that controls the activities and level of methanogens at diseased sites.

References

1. Matarazzo F, Ribeiro AC, Feres M, Faveri M, Mayer MPA. Diversity and quantitative analysis of Archaea in aggressive periodontitis and periodontally healthy subjects. J Clin Peridontol 2011;38:621-27.
2. Nishihara T, Koseki T. Microbial etiology of periodontitis. Periodontology 2000 2004;36:14-26.
3. DeLong E. Archaeal Means And Extremes. Science 1998;280:542-43.
4. Mark Pimentel, Robert P. Gunsalus , Satish S.C. Rao And Husen Zhang: Methanogens In Human Health And Disease. Am J Gastroenterol Suppl 2012;1:28–33.
5. Horz HP, Conrads G. Methanogenic Archaea And Oral Infections-Ways To Unravel The Black Box. Journal Of Oral Microbiology 2011;3:5940.
6. Marsh PD. Dental biofilm, chapter 8:Clinical Periodontology and Implant Dentistry, Sixth Edition. Edited by Niklaus P. Lang and Jan Lindhe. Published 2015 by John Wiley & Sons, Ltd.
7. Reeve JN. Archaebacteria then ... Archaes now (are there really no archaeal pathogens?). J Bacteriol 1999;181:3613-7.
8. Madigan MT, Martinko JM, Stahl DA, Clark DP. Brock Biology of Microorganisms. Edition 13, Chapter 2, Brief of microbiology evolution, pg 35-41, publication: Pearson.
9. Matarazzo F, Ribeiro AC, Faveri M, Taddei C, Martinez MB, Mayer MPA. The Domain Archaea In Human Mucosal Surfaces. Clin Microbiol Infect 2012;18:834–40.
10. Meyer, Benjamin H; And Albers, Sonja-Verena : Archaeal Cell Walls. In: Els. John Wiley & Sons, Ltd: Chichester, February 2014.
11. Albers SV, Meyer BH. The archaeal cell envelope. Nat Rev Microbiol 2011;9:414-26.
12. Duggin IG, Aylett CHS, Walsh JC, Michie KA, Wang Q, Turnbull L et al. Tubulin-Like Proteins Control Archaeal Cell Shape. Nature 2015;519:362–5.
13. Schink B. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 1997;61:262-80.
14. Li Cl, Liu Dl, Jiang Yt, Zhou Yb, Zhang Mz, Jiang W et al. Prevalence And Molecular Diversity Of Archaea In Subgingival Pockets Of Periodontitis Patients. Oral Microbiol Immunol 2009;24:343–6.
15. Belay N, Mukhopadhyay B, De Macario EC, Galask R, Daniels L. Methanogenic Bacteria In Human Vaginal Samples. Journal Of Clinical Microbiology 1990;28:1666-8.
16. Michalke K, Schmidt A, Huber B, Meyer JR, Sulkowski M, Hirner AV et al. Role Of Intestinal Microbiota In Transformation Of Bismuth And Other Metals And Metalloids Into Volatile Methyl And Hydride Derivatives In Humans And Mice. Applied And Environmental Microbiology 2008;74:3069-75.

17. Lurie-Weinberger MN, Gophna U. Archaea In And On The Human Body: Health Implications And Future Directions. Plos Pathog 2015;11:E1004833.
18. Aminov RI. Role of archaea in human disease. Front Cell Infect Microbiol. 2013;3:42.
19. Caricilli AM, Saad MJ. The role of gut microbiota on insulin resistance. Nutrients 2013;5:829-51.
20. Vianna ME, Conrads G, Gomes BP, Horz HP. Identification and quantification of archaea involved in primary endodontic infections. J Clin Microbiol 2006;44:1274-82.
21. Jiang YT, Xia WW, Li CL, Jiang W, Liang JP. Preliminary study of the presence and association of bacteria and archaea in teeth with apical periodontitis. Int Endod J 2009;42:1096-103.
22. Yamabe K, Maeda H, Kokeguchi S, Tanimoto I, Sonoi N, Asakawa S et al. Distribution Of Archaea In Japanese Patients With Periodontitis And Humoral Immune Response To The Components. Fems Microbiol Lett 2008;287:69–75.
23. Faveri M, Mayer MP, Feres M, De Figueiredo LC, Dewhirst FE, Paster BJ. Microbiological Diversity Of Generalized Aggressive Periodontitis By 16s rRNA Clonal Analysis. Oral Microbiol Immunol 2008;23:112–8.
24. Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR. Phylogenetic Analysis Of Bacterial And Archaeal Species In Symptomatic And Asymptomatic Endodontic Infections. Journal Of Medical Microbiology 2007;56:110–8.
25. Ashok N, Warad S, Singh Vp, Chaudhari H, Narayanan A, Rodrigues J. Prevalence Of Archaea In Chronic Periodontitis Patients In An Indian Population. Indian J Dent Res 2013;24:289-93.
26. Krishnan L, Sad S, Patel GB, Sprott GD. Archaeosomes induce enhanced cytotoxic T lymphocyte responses to entrapped soluble protein in the absence of interleukin 12 and protect against tumor challenge. Cancer Res 2003;63:2526-34.
27. Krishnan L, Sad S, Patel GB, Sprott GD. The Potent Adjuvant Activity Of Archaeosomes Correlates To The Recruitment And Activation Of Macrophages And Dendritic Cells In Vivo. The Journal Of Immunology 2001;166:1885–93.
28. Nguyen-Hieu T, Khelaifia S, Aboudharam G, Drancourt M. Methanogenic Archaea In Subgingival Sites: A Review. Apmis 2013;121:467–77.
29. Scott ML, Soule Michael E., Doak Saniel F. The Keystone-Species Concept In Ecology And Conservation. Bioscience 1993;43:219-24.
30. Eckburg PB, Lepp PW, Relman DA. Archaea and their potential role in human disease. Infect Immun 2003;71:591-6.

Legend Tables

Table 1: Comparison of Diverse Features between Bacteria, Archaea and Eukaryotes

S.No	Features	Domain Bacteria	Domain Archaea[10,11]	Domain Eukarya
1.	Cell size	0.5 - 4μ	0.5 - 4μ	> 4μ
2.	Membrane-enclosed with nucleus with nucleolus	Absent	Absent	Present
3.	Complex internal membrane bound structures	Absent	Absent	Present
4.	Cell Wall	Peptidoglycan with muramic acid	Various types with no muramic acid	No muramic acid, cellulosic in plants
5.	Membrane lipid	Ester linked straight chained fatty acid	Ester chained branched aliphatic chains	Ester linked straight chained fatty acid
6.	Gas vesicle	Present	Present	Absent
7.	Transfer RNA	Thymine present in most tRNA	No Thymine in T/ TψC arm of tRNA	Thymine present
8.	Polycistronic mRNA	Present	Present	Absent
9.	Post transcriptional modification of RNA	Absent	Absent	Present
10.	Ribosome size	70S	70S	80S
11.	RNA polymerase enzyme types	One type only	Several	Only three types (I,II,III)
12.	Structure of RNA polymerase	Simple, 4 subunits	Complex, 8-12 subunits	Complex, more than 12 subunits
13.	Plasmid	Present	Present	Absent
14.	Histone	Absent	Present	Present
15.	Methanogenesis	No	Yes	No
16.	Nitrogen fixation	Yes	Yes	No
17.	Chlorophyll based photosynthesis	Present in some	Absent	Present in plants
18.	Chemolithotrops	Present	Present	Absent
19.	Multicellularity	No	No	Yes
20.	Growth at temperature more than 800C	Few organisms	Most of the species	None

Table 2: Production of methane from different classes of substrate

Component	Methanogenic reaction	
Lipids	$C_{50}H_{90}O_6 + 24.5H_2O$	$\rightarrow 34.75CH_4 + 15.25CO_2$
Carbohydrates	$C_6H_{10}O_5 + H_2O$	$\rightarrow 3CH_4 + 3CO_2$
Proteins	$C_{16}H_{24}O_5N_4 + 14.5H_2O$	$\rightarrow 8.25CH_4 + 3.75CO_2 + 4NH_4 + 4HCO_3$