Diversity of microorganisms associated with pristine and extreme environment of mud volcanoes

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Abstract- Microbial diversity of pristine habitats is studied owing to two main reasons: contributing to the taxonomic literature and exploration of microbes for their potential to produce novel industrially important biomolecules. These biomolecules generally have better potential to survive under harsher conditions like pH, temperature and salt concentration. Traditionally, diversity analysis was based on cultivation dependent methods and hence was biased towards the labweeds or culturable microbes. Chances of accounting for the complete biodiversity were comparatively lower. With the advances in cultivation independent methods, a vast majority of yet uncultured bacteria and archaea have been detected. Mud volcanoes are points of release of pressure from deep layers of earth. They generally coincide with the tectonic plate boundaries. Heat that is generated due to movement of tectonic plates is released by these vents. Microorganisms from various depths of earth might get mobilized along with the flowing mud. Being an unexplored habitat with no chances of human intervention, such habitats may offer a unique source of unexplored microbes.

Index Terms- Mud volcano, microbial diversity, bacteria, archaea, hypervariable regions of 16S rRNA gene.

I. Introduction

Microbial Diversity

It is rightly said that we live on a “microbial planet” (Woese, 1999) in the “age of bacteria” (Gould, 1996). Microorganisms were active for more than 3 billion years before the development of multicellular life forms. Microbes evolved in microhabitats presented by abiotic world. Microorganisms are ubiquitous in nature including extreme environments where they behave as catalysts during the course of biogeochemical cycling. They progressively altered geochemical conditions leading to development of new environmental conditions and habitats. This led to continuing evolution of distinct microbial types and higher forms of life. Over a period of time, the release of oxygen led to gradual change in earth’s atmosphere from reducing to oxidizing. Thus energetically more efficient aerobic organisms were favored in evolution. This is probably the reason for extraordinary diversity and habitat range of microbes. At least 10^{30} microbial genomes are estimated to be present in the biosphere (Whitman, 1998). Microorganisms encompass an extraordinary diversity in their ecological functions as well as taxonomy (Dunlap. 2001).

Various habitats harboring microbes are common in nature, like mud or other sediments, marshes, water logged soils, intestinal tract, sewage, sludge, deep subsurface of earth, etc. Such habitats can be extreme with respect to pH, temperature, salinity and other environmental conditions. The prokaryotes that thrive in these habitats are termed ‘Extremophiles’.

Historic perspective on identification of microbes was based on morphological characterization. In order to account for all groups of organisms, various techniques like most probable number (MPN) were performed and then selective enrichments at various conditions like pH, salinity and temperature were used for enrichments. Later on the focus was on biochemical characterization. But in both the cases, clear cut distinction for identification purpose was difficult and hence, the focus shifted to molecular methods. Though, culture dependent and biochemical methods are accurate, inexpensive and fast, various biases compel the user to supplement the studies by cultivation independent methods. Now days, Polyphasic approach is commonly used.

II. Methods for investigating microbial diversity

Cultivation dependent diversity analysis

Bacteria in soil play pivotal role in various biogeochemical cycles and are responsible for cycling of organic molecules (Molin, 1997). As only 1% of soil bacteria can be cultured, there is a continuing need for reliable and accurate mechanisms for studying the complete diversity of microbes in soil (Watve, 2000, Alain, 2009). Innate heterogeneity of soil and spatial distribution of microbes add up to the problem (Trevors, 1998). Counting microorganisms and identifying individual species is neither practical nor feasible, as morphological variations may not be distinct enough to reflect species diversity. Hence, there is a continuing need for reliable and accurate methods for understanding the complete diversity of organisms in soil.

Cultivation independent diversity analysis

Comparing primary structure of macromolecules became easier after improvements in molecular sequencing techniques to deduce phylogenetic history (Zuckerkandl and Pauling, 1965). Cytochromes and ferredoxins were the first molecules to be analyzed for this purpose (Fitch and Marguliash 1967). Woese *et al*. demonstrated usefulness of SSU rRNA as universal phylogenetic marker (Fox et al., 1977). SSU rRNAs, molecules are characterized by the evolutionary preservation of a common

core of secondary or higher order structure. Functional pressures dictate the evolutionary preservation of such structure (Bergeys Manual of Systematic Bacteriology). Besides functional constancy, adequate size, ubiquitous distribution, genes coding for SSU have both evolutionarily conserved regions and highly variable structural elements. Varying degree of sequence conservation allows reconstruction of phylogeny for a broad range of relationships (Stackebrandt and Goebel, 1994). Even though large subunit (LSU) would provide twice the phylogenetic information than SSU, the average rate of sequence change is faster significantly than that of 16S. Thus for closer relationships, analysis of larger molecule can be quite valuable (Olsen, 1993). Genes like RNA polymerases, elongation factor G (EF-G), EFTu/1α, RecA, hsp 60, proton translocating ATPase may also be targeted.

Hypervariable regions of 16S rRNA genes and difference in diversity observed on targeting different hypervariable regions

Bacterial 16S rRNA can be divided into 9 hypervariable regions (V1 to V9) flanked by highly conserved regions (Head, 1998, Muyzer, 1993). Taking advantages of these conserved sites, universal primers have been designed that amplify the variable regions of choice. Different community profiles are seen on amplifying various hypervariable regions. So knowledge of preferred V regions to get a complete picture of community is essential. V1 region is found to be most variable but is targeted to a lesser extent owing to its small size (46 bases). V9 and V3 follow variability. So a combination of hyper variable region is targeted V3-V5 and V6-V8 portray maximum biodiversity in case of bacteria (Zhongtang, 2004). Similarly in case of archaea, the preferred hypervariable region is V3 followed by combinations of V1-V2 and V3-V4. Different organisms might be detected when different hyper variable regions targeted by PCR (Zhongtang, 2008).

Fig 1: Mud Volcanoes and hypervariable regions of the 16S rRNA gene

Limitations of Molecular Methods

PCR based molecular techniques are generally used to overcome the limitations of culture based methods. However, numerous biases and limitations have to be taken care of, like efficiency of cell lysis, method of DNA-RNA extraction, primer based PCR bias, preferential amplification of low G+C content DNA etc. If gentle methods are used for genomic DNA extraction gram negative cells lyse easily, but gram positives do not. If the method is too harsh, there are chances of shearing of DNA (Wintzingerode, 1997). If purification method for extraction of nucleic acid is not appropriate, co-precipitation of PCR inhibitors like humic acid may result in their further interference in subsequent PCR analysis. Additional purification steps can lead to loss of DNA, again introducing a bias in molecular diversity analysis (Kirk, 2004). The primers used for PCR amplifications, though universal have proven to have different affinities to templates. Different copy numbers of target genes also complicates the problem. Sequences with low G+C contents separates more efficiently in denaturing steps of PCR and therefore could be preferentially amplified (Wintzingerode, 1997).

Diversity analysis in the metagenomic era

To take care of the deviations from conserved regions, commonly used primers have been modified with degenerate positions to account for higher number of organisms (Baker and Cowan, 2004). In this case also, the primer designing is based on known cultured representatives' sequences. So, divergent 16S genes have lesser chances of being amplified (J. Rajendhran,2011). A promising alternative to these obstacles appears to be 16S based metagenomic analysis using high throughput sequencer. The most recent and powerful method of analysis is metagenomic analysis. The metagenomic analysis analyzes huge numbers of relatively short DNA sequences (100-500 bp). The raw material is generated by high throughput next generation sequencers. This is potentially the most powerful technique used (Wommack, 2008) . The validity of metagenomic analysis depends on ability to obtain representative community DNA sample (Chandler *et al*., 1998).

Archaea and bacteria

Archaea and bacteria are prokaryotes with very diverse phenotypes, nutritional modes and physiology. While the study of fascinating micro-organisms needs no special justification, these prokaryotes provide unique opportunity to gain insight into a number of fundamental conundrums in biology. Earlier view about archaea being relict organisms has now changed radically. Most of the archaea exhibit unique features like methanogenesis, hyperthermophily,halophily, capacity to reduce sulphates etc. Archaea being most ancient lineage of living organisms, set a

boundary for evolutionary diversity.Archaea also share a few characteristics with eukarya like some tRNA genes contain introns, the DNA dependant RNA polymerases are multicomponent enzymes, etc. On the basis of SSU rRNA gene analysis, the archaea consist of three phylogenetically-distinct groups: Crenarchaeota, Euryarchaeota and Korarchaeota. The diversity of prokaryotes is also of great applied importance in bioremediation and bioprospecting (Demirjian, 2001).

Mud volcanoes

Mud volcanoes are the geologic structures formed as a result of emission of gases (predominantly methane), water and clay-based material at the earth's surface or seafloor. A composite mixture of water and gas along with mud is forcefully ejected through long, narrow openings or fissures in crust resulting in out-flowing mass of mud breccias on the surface. Mud volcanoes are irregularly clustered in separated areas forming belts that coincide with the tectonic plate boundaries. The geological factors responsible for the formation of volcanoes include abnormally high pore fluid pressures (caused due to rapid sedimentation and density inversion), in situ gas generation and strong lateral or vertical compressions which allow deep lying sediments to move upward. Mud volcanoes are believed to originate from a few meters to several kilometers depth (Kopf, 2000). Pore fluid chemistry and fluid rock interaction suggest that aqueous fluids are released at mantle depth (Mottl, 1992). The phenomenon of mud volcanism is global and approximate 1950 individual volcanoes worldwide of which 60 to 65 erupt every year (Dimitrov, 2003).

As reported in literature, temperature of earth below the surface increases. So, presence of thermophilic organisms can be anticipated in the flowing mud. The water that is mobilized might have its origin in the ocean. So, presence of halophiles can be anticipated as well. Enzymes from organisms present at such locations are stable at high temperatures with optimal activity at temperatures above 70°C. They are also resistant to harsher environmental conditions. Some of these enzymes are active at temperatures as high as 110°C and above. Various strategies are used by organisms growing in such habitats to modify their proteins to confer higher thermal stability, enhanced rigidity and resistance to chemical denaturation etc.

Classification of mud volcanoes

Classification of mud volcanoes was initially based on morphological characteristics, style of eruption, size, tectonic settings and chemical composition of volcanic products (Hone, 2007). On a broader scale classification of mud volcanoes is done into three types:

Lokbatan type: Volcanoes with strong explosive nature, commonly with ignition of emitted gases. The period of activity is short, separated by long periods of inactivity. The name derives from location of such type of volcano in Lokbatan on Aspherson peninsula, Azerbaijan on 25 th October 2001.

Chikishlyar type: Volcanoes characterized by continuous, calm and relatively weak activity. Commonest feature of this type of volcanoes is having numerous vents ejecting water, gas and mud in small quantities.

Schugin type: The volcanoes whose activity is transitional between Lokbatan and Chikishlyar type are classified under Schugin type of mud volcanoes (Kalinko, 1964).

Microbial diversity of mud volcanoes round the globe and bioprospecting

Terrestrial mud volcanoes:

Terrestrial zones with mud volcanoes are hydrocarbon rich indicating a relationship between hydrocarbon generation and mud volcanic eruption. A total of 5.06 Tg CH_4 or 4.98 Tg carbon, half of which is fossil, and is emitted to the atmosphere by quiescent and eruptive mud volcano activity every year (Dimitrov, 2003).

Mud volcanoes of Andaman

The Andaman islands (92°-94°E, 6°-14°N) have experienced eruption of mud volcano previously in 1843, 1879, 1907, 1983 and 2003 in Baratang (Vignesh A, 2016). The major catastrophe caused by the magnitude 9.3 mega thrust earthquake and tsunami on 26 December 2004 resulted in shaking the oceans interior and re eruption of the mud volcano (Malik, 2006). Like all other microbial populations of mud volcanoes reported, microbial community of these mud volcanoes is dominated by members of *Proteobacteria*, *Cytophaga / Flavobacter, Archaea, methanogens*, methanotrophs, halophiles and sulfate reducing bacteria. Presence of many novel bacterial and archaeal lineages is indicated in DGGE. Moreover, many of the isolates show lesser sequence homologies to known sequences. The phylogenetic approach to study the microbial community of mud volcanoes showed presence of rare microbes which are potential source for new bioactive molecules like enzymes, biosurfactants, antimicrobial compounds, exopolysaccharide and polyhydroxyalkonates. A novel Gram-negative, oval-shaped, motile bacterium was isolated from the mud containing polar lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified lipids, one unidentified phospholipid and one unidentified aminolipid. 16S rRNA gene sequence analysis showed that strain was related most closely to the type strains of *Tepidamorphus gemmatus*, *Bauldia consociata* (P. Anil Kumar, 2012) .

Paclele Mici mud volcanoes in Carpathian Mountains, Romania:

Among the terrestrial volcanoes in Carpathian Mountains in Romania, Paclele Mici has been extensively studied for microbial diversity. At the site, thermal alteration leads to release of methane along with higher hydrocarbons and petroleum compounds. $\delta^{13}C$ value of released methane indicates a thermogenic and likely kerogenic origin (Whiticar, 1999). Considering hydrocarbons as utilizable bacterial substrate, presence of bacteria was anticipated. 16S rRNA gene based diversity analysis was performed. Bacteria were found to be abundant than archaea. Respective abundances of bacteria and archaea were estimated to be 3.4-6.1 x 10^8 and 0.25-1.6 x 10^6 cells/gm dry weights. Presence of members of order *Methanosarcinales* was confirmed. Bacterial diversity was relatively larger comprising of members of *Deltaproteobacteria*, *Gammaproteobacteria* and *Bacteroides-Cytophaga* group. The presence of sulphate reducing population is likely to confirm the

presumed marine origin of the volcanic fluid (Karine, 2006). Contribution to aerobic methanotrophy / methylotrophy and methanogenesis to the carbon cycle is significant at the site.

Mud volcanoes near Tianshan Mountains in Usu city of Xinjiang, China:

Largest group of terrestrial mud volcanoes in China is located near Tianshan mountains in the arid / semi arid regions, comprising of 40 volcanoes, of which 36 volcanoes are active. Clone libraries targeting the 16S rRNA gene were constructed and on sequencing the representative clones, 11 bacterial and 7 archaeal phylotypes were detected. Population of *Proteobacteria* dominated being 79% of the bacterial counts (mostly *Deltaproteobacteria*). *Actinobacteria* constituted 15% followed by *Fusobacteria* (6%). Relatives of uncultivated species of archaea were also detected followed by *Methanosarcinales* and *Halobacteriales* from *Euryarchaeota* (88%) group and others from *Crenarchaeaota* group (12%) (Yang, 2012).

Mud volcanoes at Dushanzi and Baiyanggou in Junggar Basin in Xinjiang, China

The first comparative analysis of the diversity of microbes of Dushanzi (DSZ) and Baiyanggou (BYG) mud volcanoes was done using 16S rRNA gene analysis. Phylogenetic analysis showed that the bacterial and archaeal community structure was remarkably different between the DSZ and BYG mud volcanoes. At BYG mud volcano, bacterial community diversity was higher than that of the DSZ mud volcano. Archaeal community diversity of BYG mud volcano was lower than that of the DSZ mud volcano. Most of the archaea recovered from DSZ identified in these mud volcanoes are anaerobic or highly thermophilic. Microbes recovered from BYG media were identified as halobacteria consistent with their particular geographical nature. Many bacterial and archaeal genera, such as *Methanomicrobia*, sulfur-oxidizing symbiont bacterium and *Alcanivorax sp.* have been detected in these two mud volcanoes (Zhiyong et al., 2016).

Mud volcanoes in San Baigio-Belpasso, Mount Etna, Italy:

Restriction fragment length polymorphism (RFLP) analysis of the bacterial clones clustered microbes into Eubacterial group. 77% affiliated to *Proteobacteria*, 19% affiliated to *Actinobacteria* and 4% affiliated to *Flexibacter-Cytophaga-Bacteriodies* division. Thus major constituents of microbial communities of saline volcanic mud were autotrophic methane oxidizers and heterotrophic hydrocarbon degraders (belonging to gamma subclass of *Proteobacteria*). Overall the community structure of San Biagio mud volcano resembles compositions of marine microbial communities (Yakimov, 2002).

Mud Volcanoes in Salse di Nirano, Northern Apennines, Italy:

Anaerobically maintained fluid samples from the Nirano mud volcano field located in the foreland of the Northern Apennines were used for microscopy. The mud was found to contain low numbers of bacterial cells. Media was devised based on composition of volcanic mud and anaerobic enrichments were set up. Enrichment of *Desulfovibrio* and *Clostridial* strains were successful. Two isolates of *Clostridium thiosulfatireducens*- and *Desulfovibrio psychrotolerans*-related strains were isolated from enrichment cultures. Bacterial biomass, extracellular polysaccharides and a mineral matrix was also isolated from aggregates of sulfate reducers. The Nirano mud volcano fluids contain culturable bacteria related to organisms typical for other sediments of saline systems and/or oil reservoirs (Sebastian, 2015).

Mud Volcanoes of Tang and Pirgal, Iran:

Analysis and evaluation of bio-emulsifier and bio-demulsifier activities of Capnophilic bacteria (microorganisms that thrive in the presence of high concentrations of carbon dioxide) isolated from volcanic mud was done. Organisms identified were various species of *Bacillus viz. B. megatarium, B. firmus, B. brevis, B. laterosporus, B. cereus), Enterobacter, Pseudomonas, Enterobacteriaceae* (Yasaman, 2017).

Submerged mud volcanoes and hydrothermal vents:

Digenetic processes occurring in deep sea continental margins result from three main microbial processes: sulphate reduction, methane generation and methane oxidation. Microbially mediated anaerobic methane oxidation is capable of consuming sulphate and requires a supply of methane. Microorganisms, however, are capable of providing these processes (Borowski, 1996).

Haakon Mosby Mud Volcano (HMMV):

HMMV is an unusual example of cold, methane venting seep because of its Arctic location, lack of association with salt tectonics or plate subduction and development within glacial marine sediment (characterized by high rate of accumulation). Microbiological studies using radioisotope showed that contemporary process of anaerobic methane oxidation occurs in sediment beginning at a depth of 80 cm and below (Pimenov, 1999). At active volcano centre (diameter of 500 meters), the main methane consuming process was bacterial aerobic oxidation. Aerobic methanotrophs belonging to *Methylobacter* and *Methylophaga* accounted for 56 % of total cells. In sediments below *Beggiatoa* mats encircling the center of HMMV, methanotrophic archaea of ANME-3 (aggregates associated with SRB of *Desulfobulbus* branch) clades dominated zone of anaerobic methane oxidation. ANME-3/DBB aggregates accounted for 94% of total biomass. Outer rim of mud volcano was colonized by tubeworms *Siboglinidae*. Here both aerobic and anaerobic methane oxidizers were detected (Losekann, 2007). Thus, high methane availability and various fluid flow regimes provide distinct niches for aerobic and anaerobic methanotrophs (Niemann, 2006).

Mud volcanoes in Amsterdam, Eastern Mediterranean sea

The Amsterdam mud volcano in the Anaximander Mountains (south of Turkey), is characterized by intense active methane seepage, produced partly by methanogens. ^{14}C-radiotracer measurements have showed that substrates like methylamines/methanol; H_2/CO_2 and acetate were used for methanogenesis. Methylotrophic methanogenesis was measured using archaeal 16S PCR-DGGE and *mcrA* gene libraries, and M*ethanosarcinales* affiliated sequences were detected.

Enrichments of methanogens showed the presence of *Methanococcoides* (Cassandre, 2012).

Hydrothermal mud vent underneath deep sea anoxic brine Lake Urania:

The deep sea hydrothermal mud vent of Urania can be considered to be most hostile environment for microbial growth owing to its depth (>3.3 meters beneath sea surface) and hypersalinity (5 to 10 times more salinity than seawater) along with deficiency of oxygen (due to lower penetration and temperature nearly equal to 45°C). Great genetic diversity was observed based on rRNA gene clone library analysis. The population was mostly dominated by members of yet uncultured organisms. Among the cultured representatives epsilon-*Proteobacteria* dominated followed by 18% population of delta-*Proteobacteria* (suggesting sulfate reduction as dominant process). Representatives of alpha-*Proteobacteria*, beta-*Proteobacteria*, *Actinobacteria*, *Bacteriods*, *Deinococcus-Thermus* group were also detected.

Lower abundance of archaea was also noted of which dominating members belonged to Euryarchaeotes. MSBL-1 candidate (Mediterranean Sea Brine Lake Candidate division 1 of Euryarchaeotes) found to be 96%. DHVE-1 (Deep Sea Hydrothermal Vent group 1 of Euryarchaeotes) and ANME-1 were found to be as single colony representatives. Possibility of presence of novel microbes also exists. Diverse metabolic processes like aerobic / anaerobic heterotrophy, sulphide and methane dependent chemotrophy along with anaerobic oxidation of methane, sulphate and metal reduction were found to be active (Yakimov, 2007).

Hydrothermal mud vent underneath deep sea Mid-Atlantic Ridge (MAR):

Molecular investigation of microbial flora of an active black smoker at the Mid-Atlantic Ridge (MAR), in the rainbow field at 2275 meters depth was carried out. Microbial diversity detected using DGGE targeting the 16S rRNA gene showed presence of *Pyrococcus*, *Marinitoga*, *Bacillus*, *Thermococcus*, gamma-*Proteobacteria* and epsilon-*Proteobacteria*. The anaerobic bioreactor operating at temperature 90°C, pH 6.5 and sulphur as terminal electron acceptor was used. Of the detected diversity only *Thermococcus* could be cultivated (Postec, 2005).

Hydrothermal mud vents off Panarea Island, Italy

Total bacterial and archaeal population abundance at two shallow hydrothermal vents off Panarea island, Eolian Islands in Italy was found to be 10^5 cells/ml. Presence of sulfur oxidizing bacteria was detected by PCR-DGGE analysis and cultivation dependent techniques. Phototrophs and chemolithotrophs were also detected. *Bacillus* and *Geobacillus* dominated the community. Members of epsilon-*Proteobacteria* like *Sulfurimonas denitrificans*, gamma-*Proteobacteria* like *Pseudomonas* and alpha-*Proteobacteria* like *Caulobacter* were detected. Members belonging to unculturables were found to be present in larger numbers (Maugeri 2009).

Hydrothermal mud vents at Castro Seamount, Azores, Portugal

Bacterial diversity from shallow vents of D. Joao de Castro Seamount (DJCS) was studied using 16S rRNA gene analysis, FAME and RFLP. Presence of groups of bacteria like *Bacillus*, *Staphylococcus*, *Micrococcus*, *Halomonas*, *Pseudoalteromonas*, *Alcaligenes* and *Brevibacterium* was noted. Biomolecules produced by isolates showed optimum activity at higher temperatures nearly 70°C. Adaptive mechanisms to detoxify metals by H_2S production, catalase and oxidase production for decomposition of H_2O_2 and O_2 and capacity to survive in unfavorable conditions by sporulation was observed (Mohandass, 2012).

Hydrothermal mud vents at Loihi Seamount, Hawaii

The SSU rRNA genes were targeted for investigating the bacterial diversity associated with microbial mats in Pele's vents. Cluster of phylogenetically related OTUs related to *Thiovulum* species within the epsilon sub class of *Proteobacteria* accounted for 60.5%. A second cluster from gamma subclass of *Proteobacteria* affiliated to *Xanthomonas* sp. accounted for 27.1%. *Alteromonas* group and *Thiothrix* group accounted for 2.1% with endosymbiotic bacteria from *Bathymodiolus thermophilus* and *Calyptogena magnifica*. Members of *Myxobacterium* group were also detected (Moyer, 1995).

Subseafloor alkaline serpentine mud volcano of Mariana Forearc

In the ocean drilling programme at the serpentine mud volcano, south Chamorro seamount in Mariana Forearc, a novel alkalophilic, mesophilic bacteria was isolated. Growth temperature range of the isolates was 10°C to 50°C, pH 6.5 to 11.4, NaCl concentration of 0 to 21 % was noted. Facultative anaerobic growth, utilizing various complex substrates such as, hydrocarbons, carbohydrates, amino acids and organic acids was observed. The G+C contents was 57.5 mol% and phylogenetically the isolate belonging to *Marinobacter* genus and was named as *Marinobacter alkaliphilus* (Takai, 2005).

Submarine Volcano at Kolumbo, Hellenic Volcanic Arc, Greece

Illumina sequencing of bacterial and archaeal communities on active and inactive sulfide chimneys collected from the Kolumbo hydrothermal field was performed. A total of 56 bacterial and 3 archaeal phyla, 133 bacterial and 16 archaeal classes were detected. Thermophilic members of *Epsilonproteobacteria*, *Aquificae* and *Deltaproteobacteria* were dominant in active chimney communities. Inactive chimney communities were dominated by an OTU closely related to the archaeon *Nitrosopumilus* sp., and by members of *Gammaproteobacteria*, *Deltaproteobacteria*, *Planctomycetes* and *Bacteroidetes*. Overall, the inactive sulfide chimneys showed highly diverse and uniform microbial communities, in contrast to the active chimney communities (Christos A. Christakis, 2017).

Bacterial diversity of mud volcanoes around the globe

No	Mud volcano	Reported diversity	Reference
1	Kolumbo submarine volcano,	*Nitrosopumilus sp.*, *Gammaproteobacteria*, *Deltaproteobacteria*,	Christos A. Christakis, 2017

	South Aegean Sea, Greece	*Planctomycetes* and *Bacteroidetes.*	
2	Tang and Pirgal mud volcano, Iran	*Bacillus (megatarium, firmus, brevis, laterosporus,cereus), Enterobacter, Pseudomonas, Enterobacteriaceae.*	Yasaman, 2017
3	Dushanzi and Baiyanggou in Junggar Basin, Xinjiang, China	*Halobacteria, Methanomicrobia, Alcanivorax sp..*	Zhiyong ,2016
4	Salse di Nirano, Northern Apennines, Italy	*Clostridium, Desulfovibrio.*	Sebastian Kokoschka, 2015
5	Xinjiang , China	*Actinobacteria, Crenarchaeotes Deltaproteobacteria, Euryarchaeotes Fusobacteria, Halobacteriales Methanosarcinales.*	Yang,2012
6	Baratang Island, Andaman, India	*Lutibaculum baratangense.*	P. Anil Kumar, 2012
7	Mud volcanoes in Amsterdam, Eastern Mediterranean sea, Europe	*Methanosarcinales, Methanococcoides.*	Cassandre,2012
8	Hydrothermal mud vents at Castro Seamount , Portugal	*Alcaligenes, Bacillus, Brevibacterium Halomonas, Micrococcus, Pseudoalteromonas, Staphylococcus.*	Mohandass, 2012
9	Hydrothermal mud vents off Panaria islands , Italy	*Alphaproteobacteria, Bacillus, Epsilonproteobacteria, Gammaproteobacteria, Geobacillus.*	Maugeri , 2009
10	Haakon Mosby mud volcano, Barents sea, Norway and Russia (Basin countries)	Aerobic and Anaerobic methane oxidizers, *Beggiatoa,, Desulphobulbus, Methylophaga.*	Losekann, 2007
11	Anoxic brine mud vent, Urania	*ANME* 1, *Alphaproteobacteria Actinobacteria, Bacteriods , DHVE 1, MSBL1, Deinococcus-Thermus Deltaproteobacteria.*	Yakimov, 2007
12	Paclele Mici Mud Volcano, Romania	*Bacteroides-Cytophaga group, Deltaproteobacteria, Gammaproteobacteria, Methanosarcinales.*	Karine ,2006
13	Hydrothermal mud vent at Mid Atlantic ridge, Iceland	*Bacillus, Epsilonproteobacteria, Gammaproteobacteria, Marinitoga, Pyrococcus, Thermococcus.*	Postec, 2005
14	Alkaline serpentine MV of Mariana Forearc	*Marinobacter.*	Takai, 2005
15	San Baigio – Belpasso, Italy	*Actinobacteria, Flexibacter-Cytophaga-Bacteroides, Gammaproteobacteria.*	Yakimov, 2002
16	Hydrothermal mud vents at Loihi Seamount , Hawaii	*Alteromonas, Bathymodiolus, Calyptogena, Epsilonproteobacteria, Gammaproteobacteria, Myxobacteria, Thiovulvum.*	Moyer, 1995

DHVE: Deep Sea Hydrothermal Vent group 1; MSBL: Mediterranean Sea brine Lake Candidate division 1.

III. Conclusion

Over the last decade or so the speed of detection of microbes has increased drastically, as the focus has shifted from traditional cultivation based approach to cultivation independent approach. The advent of next generation sequencing technology has further revolutionalised the biodiversity studies. Unique ecosystems like mud volcanoes which were difficult to study previously have now been studied to a greater extent. New groups of bacteria and archaea are being detected and tried to cultivate. Coexistence of obligate anaerobes like methanogens with microaerophilic microbes like *Pseudomonads*, autotrophic organisms like *Thiobacillus* with heterotrophs like *Bacillus* definitely makes the ecosystem a unique location. This coexistence in nature may exist in micro-environmental form. Further investigation on these lines is essential to completely understand the complex interactions amongst these microbes.

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