

## Microbial communities of the mud volcanoes: A review

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### Abstract

Mud volcanoes are formations created by geo excreted liquids and gases. They serve as an important natural laboratory for evaluation of geological, geophysical and biogeochemical features, in addition to biological and microbial community structures. They are associated with subduction zones/tectonic activity, petroleum deposits and organic belts. Microbial investigations have been carried out on the diversity and spatial distribution of prokaryotic communities along the vertical profiles of deep-sea mud volcanoes throughout the world. Microbial communities, so far, recognized from the mud volcanoes are *Eubacteria*, *Proteobacteria*, *Actinobacteria*, *Flexibacter* - *Cytophaga* - *Bacteroides*, *aerobic methanotrophic bacteria (Methylococcales)*, *anaerobic methanotrophic archaea*, etc. In India, actinobacterial diversity has been studied from the mud volcano samples of the Baratang Island of the Andamans which showed a good heterogeneity of culturable actinobacterial strains of *Streptomyces* and *Nocardiopsis*, the former being the dominant genus. There is a lot of scope to carry out further research on many aspects of the microbial communities of mud volcanoes for both environmental and economic benefits.

**Keywords:** Actinobacteria, frenulates, microbial community, Mud Volcano, nematodes.

### INTRODUCTION

Mud Volcanoes are morphologically very diverse, ranging from plano conical shapes to irregular, negative funnel shaped structures. They differ in size, structures and are spread upto 100 km<sup>2</sup> in area, to small landforms of 100 m<sup>2</sup>. Due to their surface morphologies and resemblance to the volcanic activities, they are said to have volcanic characteristics. Sometimes, they erupt violently, shooting mud and ash upto a height of a few kilometers. The gases emitted might burn producing enormous flames but, mud volcanoes appear to be gentle, growing and expanding by oozing out semi liquid mud volcano breccia during most of their lifetimes (Dimitrov, 2002). Mud volcano is a unique system in itself. Apart from the fact that they get excreted out from the earth's layers, they also contain many components like N<sub>2</sub>, H<sub>2</sub>S, NH<sub>3</sub>, CO<sub>2</sub> and CH<sub>4</sub> which help the organisms depending on such gases to thrive there.

Mixture of gases released are mainly of hydrocarbons (dominantly CH<sub>4</sub>) or CO<sub>2</sub>, which can be followed by small quantities of N<sub>2</sub>, H<sub>2</sub>S, NH<sub>3</sub> and noble gases. The significance of mud volcanoes as natural sources of atmospheric methane (CH<sub>4</sub>) is already recognized (Etiope *et al.*, 2002, 2004). The mud and fluids have a deep origin, but are sometimes stored in intermediate depth mud chambers. In eastern Romania, studies on mud volcanoes documented CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub> emissions with crustal origin (Etiope *et al.*, 2004). Previous studies also exist on the mud volcano of Banat by Cretan (2007) and Uruioc *et al.* (2007, 2011) in southwest Romania.

Microbial mats in marine cold seeps are also known to be associated with ascending sulphide and methane rich

fluids. Hence, they could be visible indicators of Anaerobic Oxidation of Methane (AOM) and methane cycling processes in underlying sediments (Pachiadaki *et al.*, 2011).

Study of mud volcanoes, cold seeps and the necessity of protecting and preserving them within the sustainable development has now become important to many scientists as it is not only a source of direct information through the fluid material carried by water, gases, mineral suspensions and enthralling microbial diversity but also the mud volcanoes represent a touristic interest area (Uruioc *et al.*, 2011).

### MICROBIAL DIVERSITY IN THE MUD VOLCANOES

In the mud volcano of San Biagio - Belpasso, Mt. Etna (Italy) Yakimov *et al.* (2002) investigated the microbial community; 140 bacterial clones were studied using RFLP analysis. Out of which, 28 sequences were *Proteobacteria*, two sequences were *Actinobacteria* and three sequences were from *Flexibacter-Cytophaga-Bacteroides* division. These findings suggest that the major constituents of the microbial communities of the saline volcanic muds are gamma subclass of *Proteobacteria*, which are phylogenetically affiliated to autotrophic methane oxidizers and heterotrophic hydrocarbon degraders.

Stadnitskaia *et al.* (2005) worked on NIOZ, Odessa, and Kazakov mud volcanoes (NE Black Sea) and their detailed biogeochemical investigations were done. Four methane related carbonate crusts covered with microbial mats and mud breccia were collected from these mud volcanoes and this is the first finding of methanotrophic microbial mats associated with the authigenic carbonates in the deep Black Sea. *Concentrations and*

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values of methane of specific archaeal and bacterial lipid were measured using  $\delta^{13}\text{C}$  methods and their 16S rRNA gene sequences were also determined;  $\delta^{13}\text{C}$  results of the microbial lipids showed that methane is the main carbon source for the microorganisms for carbonate formation. Utilisation of methane through AOM process was noticed in the upper sediments which probably induced the carbonate precipitation below the seafloor. Results showed that there is a crucial role of AOM process in the formation of authigenic carbonates in the methane seep environments.

Alain *et al.* (2006) carried out some microbiological studies in the methane and hydrocarbon discharging Mici mud volcano of the Carpathian Mountains, Romania Paclele. In this study, PCR analysis showed that bacteria were more abundant than archaea. In their study, phylogenetic analysis of 16S rDNA clones showed the following:

- Bacterial and archaeal lineages were generally associated with methane cycle (*methanogens*, *aerobic* and *anaerobic methanotrophs*), and sulfur cycle (sulfate reducers) and the groups were linked to the anaerobic degradation of alkanes or aromatic hydrocarbons
- Presence of sulfate reducers, *methanogens* and *methanotrophs* in this habitat was confirmed by concurrent surveys of lipid biomarkers and their isotopic signatures

Martinez *et al.* (2006) studied an active seafloor mud volcano site in the Gulf of Mexico for the changes in the microbial community vertically and also identified metabolically active microbial populations from three different sediment depths (0 - 2, 6 - 8 and 10 - 12 cm); 12 different 16S clone libraries, representing three sediment depths were constructed and 154 rDNA (DNA derived), 142 crDNA (RNA derived) Bacterial clones, 134 rDNA and 146 crDNA Archaeal clones were obtained. When 576 clones were analysed, relative to the sediment depths, distinct differences were revealed in the composition and patterns of metabolically active microbial phylotypes.

Niemann *et al.* (2006) studied the novel microbial communities of the Haakon Mosby mud volcano and their role in methane sink. Three key communities were found: aerobic methanotrophic bacteria (*Methyloccoccales*), anaerobic methanotrophic archaea (ANME - 2) booming below the sibloglinid tubeworms and a novel clade of Archaea (ANME - 3) associated with the bacterial mats.

Omoregie *et al.* (2008) determined the biogeochemistry and community composition of novel, brightly coloured, white and orange, iron and sulphur precipitating microbial mats of a brine seep at the Chefren mud volcano (Nile Deep Sea Fan, Eastern Mediterranean). These mats were intermixed with one another and their

basic sediment biogeochemistries differed significantly. Microscopical studies revealed that the white mats were granules composed of elemental S filaments, similar to those produced by the sulphide oxidizing *α-Proteobacterium candidatus*, *Arcobacter sulfidicus*. Fluorescence *in situ* hybridization indicated that the microorganisms targeted by a *Ca. Arcobacter sulfidicus* specific oligonucleotide probe constituted up to 24% of the total cells within these mats; 16S rRNA gene sequences were used to identify several organisms which were closely related to *Ca. Arcobacter sulfidicus*. Whereas, the orange mat which consisted mostly of bright orange flakes was similar to the neutrophilic Fe (II) oxidizing *β-Proteobacterium leptothrix ochracea*. None of the 16S rRNA gene sequences obtained from these samples were closely related to sequences of known neutrophilic aerobic Fe (II) oxidizing bacteria. Sediments of both the types of mats showed relatively high sulfate reduction rates which were partially fueled by the anaerobic oxidation of methane. The study showed that both the mats and the sediments below had diverse microbial communities and mineral precipitates, which is most likely due to the differences in fluid flow patterns.

Thirteen morphologically distinct actinobacterial strains were isolated from the mud volcano of the Baratang island, the Andamans by Aarthi (2007) and among them, nine strains belonged to *Streptomyces* and four belonged to *Nocardioopsis*. Out of which, two *Streptomyces* strains were tested positive for bioethanol production. Similarly, Ilayaraja *et al.* (2014) isolated 6 strains of thermophilic *actinobacteria* from the same mud volcano of the Baratang island, the Andamans. These strains were tested for carbohydrate degrading enzyme production and 2 strains showed cellulase and xylanase activity. RDP database analysis of the uncultivable microbial diversity revealed 64 genera. A few thermophilic *actinobacteria* from the mud volcanic sediments of the Baratang Island showed cytotoxic activity (Aarthi, 2009). Out of the 19 strains, three strains belonged to *Streptomyces* and possessed cytotoxicity against Hep2 cancer cell line.

A Gram negative, oval to rod shaped, motile bacterium, strain AMV1(T), was isolated from a mud volcano soil sample of Baratang Island, Andamans, India by Anil Kumar *et al.* (2012); Phylogenetic analysis showed that the strain was clustered with *Tepidamorphus gemmatus* and species of the genera of *Amorphus*, *Rhodobium* and *Afifella*; 16S rRNA gene sequence analysis showed that the strain was related most closely to the strains of *Tepidamorphus gemmatus* (95.0%), *Bauldia* (94.5%), *Afifella pfennigii* (94.4%) and *Amorphus coralli* (94.0%) of the order Rhizobiales (class: *α-Proteobacteria*). Both phenotypic and phylogenetic characteristics suggested that the strain was a representative of a novel species

of a new genus and hence the name *Lutibaculum baratangense* gen. nov., sp. nov. was proposed.

Pachiadaki *et al.* (2010) studied the prokaryotic community structure and its diversity using 16S rRNA gene sequencing from different sediment depths of every 5 cm till 30 cm in the Kazan Mud Volcano, East Mediterranean Sea. Totally, 242 archaeal and 374 bacterial clones were analysed, and were assigned to 38 and 205 unique phylotypes respectively. Most of the archaeal phylotypes were related to anaerobic methanotrophs. *Proteobacteria* was found to be the most abundant and diverse bacterial group, with  $\gamma$ -*Proteobacteria* dominating in most of the sediment layers and they were related to phylotypes involved in methane cycling.  $\delta$ -*proteobacteria* included several of the sulphate reducers related to anaerobic oxidation of methane. The rest of the bacterial phylotypes belonged to 15 known phyla and three affiliated groups, with representatives from similar habitats.

Pachiadaki *et al.* (2011) investigated the diversity and spatial distribution of prokaryotic communities along a sediment vertical profile of a deep sea mud volcano, Amsterdam mud volcano, East Mediterranean Sea, based on the 16S rRNA gene diversity. A total of 339 and 526 sequences were retrieved, corresponding to 25 and 213 unique phylotypes of *Archaea* and *Bacteria*, respectively, at all the depths. *Archaea* was dominant with anaerobic methanotrophs *ANME-1*, *ANME-2* and *ANME-3* groups and were related to phylotypes involved in anaerobic oxidation of methane from similar habitats. The much more complex bacterial community consisted of 20 phylogenetic groups at the phylum/candidate division level. *Proteobacteria*, in particular  $\delta$ -*Proteobacteria* formed the dominant group. In most sediment layers, the dominant phylotypes of both the *Archaea* and *Bacteria* communities were found in neighbouring layers, suggesting the existence of overlap in the species richness of microbes.

Prokaryotic diversity of an active mud volcano of the Ust'-Kamennoy city of Xinjiang, China was investigated by Yang *et al.* (2012). Bacterial and archaeal clone libraries of 16S rRNA gene were constructed; 11 and 7 distinct phylotypes were found from a total of 100 bacterial and archaeal analysed clones. The bacterial phylotypes were classified into three Phyla (*Proteobacteria*, *Actinobacteria* and *Fusobacteria*). Of these, *Proteobacteria* was the most abundant bacterial group, with  $\alpha$ -*Proteobacteria* dominating the sediment community, and these were affiliated to the Order *Desulfuromonadales*. The archaeal phylotypes were all closely related to uncultivated species, and the majority of the members were related to the Orders, *Methanosarcinales* and *Halobacteriales* originating from methane hydrate bearing or alkaline sediments. The rest of the archaeal phylotypes belonged to the Phylum *Crenarchaeota*, with representatives from

similar habitats. These findings suggest that a large number of novel microbial groups and potential methanogenesis might exist in this unique ecosystem.

Not only microbes but also other interesting organisms have been recorded from the mud volcanoes. Hilario *et al.* (2010) investigated the *frenulates* and found that they are the most poorly known members of the family *Siboglinidae* (*Polychaeta: Canalipalpata*). These thread like worms occur worldwide, reducing the marine sediments and are usually overlooked in benthic samples or poorly preserved for adequate taxonomic evaluations. There were reports from 13 mud volcanoes in the Gulf of Cadiz (350 - 3902 m depth), off Southern Iberia with the notable diversity of *frenulates*. Fifteen evolutionary lineages inhabiting the Gulf of Cadiz, were distinguished by Mitochondrial cytochrome - *c* - oxidase subunit 1 (*COI*) sequences out of which only four of the lineages could be assigned to currently recognized Atlantic species and the remaining 11 may be new to science. It is hypothesised that the diversity of *frenulates* in this small geographical region is unique because of the environmental heterogeneity associated with the bathymetric and geochemical settings of these mud volcanoes.

## Conclusion

Studies on mud volcanoes and the associated organisms have been increasing in the recent past. It is interesting to note that the novel microbial communities and other organisms can survive in such extreme conditions. Vast diversity of the microorganisms found there can even have keys to many unknown facts and there is a lot more to understand about these organisms, their ecosystem functions and usefulness to mankind. Most of the works done so far are on the microorganisms, their diversity and composition but there are only a few works on the ecological aspects. So, there is a lot of scope to carry out further research for the better understanding of the microbial communities of the mud volcanoes and their uses in various fields.

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