COMPARATIVE STUDY OF BACTERIAL POPULATION OF AN OLIGOTROPHIC ENVIRONMENT OF GARHWAL HIMALAYA

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ABSTRACT

Caves are nutrient-poor ecosystem due to lack of photosynthesis in major parts as either they have low light intensity or total darkness except at the entrance. However, the caves still harbor a rich microbial diversity. They overcome nutrient limitations by forming collective structures and having mutualistic relations with each other. These microorganisms acquire energy from minerals present in the caves. In the present study, stalactite samples collected from Bhadra (Gumki) cave near the Kurur village (District Chamoli, Uttarakhand, India) were examined for microbial diversity at different nutrition levels. Three different media were used for recovery of bacterial flora of the cave. A rich bacterial diversity was observed with the dominance of *Bacillus* (30.76%) followed by *Paeni bacillus* (21.15), *Staphylococcus* (17.30), *Lysini bacillus* (11.53), *Vigri bacillus* (5.76), *Salimicrobium* (3.84%), *Anaero bacillius* (3.84%), *Clostridium* (3.84%) and *Macrococus* (1.92%). These bacterial isolates can serve as promising candidates for biomineralization.

Keywords: Garhwal Himalaya, Cave, Microbial diversity, Biomineralization, Stalactites.

INTRODUCTION

Caves represent an extreme environment in which the resident organisms experience steady or fluctuating exposure of one or more environmental factors such as salinity, osmolarity, desiccation, UV radiation, barometric pressure, pH, temperature, nutrient limitation and trophic dependence (Boston *et al*., 2001; Seufferheld *et al*., 2008). The nutrient availability in caves to support heterotrophic microbial growth is very poor which approximately a thousand-fold is lower than the most starved terrestrial environment. The absence of light in majority parts of cave checks the growth of phototropic microorganisms and plants (Gurtner *et al*., 2004). The autotrophic bacteria of caves are the primary producers which support the growth of chemotrophic bacteria (Canaveras *et al*., 2001). The resident microorganisms of oligotrophic environments have evolved several scavenging mechanisms for pulling scarce nutrients into the cell for survival (Koch 1997; Pan *et al.,* 2007).

The microbial community of cave acquires energy from oxidation and reduction of metal, transformation of aromatic compound, fixation of gases and available nutrients from cave atmosphere (Barton *et al.,* 2007). These chemoautotrophic bacteria need only simple inorganic compounds for survival. Pemberton *et al*., 2005 studied the effects of nutritional limitation inside the cave on culturable diversity by using various media ranging from high concentration to low concentration of nutrients. A greater diversity was observed on nutrient deficient medium. This report suggests that cave ecosystem serves a nutrient deficient environment and microorganisms tends to grow in this condition.

The mineral precipitating ability of microbial community of cave plays an important role in the structuring of speleothems (cave structures). The calcium carbonate precipitation has been widely reported by bacteria from different caves *viz*., *Rhodococcus* sp. fromGrotta dei caervi (Groth *et al*., 2001), *Bacillus thuringenesis* and *B. pumilis* from Sahastradhara cave (Baskar *et al*., 2006), *Bacillus* sp. from cave of central China (Wang *et al*., 2010), *Arthrobactor* and *Rhodococcus* sp. from Pristine krastic Herrenberg cave (Rusznyak *et al*., 2012), *Lysini bacillus* sp. and *Bacillus* sp. from caves of Meghalaya (Banerjee *et al*., 2014) and *Bacillus subtilis* from Rani cave (Chalia *et al*., 2016).

In Garhwal Himalayan region there are many caves with virgin microbial diversity. It is thus important to explore these caves as anthropogenic activities may adversely affect these valuable bioresources. Thus this investigation is an attempt to study microbial diversity of stalactite and to determine whether the nutrition limitations in cave ecosystem fluctuate the culturable diversity.

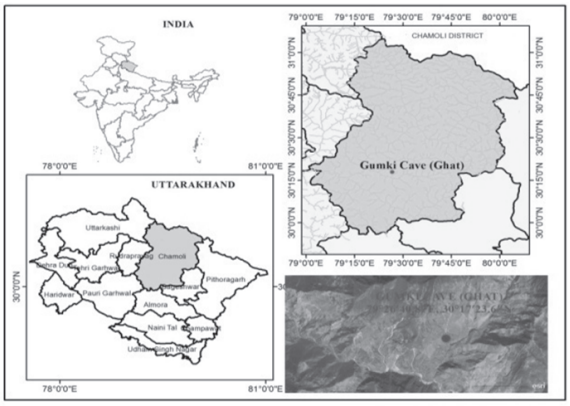

Fig. 1. Location of sampling sit

METHODOLOGY

The Gumki Cave (30°28' N, 79°44'E) is located near Kurur village (District Chamoli) Uttarakhand India (Fig. 1). The length of cave is still unknown. According to local folks the cave is more than 2 km long. During monsoon season, stream of water flows from the top of cave as well as from the inside of cave. Climate of this region varies from subtropical monsoon to monsoon upland type. Vegetation above the cave is dominated by oak (*Quercus semecarpifolia*), buransh (*Rhododendron arboreum*), melu (*Pyrus pashia*), angyar (*Fraxinus micrantha*), sanayu (*Vitex negundo*), amesh (*Lonicera quinquelocularis*) and small shrubs. This cave has not yet been explored from microbiological, archeological and geological viewpoint due to its remote location. The cave doesn't have much human visitation and therefore it is anticipated that it still preserves the indigenous microbial population. Stalactite samples were collected aseptically in autoclaved bags at the time of monsoon (July). The samples were air dried for 2 days and then crushed into fine powder for further processing. 1 gm of crushed sample of stalactite was suspended in 9 ml of sterile phosphate buffered saline and was diluted ten-fold. Three different media were used *i.e.* Luria Bertani agar media (LB) g/L (Casein enzymic hydrolysate– 10g, Yeast extract 5g, NaCl 10g and Agar- 15g), Angle's media (AM) g/L (Glucose- 10 g, KH_2PO_4- 1.5g, K_2HPO_4– 2.0g, $MgSO_4$, $7H_2O$- 0.05g, $NaNO_3$- 0.5 g, $FeSO_4.7H_2O$ – 0.01g, Yeast extract- 0.5 g, NaCl – 2g, $CaCO_3$- 0.02g and Agar – 15g) and Distilled Water (DW) media. Luria Bertani agar medium was nutrient rich medium while Angle's medium was minimal medium and Distilled Water medium was nutrient poor medium. 0.1 ml of diluted stalactite sample was plated onto all three medium by spread plating. The experiment was done in triplicates. After incubation at 25°C, the isolated bacterial colonies were purified and preserved in slants and glycerol stocks. The isolates were identified on the basis of various biochemical tests *viz.*, Indole production, Methyl Red-Voges Proskauer Test, Citrate Utilization Test, Triple sugar iron agar test, Nitrate reduction test, Oxidase, Catalase, Urease production and Sugar utilization tests (Cappuccino *et al.*, 2007). ABIS online software was used for identification of bacterial isolates (Costin *et al.*, 2015).

RESULTS AND DISCUSSION

The incubation time for recovery of bacterial isolates in all three media was observed to be different. The colonies appeared in lesser time on nutrient rich medium than on nutrient deficient media (Distilled Water agar medium). In LB medium, the colonies appeared in 24-48h, whereas in AM and DW media, the colonies appeared in 7-14days. Similar observations have also been reported by Pemberton *et al*., 2005. A rich microbial diversity was observed on the stalactite sample. The isolates were observed to be belonging to a number of genera viz., *Bacillus, Paeni bacillus, Staphylococcus, Lysini bacillus, Vigri bacillus, Salimicrobium, Anaero bacillius, Clostridium* and *Macrococus* (Fig. 2). These isolates were identified using ABIS bacterial identification software which has been used by a number of researchers for identification viz.,

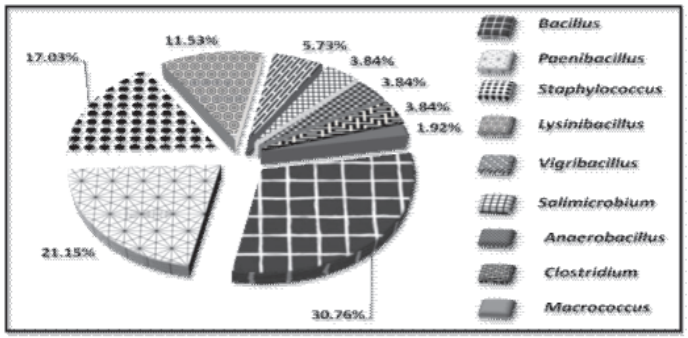

Fig. 2. Distribution of isolated bacterial genera in stalactite sample

Polyhdroxyalkanoates producing bacteria from pulp, paper, and cardboard industry (Bhuwal *et al*., 2013) caffeine degrading isolates from the soil of coffee factory (Thangaraj *et al*., 2013) lactic acid bacteria (Dumitru *et al*., 2017), plant growth promoting rhizobacteria from coastal region (Pahari *et al*., 2017) halotolerant soil bacteria from coastal Patenga area (Rahman *et al*., 2017) and lipase producing bacteria from Windrow compost (Shaini *et al.*, 2016). *Bacillus* was observed to be the dominant genera followed by *Paenibacillus* and *Staphylococcus*. The predominance of *Bacillus* has been reported by a number of workers. *Bacillus thuringiensis* and *Bacillus pumilus* were reported from stalactite of Sahastradhara cave by Baskar *et al.,* 2006. Baskar *et al.,* 2009 reported *B. licheniformis* and *B. cereus* from stalactite samples. Laiz *et al*., 2000 reported species of *Bacillus* and *Paeni bacillus* from stalactites samples of *Grotta dei cervi*, Italy (Banerjee *et al.,* 2014). The calcifying bacteria belong to genera *Bacillus* and *Lysini bacillus* in caves of Meghalaya. A total of 52 bacterial isolates were recovered from stalactite samples. Out of these isolates 14 were recovered from Luria Bertani agar medium, 17 from Angle's media and 21 from Distilled Water agar medium. It can thus be apparently concluded that the diversity of microorganisms increased with decrease in the level of carbon and energy source. These results demonstrated that the organisms in stalactite were oligotrophic. A variation in bacterial genera was also observed in all three medium (Fig. 3). *Bacillus, Paeni bacillus* and *Staphylococcus* were present in all three media whereas *Vigri bacillus* was present in LB and AM media and *Lysini bacillus* was present in AM and DW media only. The ability of *Bacillus lichen informs* to survive in starved conditions was studied by Voigt *et al.,* 2006.

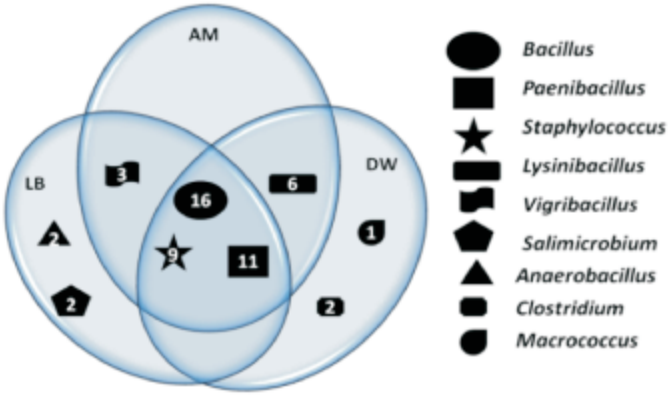

Fig. 3 Distribution of recovered bacterial genera in different levels of nutrient

They demonstrated that the bacteria secretes some specific proteins due to limitation of energy sources which might be responsible for adaptation in nutrition deficient conditions. *Anaerobacillus* and *Salimicrobium* were present only in LB medium while *Clostridium* and *Macrococcus* were present only in DW medium (Zehr *et al*., 1998). *Clostridium* in association with planktonic crustacean from oligotrophic oceanic water. The appearance of some bacterial genera on all medium while some on specific medium can be attributed to the fact that these bacteria have evolved mechanisms to adapt in nutrient rich or deficient conditions and display higher ecological amplitude.

CONCLUSIONS

Oligotrophic microorganisms have a number of biotechnological, ecological, medical and environmental applications which make their conservation essential. The present study gave an insight into the oligotrophic bacterial community inhabiting the cave ecosystem. The nutrient deficient medium contained highest number of isolates as compared to nutrient rich medium and mineral medium. The present study demonstrated that some bacterial population appeared on all three medium while some appeared on specific medium. This is a preliminary study which gives insights into the bacterial diversity of this unexplored cave. The isolates recovered will be screened for their functional potential.

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