Published by: National Cave Research and Protection Organization, India

Ambient Science, 2014: Vol. 01(2); 09-16
DOI:10.21276/ambi.2014.01.2.rv01

**ambient
SCIENCE**
Vol. 1(2):09-16
Year 2014

REVIEW ARTICLE

A Summary of Some Microbes Identified from Different Indian Caves and Their Possible Role in Mineral Formations

Sushmitha Baskar¹, Ramanathan Baskar^{2*}

¹Environmental Studies, School of Agriculture, Indira Gandhi National Open University (IGNOU), Maidan Garhi, New Delhi 110068, India

²Department of Environmental Science and Engineering, Guru Jambheshwar University of Science and Technology, Hisar 125001, Haryana, India

Key words: Geomicrobiology, Speleothems, Biominerals

Abstract

Microbe-mineral interactions in caves can lead to secondary mineral formations such as speleothems, moonmilk etc. In the present review, we provide a summary of geomicrobiological work done so far in some Indian caves. Microbially induced mineralization is documented in cave ecosystems and microbial metabolisms help in mineral formations either through direct/active or indirect/passive processes. Laboratory based experiments conducted on speleothems from Sahastradhara caves (Dehradun); Borra Cave (Andhra Pradesh); Krem Phyllut, Krem Mawsmi, Krem Mawmluh and Rongai Dobhakol (Meghalaya) have clearly demonstrated that bacterial strains isolated from these caves have the capability to precipitate minerals under controlled conditions.

Introduction

It is well established that the earth's subsurface contains a significant fraction of microbial life (Roussel *et al.*, 2008). Of these, the caves are prominent features of karst terrains that host a wide spectrum of interesting life forms: microorganisms (bacteria, fungi); cave flora (lichens, algae, mosses); cave fauna (mainly- snails, worms, spiders, leeches, insects, millipedes, centipedes, scorpions, fishes, salamanders and bats). Cave geomicrobiology is concerned with the diversity of microorganisms and their interactions with minerals, which can provide us clues on past/ongoing geomicrobiological interactions. Cave environments are usually resource limited and are extreme environments for life. Cave ecosystems also

*Corresponding Author: rbaskargjuhisar@yahoo.com

depend on allochthonous organic matter for metabolism (Poulson & Lavoie, 2000; Simon *et al.*, 2003; Engel *et al.*, 2003, 2004; Biswas, 2009; Biswas & Ramteke, 2008; Baskar *et al.*, 2009, 2012, 2011, 2014).

The first reports on microbial inclusions within rocks to speculate the possible role of microorganisms in carbonates was conducted five decades ago (Shoji & Folk, 1964). Thereafter, a number of cave geomicrobiological studies have been reported world over (Melim *et al.*, 2001; Boston *et al.*, 2001; Hammes & Verstraete, 2002; Frisia *et al.*, 2002; Engel *et al.*, 2003, 2004; Cacchio *et al.*, 2004; Barton *et al.*, 2004; Canaveras *et al.*, 2006; Meisinger *et al.*, 2007; Engel *et al.*, 2010, 2013; Baskar *et al.*, 2005, 2006, 2009a, 2012, 2014). Reviews on cave geomicrobiology have been published (Northup & Lavoie, 2001; Northup *et al.*, 1997; Barton & Northup, 2007; Barton 2006; Ehrlich 1998; Banfield *et al.*, 1998; Jones 2001; Newman & Banfield, 2002; Barton & Jurado, 2007, Whitman *et al.*, 1998; Baskar *et al.*, 2009b, c). Microorganisms by way of their metabolic interactions with rocks and minerals for their energy requirement play an important role in reshaping the mineral environment of caves and contribute significantly to various cave deposits, speleothems. Several lab based studies have clearly demonstrated that these bacterial species isolated from caves are capable of precipitating minerals under controlled conditions (Danielli & Edington, 1983; Rivadeneyra *et al.*, 1993; Baskar *et al.*, 2006, 2009, 2011, 2014).

More than 1545 cave sites (Deshmukh, 1994) have been reported on the Indian sub-continent. Unfortunately, till date very little efforts have been made to understand these poor biospheres. Most of the works were restricted to understand the animal biodiversity (Biswas, 2010, Harries *et al.*, 2008). In this short communication, a summary of the geomicrobiological work done so far by our team has been discussed to understand cave mineral-microbe interactions in some of the Indian caves.

Sahastradhara caves; Uttarakhand:

The cave systems at Sahastradhara, are situated in the Dehradun Valley, formed within the Siwalik formations in Garhwal Himalaya, situated on the Krol carbonates. These caves are smaller in size (10 m long, 2 m wide), and are well known for their pH neutral springs believed to cure skin diseases. In the studied speleothems, calcite was the dominant mineral consisting of microcrystalline calcite attributable to mineralized bacterial cells. Microbial cell enumeration by DNA-specific DAPI staining (Amann *et al.*, 1995) revealed high numbers (9×10^5 cells, g sed⁻¹ dry wt; Table 1). Further, *in vitro* culture experiments with *Bacillus thuringiensis* and *Bacillus pumilis* (using 16S rRNA gene amplification and sequencing) strains isolated from the stalactites showed their capability to form crystalline CaCO₃.

Fig. 1; SEM of Sahastradhara moonmilk showing spiral, tubular filaments; calcite formed in close association with microbial filaments .

crystals (Baskar *et al.*, 2006). Further, moonmilk from Sahastradhara also showed bacterial involvement in their formation (Baskar *et al.*, 2014; Fig. 1). Bacterial species identified from moonmilk that initiated crystal precipitation were: *Bacillus pumilis*, *Bacillus cereus*, *Bacillus anthracis*, *Bacillus lentus*, *Bacillus circulans*, *Bacillus sphaericus*, Actinomycetes (Table 1).

Fig.- 2: Whitish green cave wall deposits, Borra Cave, Andhra Pradesh. Fig. 3: Iron biofilm on a neutrophilic spring, Borra cave, Andhra Pradesh. Fig. 4: SEM showing sheaths of *Leptothrix* sp. with Fe minerals deposited on the sheaths. Fig. 5: SEM showing EPS (exopolysaccharides), long fibres and sheaths of *Leptothrix* sp. with Fe minerals

Borra Caves; Andhra Pradesh:

They are situated in the Araku Valley, 95 km from Visakhapatnam (18°15'N; 83°3'E) on the east coast of India (Bas *et al.*, 2002). The cave is 2 km long, 12 m high and located 1,300 m above sea level. The cave hosts a variety of stalactites, stalagmites, cave wall formations (Fig. 2) and springs with thick biofilms (Baskar *et al.*, 2012; Fig. 3). SEM of speleothems revealed the presence of calcified prokaryotic-like cells, micro-rods, and needle calcite. The textures preserved in the speleothems indicate the presence of microbes and their possible role in speleogenesis. DNA sequencing analyses and electron microscopy of an Fe-rich organic mat associated with a neutrophilic spring confirmed high abundances of *Leptothrix*-like sheaths, poorly crystalline Fe-precipitates, a large number of hollow tubes and broken dissociated sheaths embedded in extracellular polymeric substances (EPS) (Fig. 4,5). The DNA sequencing analyses of the 16S rRNA gene diversity revealed bacteria assigned to eight different phyla: Proteobacteria (62%), Chloroflexi (8%), Bacteroidetes (7%),

Planctomycetes (1%), Actinobacteria (5%), Acidobacteria (6%), Nitrospira (1%) and Firmicutes (5%). The dominant class was the Betaproteobacteria that accounted for 28% of the sequences. Further, sequences affiliated with Leptothrix, Siderooxidans, Crenothrix, Comamonadaceae, Dechloromonas were also present (Baskar *et al.*, 2012). Our SEM images suggest that the samples consist of amorphous to poorly crystalline siliceous Fe oxyhydroxide (Fig. 4,5,6) with characteristics similar to ferrihydrite which are in accordance with observations by several previous studies (e.g., Banfield *et al.* 2000; Barton *et al.*, 2007). Our results allow us to infer a possible relationship between the microbial community diversity to the microbial sheaths, EPS, and the iron precipitates formed.

Fig. 6: EDX of the minerals deposited on the sheaths showing Fe peaks

Krem Mawsmi, Krem Phyllut, Krem Mawmluh; Meghalaya:

These caves are situated in the Khasi Hills, an uplifted Precambrian crystalline complex forming the northeastern extension of the Indian Peninsular Shield. Meghalaya has huge deposits of limestone and abundant rainfall, which is the main reason for the Karst cave formations. The important limestone deposits are in Cherrapunji located in the Mawmluh-Mawsmi Hills. Krem Mawsmi caves are located amidst a thickly forested zone and are 160 m long, 15 m high, width 410 m. Krem Phyllut has a large section of fossil passage, was relatively long with a total length of 1,003 m, width 4.5 m, height 15 m and had three entrances. Krem Mawmluh (Mawkhyrdop) is located near Sohra 58 km from Shillong, It has a “Bat Hall” which leads to a passageway full of mollusc fossils. This main passage continues to a width of 28 m and 25 m height at one place called the “Feet Up Double Doughnut Passage” leading to the big “Gold Fish Pond” (Kharpran Dally, 2006). The floor in the passageway above the Gold Fish Pond is full of sinkholes resembling Swiss cheese, and numerous moonmilk deposits occur in this part of the cave spread over 300 m. The cell enumeration in the three caves showed good numbers in different enrichment agars tested (Table 1). The bacteria that were capable of forming calcite *in vitro* include: *Bacillus licheniformis* *Bacillus cereus* *Bacillus pumilis* *Bacillus mycoides* *Micrococcus luteus*, *Streptomyces*, *Bacillus* spp (Baskar *et al.*, 2009a, 2011). These results are comparable to other moonmilk and speleothem culture studies (Mulec *et al.*, 2002; Curry *et al.*, 2009). The fossil filaments and textures observed in our study contain excess carbon, which indicate an organic composition which is in accordance with results obtained by Melim *et al.*, (2009).

Rongai Dobhakol Cave; Meghalaya:

It is in the Garo Hills which form a western part of the Meghalaya Plateau where the area, lying to the south of the Tura Ranges, represents an extension of the Assam shelf and is characterized by thick Cenozoic deposits (Srivastava *et al.*, 2008). Rongai Dobhakol is situated in a dense forest in the Balpakram National park region which is a karst area in Eocene Siju (Upper Sylhet) limestone and lies at a distance of 167 km along the road from

Tura. There is a plethora of speleothems and some splendid irregular shaped stalactites and stalagmites. The total population densities on nutrient agar was 4.2×10^5 (cells, g sed⁻¹ dry wt; Table-1) and most isolates were aerobic rod-shaped bacteria, of which 50% were Gram positive. Detailed experiments of pathways associated with microbial oxidation, hydrolysis, dissimilatory sulphate reduction that lead to mineral formation is presently being carried out with isolated strains *in vitro*.

Conclusion:

This short review summarizes the studies performed by our group on the Indian caves to understand microbe-mineral interactions. The studies have primarily focussed on geochemical, mineralogical, conventional and molecular microbiological approaches to understand the extent of microbial involvement in cave formations. In all of these caves, it has been clearly established that microbes through their metabolic functions for their energy requirement, reshape the mineral environment of caves. Minerals precipitate onto bacterial cell surfaces either through direct/enzymatic processes (biologically controlled mineralization) or indirect/passive processes (biologically induced mineralization) (Konhauser, 1998; Northup & Lavoie, 2001). Our *in vitro* studies establish that passive processes i.e. biochemical factors affected by bacterial metabolism influence the precipitation process and also the shape and morphology of the carbonate precipitates attached to the mineral surface. Further, metagenomic studies along with lab based microbe-mineral experiments will help to understand mineral-microbe interactions. Thus, cave environments, offer invaluable scientific information about biosignatures detected, which can be applied to search for subsurface life in other planets (Boston *et al.*, 2000).

Acknowledgements:

We thank ETH, Zurich, Switzerland; Centre for Geobiology, University of Bergen, Norway and Stockholm University, Sweden for generous laboratory facilities. RB thanks UGC for financial assistance (UGC-MRP).

References:

- Amann R.I., Ludwig W. & Schleifer K.H. (1995): Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59: 143-169.
- Banfield J.F., Welch S.A. & Edwards K.J. (1998): Microbes as geochemical agents. *The geochemical news*, 96: 11-17.
- Banfield J.F., Welch S.A., Zhang H., Ebert T.T. & Penn R.L. (2000): Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. *Science*, 289: 75-1754.
- Barton H.A. (2006): Introduction to cave microbiology: A review for the non-specialist. *J. Cave Karst Stud.*, 68, 43-54.
- Barton H.A. & Jurado V. (2007): What's up down there? Microbial diversity in caves. *Microbe*, 2: 132-138.
- Barton H.A. & Northup D.E. (2007): Geomicrobiology in cave environments: past, current and future perspectives: *J. Cave Karst Stud.*, 69(1): 163-178.
- Barton H.A., Taylor M.R., Kreate M.P., Springer A.C., Oehrlé S.A. & Bertog J.L. (2007): The impact of host rock geochemistry on bacterial community structure in oligotrophic cave environments. *Intl J. Speleol.* 36: 93-104.
- Barton H.A., Taylor M.R. & Pace N.R. (2004): Molecular phylogenetic analysis of a bacterial community in an oligotrophic cave environment: *Geomicrobiol. J.*, 21: 11-20.
- Bas M.J.L., Subbarao K.V. & Walsh J.N. (2002): Metacarbonatite or marble? the case of the carbonate, pyroxenite,

- calcite apatite rock complex at Borra, Eastern Ghats, India. *J. Asian Earth Sci.*, 20: 127-140.
- Baskar S., Baskar R.; Mauclaire L. & McKenzie J.A. (2005): Role of microbial community in stalctite formation, Sahastradhara caves, Dehradun, India. *Curr. Sci. India*, 88, 1305-1308
- Baskar S., Baskar R., Mauclaire L. & McKenzie J.A. (2006): Microbially induced calcite precipitation in culture experiments: possible origin for stalactites in Sahastradhara caves, Dehradun, India. *Curr. Sci. India*, 90, 58-64.
- Baskar S., Baskar R., Lee N., Kaushik A. & Theophilus P.K. (2008): Precipitation of iron in the microbial mats of the spring waters of Borra Caves, Vishakapatnam, India: Some geomicrobiological aspects. **Environmental Geology**, Springer Publication, Vol. 56, No.2 237-243.
- Baskar S., Baskar R., Lee N. & Theophilus P.K. (2009a): Speleothem formations of Mawsmi caves and Krem Phyllut caves, Meghalaya, India: Some evidences for biogenic activities. In: **Environmental Earth Science** (Springer Publication), Volume 57, Number 5, 1169-1186.
- Baskar S. & Baskar R. (2009b). Geobiology and Geomicrobiology: Importance and need for studies in the Indian context, *Curr. Sci. India*, 96(1): 200-201.
- Baskar S., Baskar R. & Barton H.A. (2009c): Cave Geomicrobiology as a thrust area of research in the Indian context. *Curr. Sci. India*, 97(5): 621-622.
- Baskar S., Baskar R. & Routh J. (2011): Biogenic Evidences of Moonmilk deposits in the Krem Mawmluh Cave, Meghalaya, India. *Geomicrobiol. J.*, 28(3): 252-265.
- Baskar S., Baskar R., Thorseth I.H., Øvreas L. & Pedersen R.B. (2012): Microbial iron precipitation associated with a neutrophilic spring of Borra caves, Vishakapatnam, India. *Astrobiology Journal*, 12(4): 327-346.
- Baskar S., Baskar R. & Routh J. (2014): Speleothems from Sahastradhara caves in Siwalik Himalaya, India: Possible biogenic inputs. *Geomicrobiol. J.*, 31(8): 664-681.
- Biswas J. (2009): The biodiversity of Krem Mawkhrydop of Meghalaya, India, on the verge of extinction. *Curr. Sci. India*, 96: 904-910.
- Biswas J. (2010): Kotumsar cave biodiversity: a review of cavernicoles and their troglomorphic traits. *Biodivers. Conserv.* 19(1): 279-285.
- Biswas J. & Ramteke A.K. (2008): Timed feeding synchronizes circadian rhythm in vertical swimming activity in cave loach, *Nemacheilus vezardi*. *Biol. Rhythm Res.*, 39 (5), 405-412.
- Boston P.J., Spilde M.N., Northup D.E., Melim L.A., Soroka D.S., Kleina L.G., Lavoie K.H., Hose L.D., Mallory L.M., Dahm C.N., Crossey L.J. & Schelble R.T. (2001): Cave biosignature suites: Microbes, minerals and Mars. *Astrobiology*, 1(1): 25-55.
- Boston P.J., Soroka D.S., Kleina L.G., Lavoie K.H., Spilde M.N., Northup D.E. & Hose L.D. (2000): A garden inside out: microbial mats in springs, wall muds, and ceiling formations of a sulfur-dominated cave, Cueva de Villa Luz, Tabasco, Mexico. *J. Cave Karst Stud.*, 62(3): 198-199.
- Cacchio P., Contento R., Ercole C., Cappuccio G., Martinez M.P. & Lepidi A. (2004): Involvement of microorganisms in the formation of carbonate speleothems in the Cervo Cave (L'Aquila - Italy). *Geomicrobiol. J.*, 21: 497-509.
- Canaveras J.C., Cuezva S., Sanchez-Moral S., Lario J., Laiz L., Gonzalez J.M. & Saiz-Jimenez C. (2006): On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften*, 93: 27-32.
- Curry M.D., Boston P.J., Spilde M.N., Baichtal J.F. & Campbell A.R. (2009): Cottonballs, a unique subaqueous moonmilk, and abundant subaerial moonmilk in Cataract Cave, Tongass National Forest, Alaska. *Inter J Speleol.* 38(2): 111-128.
- Danielli H.M.C. & Edington M.A. (1983): Bacterial calcification in limestone caves. *Geomicrobiol. J.*, 3: 116.
- Deshmukh M. (1994): Influence of geology on the localization of ancient caves. *J. Geol. Soc. India*, 44: 213-217.
- Ehrlich H.L. (1998): Geomicrobiology: its significance for geology *Earth-Science Reviews*, 45: 45-60.
- Engel S.E., Lee N., Porter M.L., Stern A.L., Bennett P.C. & Wagner M. (2003): Filamentous Epsilonproteobacteria dominate microbial mats from sulfidic cave springs. *Appl Environ Microbiol.*, 69: 5503.
- Engel A.S., Porter M.L., Stern L.A., Quinlan S. & Bennett P.C. (2004): Bacterial diversity and ecosystem function of

- filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic "Epsilonproteobacteria". *FEMS Microbiol Ecol.*, 51, 31-53
- Engel A.S., Meisinger D.B., Porter M.L., Payn R.A., Schmid M., Schleifer K.H. & Lee N.M. (2010): Linking Phylogenetic and Functional Diversity to Nutrient Spiralling in Microbial Mats from Lower Kane Cave (USA). *The ISME Journal*, 4: 98-110.
- Engel A.S., Paoletti M.G., Beggio M., Dorigo L., Pamio A., Gomero T., Furlan C., Brilli M., Bertoni R., Dreon A.L. & Squartini A. (2013): Comparative microbial community composition from secondary carbonate (moonmilk) deposits: implications for the Cansiliella servadeii cave hydropetric food web. *Int J Speleol.*, 42 (3): 181-92.
- Frisia S., Borsato A., Fairchild I.J., McDermott F. & Selmo E.M. (2002): Aragonite-calcite relationships in speleothems (Grotte De Clamouse, France): Environment, fabrics and carbonate geochemistry. *J. Sediment. Res.*, 72: 687-699.
- Hammes F. & Verstraete W. (2002): Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environment. Sci. Biotechnol.*, 1: 3-7.
- Harries D.B., Ware F.J., Fisher C.W., Biswas J. & Kharpran-Daly B.D. (2008): A review of the biospeleology of Meghalaya, India. *J. Cave Karst Stud.*, 70: 163-176.
- Jones B. (2001): Microbial activity in Caves - A Geological Perspective. *Geomicrobiol. J.*, 18: 345-357.
- Kharpran-Dally B.D. (2006): **The Caves of Meghalaya**. Published by the Directorate of information and public relations. Government of Meghalaya pp. 90.
- Konhauser K.O. (1998): Diversity of bacterial iron mineralization. *Earth Sci Rev.*, 43: 91-121.
- Meisinger D.B., Zimmermann J., Ludwig W., Schleifer K.H., Wanner G. Schmid M., Bennett P.C., Engel A.S. & Lee N.M. (2007): In situ detection of novel Acidobacteria in microbial mats from a chemolithoautotrophically-based cave system (Lower Kane Cave, WY, USA). *Environmental Microbiology*, 9(6) 1523-1534.
- Melim L.A.; Shinglman K.M., Boston P.J., Northup D.E., Spilde M.N. & Queen J.M. (2001): Evidence for microbial involvement in pool finger precipitations, Hidden cave, New Mexico. *Geomicrobiol. J.*, 18: 311-329.
- Melim L.A., Liescheidt R., Northup D.E., Spilde M.N., Boston P.J. & Queen J.M. (2009): A Biosignature Suite from Cave Pool Precipitates, Cottonwood Cave, New Mexico. *Astrobiology*, 9(9):907-917.
- Mulec J., Zalar P., Zupan Hajna N. & Rupnik M. (2002): Screening for Culturable Microorganisms from Cave Environments (Slovenia). *Acta Carsologica*, 31(2): 177-187.
- Newman D.K. & Banfield J.F. (2002): Geomicrobiology: molecular-scale interactions underpin biogeochemical systems. *Science*, 296: 1071-1077.
- Northup D.E. & Lavoie K.H. (2001): Geomicrobiology of caves: a review. *Geomicrobiol. J.*, 18: 199-222.
- Northup D.E., Reysenbach A.L. & Pace N.R. (1997): Microorganisms and speleothems. In: CA. Hill, P. Forti (eds) **Cave minerals of the world**. National Speleological Society, Huntsville, 261-266.
- Poulson T.L. & Lavoie K.H. (2000): The trophic basis of subsurface ecosystems. In: DC. Wilkens, DC. Culver, WF. Humphreys (eds) **Ecosystems of the world** 30. Elsevier, Amsterdam, 231-249
- Rivadeneira M.A., Delgado R., Delgado G., Del Moral A., Ferrer M.R. & Ramos-Cormenza A. (1993): Precipitation of carbonate by Bacillus sp. isolated from saline soils. *Geomicrobiol. J.*, 11: 175-184
- Roussel E.G., Cambon Bonavita M.A., Querellou J., Cragg B.A., Webster G., Prieur D. & Parkes R.J. (2008): Extending the sub-seafloor biosphere. *Science*, 320: 1046.
- Shoji R. & Folk R.L. (1964): Surface morphology of some limestone types as revealed by electron microscope: *Journal of Sedimentary Research*, 34, 144-155.
- Simon K.S., Benfield E.F. & Macko S.A. (2003): Food web structure and the role of epilithic biofilms in cave streams. *Ecology*, 84, 2395-2406.
- Srivastava D.K., Singh A.P., Tiwari R.P. & Jauhri A.K. (2008): Cassiduloids (Echinoidea) from the Siju Formation (late Lutetian-early Bartonian) of the South Garo Hills, Meghalaya, India. *Revue de Paléobiologie, Genève*, 27 (2): 511-523.

Whitman W.B., Coleman D.C. & Wiebe W.J. (1998): Prokaryotes: The unseen majority. *Proc. Natl. Acad. Sci. USA*, 95: 6578-6583

Table 1: Bacterial diversity and species identified in different Indian caves

S.No.	Name of cave, / State	Type of deposit	Cell enumeration	Bacterial count cfu/g	Identified species/ phylotype	Reference
1	Sahastradhara /Uttarakhand	Stalactite	DAPI	9×10^5	<i>Bacillus thuringiensis</i> <i>Bacillus pumilis</i>	Baskar et al. 2006
2	Sahastradhara /Uttarakhand	Moonmilk	DAPI	6.3×10^5	<i>Bacillus pumilis</i> , <i>B. cereus</i> <i>B. anthracis</i> <i>B. lentus</i> , <i>B. circulans</i> <i>B. sphaericus</i> <i>Actinomycetes</i>	Baskar et al. 2014
3	Borra /Andhra Pradesh	Biofilm	SYBR Gold	6.3×10^5	<i>Betaproteobacteria</i> <i>Leptothrix</i> spp. <i>Alpha</i> , <i>gamma</i> , <i>deltaproteobacteria</i> ,	Baskar et al. 2012
			DAPI	5.2×10^5	<i>Planctomycetes</i> , <i>Actinobacteria</i> , <i>Acidobacteria</i> <i>Nitrospira</i> , <i>Firmicutes</i> , <i>Firmicutes</i> , <i>Chloroflexi</i> , <i>Bacterioidetes</i>	
4	Krem Mawsmi /Meghalaya	Stalactite	Plate count	4×10^3 (NA) 1.2×10^3 (SA)	<i>Bacillus licheniformis</i> , <i>B. cereus</i> <i>Actinomycetes</i> <i>Streptomyces</i>	Baskar et al. 2009
5	Krem Phyllut /Meghalaya	Stalactite	Plate count	1.6×10^2 (TA) 3.2×10^3 (NA)	<i>Bacillus licheniformis</i> <i>Bacillus cereus</i>	Baskar et al. 2009
6	Krem Phyllut /Meghalaya	Cave wall deposit	Plate count	3.5×10^3 (NA)	<i>Bacillus mycoides</i>	Baskar et al. 2009
7	Krem Mawmluh /Meghalaya	Moonmilk	Plate count	5.6×10^5 (NA) 6.2×10^4 (B4) 3×10^3 (SA) 3.5×10^3 (KIA) 3×10^3 (MNA) 3.5×10^2 (TA)	<i>Bacillus licheniformis</i> <i>B. cereus</i> , <i>Actinomycetes</i> <i>Bacillus pumilis</i>	Baskar et al. 2011
8	Krem Mawmluh Meghalaya	Moonmilk water	Plate count	6.3×10^5 (NA)	<i>Micrococcus luteus</i> <i>Bacillus</i> spp.	Baskar et al. 2011

Identification technique: S.No.- 1 to 3: Molecular, DNA sequencing; 4 to 8: Conventional

NA-Nutrient agar; **B4**-B4 agar, **MNA**-Manganese agar, **SA**-Sulphite agar, **KIA**-Kliger iron agar, **TA**-Thiosulphate agar, **SYBR**-Gold-Cyanin dye nucleic acid stain; **DAPI**-4',6-diamidino-2-phenylindole fluorescent DNA stain