NOTES ON NATURAL HISTORY

Microbes Identified from Some Caves of Bastar, Chhattisgarh and their Biocalcifying Abilities

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Study Area: in and around Kanger Valley National Park, Bastar, Chhattisgarh, India
Coordination: 18°45'N 82°10'E

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Introduction:

Cave environments host diverse microorganisms and they are sites of active mineral precipitation. The subject matter of cave geomicrobiology deals with the interactions of microbes with minerals present in caves. Microbes and their metabolisms are important drivers of biogeochemical processes. Several microorganisms have been identified in caves such as- Actinobacteria sp., Leptothrix sp., Myxobacteria sp., Pseudomonas sp., Bacillus sp. etc. (Cañaveras *et al.*, 2001; Northup *et al.*, 2011; Baskar *et al.*, 2012, 2016) of which some are useful in bioremediation applications.

Researchers have demonstrated the ability of microbes isolated from the caves and marine environments to form biominerals under controlled conditions (Danielli & Edington, 1983; Rivadeneyra *et al.*, 1993; Baskar *et al.*, 2006, 2014, 2016).

Though the Indian sub-continent hosts a number of caves, only a handful reports have focussed on the geomicrobiology of caves. Paleoclimatological studies, biospeleology and some attempts on cave microbiology have been made in some caves of Chhattisgarh (Biswas, 1992; 2010; Sinha *et al.*, 2011; Yadava *et al.*, 2007; Biswas & Shrotriya, 2011; Rajput *et al.*, 2012a,b, 2014; Rajput & Biswas, 2012; Biswas *et al.*, 2011; 2015). The present study reports on the geomicrobiological studies of some caves from Chhattisgarh. The main objective of the study is to understand the metabolic capability of the isolated microbes from these caves in precipitating calcite *in vitro*.

Rani Cave: the cave (~50 m in length) is situated in Chitapur village of block Tokapal, district Bastar, Chhattisgarh. *In vitro* culture experiments, using three bacterial strains CSJC1, CSJC2, CSJC3 isolated from speleothems from Rani Cave, were examined for their biomineralization potential. The speleothems showed high microbial cell numbers on nutrient agar (9×10^4 CFU/g). The BLASTn sequence search of 16S rRNA

sequences with the NCBI database yielded similarity scores of ~99% with the respective organisms and the strains were identified as CSJC1– *Bacillus* sp., CSJC2 – *Cupriavidus* sp., CSJC3 – *Bacillus* sp. All the strains precipitated calcite *in vitro* (Fig.-1, Table-1).

Kotumsar Cave: this cave is situated in the Kanger Valley National Park in Bastar district, Chhattisgarh and is the major tourist attraction point of the state. The cave is reported to be one of the biologically well-explored caves in India. It has a number of highly endemic species on the verge of extinction (Biswas, 2010). These speleothems showed high microbial cell enumerations on B4 agar (3.4×10^5 CFU/g) followed by sulphite agar (7.2×10^2 CFU/g). The BLASTn sequence search of 16S rRNA sequences with the NCBI database to establish the identity of S14 strain yielded similarity scores of ~99% with the respective organisms and the strain was identified as *Rhodococcus* sp. (Table-1). Culture experiments indicated that the rate of precipitation was controlled by factors like pH, temperature and bacterial growth. *Rhodococcus* sp. S14 strain induced the formation of calcite *in vitro*. The biominerals produced were calcified spherulites with pores. It had the appearance of coccoliths.

Jhumar Cave: it is situated near the Pedawada village of block Darbha, district Bastar, Chhattisgarh. It is located in the periphery of Kanger Valley National Park. These speleothems showed high microbial cell enumerations on Iron agar (9.0×10^4 CFU/g) followed by sulphite agar (4.0×10^3 CFU/g). Three strains S1, S2 and S3 from JMST1, JMST2 and JMST3 samples were tested under controlled conditions of temperatures (18-25°C) for precipitation of crystals. During calcite precipitation, the pH of medium increased. The strains precipitated calcite of 0.55 g, 0.43 g and 0.47 g respectively in the 30 days experiment period. The strains were identified were *Achromobacter insolitus*, *Bacillus subtilis*, and *Bacillus flexus* (Table-1, Fig.-1).

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Table 1: Microbial diversity and biocalcifying abilities of strains identified in caves in Chhattisgarh

Name of cave	Type of deposit	Bacterial count cfu/g	Identified species/ phylotype	Identification technique	Biocalcification ability	Biomineral formed
Rani	Cave wall	Nutrient agar 9×10^4	<i>Cupriavidus</i> sp., <i>Bacillus</i> sp.	DNA sequencing	Yes	Calcite
Kotumsar	Cave wall	B4 agar 3.4×10^5 Sulphite agar 7.2×10^2	<i>Rhodococcus</i> sp., <i>Bacillus</i> sp., <i>Micrococcus</i> sp., <i>Staphylococcus</i> sp.,	DNA sequencing	Yes	Calcite
Jhumar	Stalactite	Iron agar 9.0×10^4 Sulphite agar 4.0×10^3	<i>Achromobacter insolitus</i> , <i>Bacillus subtilis</i> , <i>Bacillus flexus</i>	DNA sequencing	Yes	Calcite

Figure-1: (a) Dumbbell shaped CaCO_3 precipitated by *Cupriavidus* sp. isolated from Rani and Jhumar cave speleothems (b) Calcite precipitated by *Achromobacter insolitus* isolated from Jhumar cave.

Conclusion:

This note summarizes the studies performed on some caves from Chattisgarh to throw light on microbe-mineral interactions. In this study, the isolated microbes through their metabolic activities have resulted in biocalcification. It can help in understanding biomineralization in caves. In vitro studies suggest that these isolated microbes help in biomineralization through biologically induced mechanisms. It establishes that bacterial metabolism does influence the calcite precipitation process.

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