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Bacterial diversity of Mandeepkhol cave, Rajnandgaon district of C.G., India.

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Abstract

Caves provide a niche to several kinds of microorganisms. Nowadays cave ecosystem is attracting microbiologists to study the microbial diversity present inside the cave. These includes Bacteria, fungi, actinomycetes etc. In Chhattisgarh state of India there is a huge cave located in deep forest in Salewara mountain range known as Mandeepkhol cave which is nearly 100 km away from Durg dictrict. The longitude of cave is and latitude is. The present paper deals with the bacterial diversity found inside the cave. Thirty two different bacterial species were isolated from different parts of cave. Bacteria were got identified by help of morphological and microscopic characteristics.

Introduction

Caves, caverns and other karst formations often have unique microbial ecosystems [Barton and Northup (2007), Barton (2006)]. Caves are divided into different zones based on the prevailing light and temperature (Mohr and Poulson). According to Poulson and White, each cave has three zones: (i) twilight zone which is located at the entrance area; (ii) middle zone in which relative darkness prevails with fluctuating temperature; and (iii) dark zone in which total darkness and constant temperature prevails (Koilraj and Marimuthu).

There has been a renewed focus from both scientific and economic points of view on microbial diversity in caves in recent times The study of cave microbiology deals with the microscopic creatures/ organisms that are inhabitants of caves. The lack of sunlight precludes photosynthesis, except at cave openings. It has been noticed that most cave ecosystems are heterotrophic, and so depend upon organic materials that fall in through cave openings, carried by water, or deposited by cave animals that travel to the surface. Microorganisms also have been implicated in the formation of many speleothems. Microorganisms play a crucial role in maintaining the delicate ecological balance of the earth. Standard culturing techniques used to cultivate microorganisms from caves, have met with limited success (Amann et al. 1995; Hugenholtz et al. 1998). Many novel prokaryotic species have been detected as a result of this new technology. These microorganisms are important participants in the precipitation and dissolution of minerals, in caves (Northup and Lavoie 2001) and on the surface (Ehrlich 1999). By interacting with minerals, microbial species play an important role in reshaping the mineral environment of caves, and may help to form features such as stalactites and stalagmites. They help in recycling of organic matters. They are helpful in preservation of ancient marble monuments and statues.

Bacteria have been found in some of the most extreme areas including deep-sea thermal vents, within rock cores, and in caves. Bacterial communities in caves acquire energy by several means, including by breaking down aromatic compounds, fixing gases, and oxidizing reduced metals within rocks. Bacteria also has a capacity to decompose major plant components particularly lignin and cellulose (the major components of plant cell walls).Bacteria are dominant decomposers and nutrient recyclers of forest litter and debris. Plants locked complex organic materials are needed to be degraded up to the level of simple monomers so that it can be reused

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by other organisms thereby helping in cycling of organic nutrients. In nature biodegradation is mainly carried out by thermophilic bacteria, actinomycetes and fungi in anerobic microenvironment. The aim of this paper is to find out the diversity of mesophilic Bacteria from Mandeepkhol cave which is a Dark cave with a constant temperature around 26ºC- 28ºC.

Cave Ecosystem

Mandeepkhol cave is located in the mountain range of Salewara. It is nearly 100 kilometres from district Durg. The cave is located in deep forest and is not easily approachable. It is about 30 kms from nearest township of Gandai. The cave is basically an adit cave that has a narrow entrance. The twilight zone of the cave is hardly 2-3 metres and the remaining part is completely dark. The temperature of that cave remains constant between 26ºC- 28ºC through out the year. Entrance is connected to mountain valleys. During rainy season a huge amount of water in form of stream flow in these valleys enters into the cave through these narrow entrance and brings twigs, logs and lot of dried leaves inside the cave and deposits there. The mandeepkhol cave provide shelter to large number of bats which hang from the roofs. The excreta of these bats get deposited over the floor in a very large quantity that contains high percentage of cellulose and lignin.

METHODOLOGY

Isolation of Bacteria

Samples for bacterial isolates were collected from the Cave during summer season, post rainy season and winter season. Guano deposits, log and twig deposits, leaf litters separately were collected aseptically in polythene bags.

The isolation of bacteria were done using nutrient agar media. The samples were serially diluted by method of serial dilution. Dilution of 10^{-6} and 10^{-7} were taken. Few drops of samples were poured into petriplates containing nutrient agar media using micropipette. The petriplates were kept for incubation in incubator for 28ºC ± 2ºC. After incubation bacterial colonies were counted and identified. Identification of bacteria were done by analyzing both morphological and microscopic characters. Bacteria which has shown maximum diversity were got identified in authentic lab, GeneI.

Results and discussions

1. **Summer Season**

A total of 25 isolates were isolated during summer season. From among different sources maximum diversity was found from Guano deposits (20 species) followed by leaf litter deposits (19 species) and log and twig deposits (14 species), (Table- 4).

Cream colony I, *Brevundimonas diminuta*, *Bacillus thuringiensis*, *Bacillus cereus*, Cream rough colony II, Cream rough colony III, Orange colony, Transparent colony 4 were dominant and isolated from all three samples.

2. **Post Rainy Season**

A total of 32 isolates were isolated during post rainy season. From among different sources maximum diversity was found from Guano deposits (25 species) followed by leaf litter deposits (23species) and log and twig deposits (19 species), (Table- 4).

Cream colony I, *Brevundimonas diminuta*, *Bacillus thuringiensis*, *Bacillus cereus*, Transparent colony II, Whitish cream colony, Creamish yellow colony II, Lemon yellow colony I and Transparent colony 4 were dominant and isolated from all three samples.

3. **Winter Season**

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A total of 28 isolates were isolated during post rainy season. From among different source maximum diversity was found from Guano deposits (22 species) followed by leaf litter deposits (21species) and log and twig deposits (13 species), (Table- 4).

Cream colony I, *Brevundimonas diminuta*, *Bacillus thuringiensis*, *Bacillus cereus*, Transparent colony 4, Light yellow colony II, White colony III, Yellowish rough colony were dominant and isolated from all three samples.

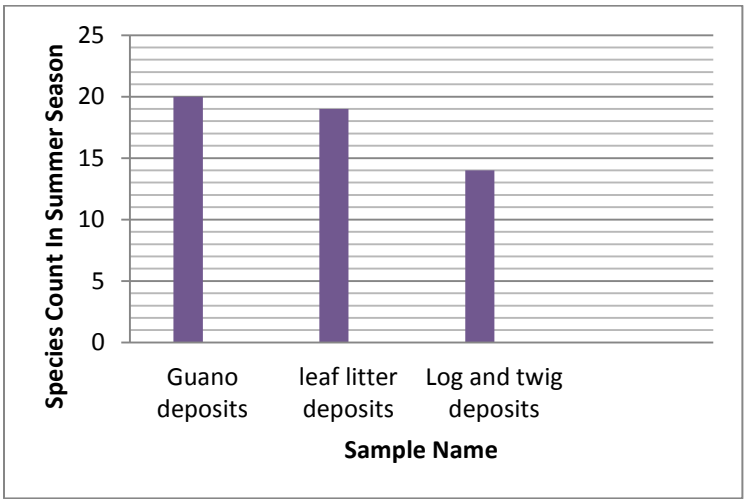

Fig-1: Species count in summer season from different sources.

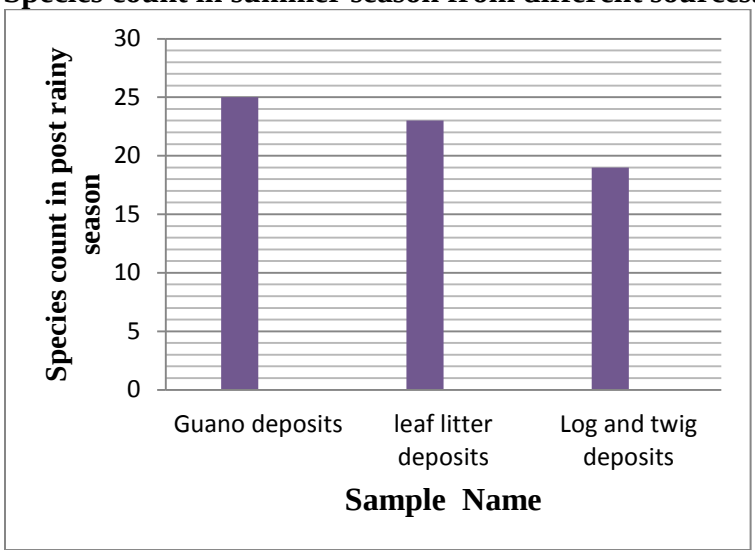

Fig-2: Species count in post rainy season from different sources.

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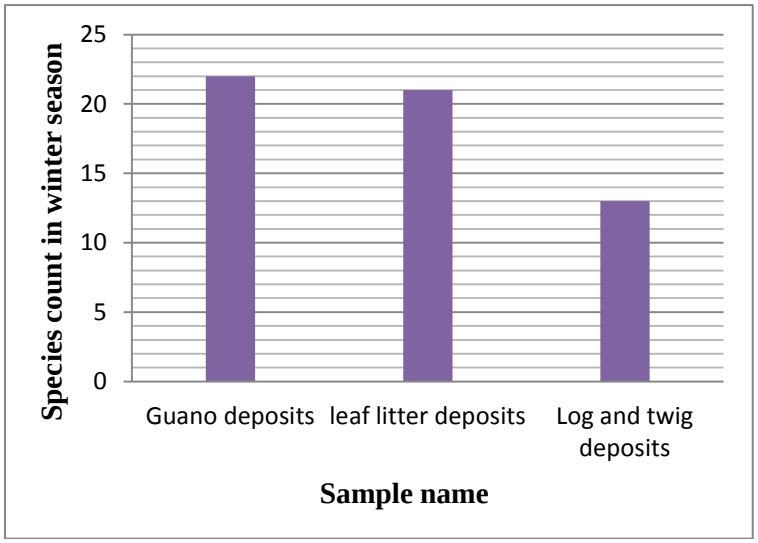

Fig-3: Species count in winter season from different sources.

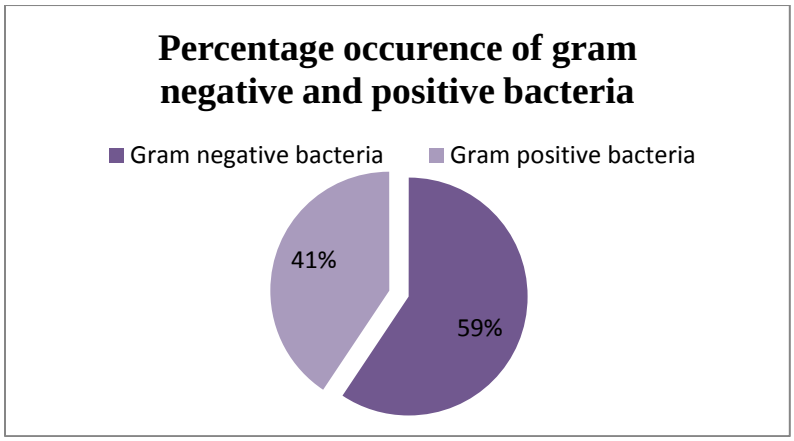

Fig-4: Percentage occurrence of gram negative and positive bacteria.

Percentage Frequency

During study maximum percentage frequency was shown by *Bacillus cereus*, *Bacillus thuringiensis*, *Brevundimonas diminuta*, Cream colony I and Transparent colony 4 (100%) followed by Yellowish rough colony, Yellowish smooth colony, Whitish cream colony, Transparent colony II and Cream rough colony III (78%), Cream yellow colony I, Lemon Yellow colony II, Light yellow colony II, Cream rough colony II and Creamish yellow colony (66%). Result Bacterial species had shown below than 50% of frequency.

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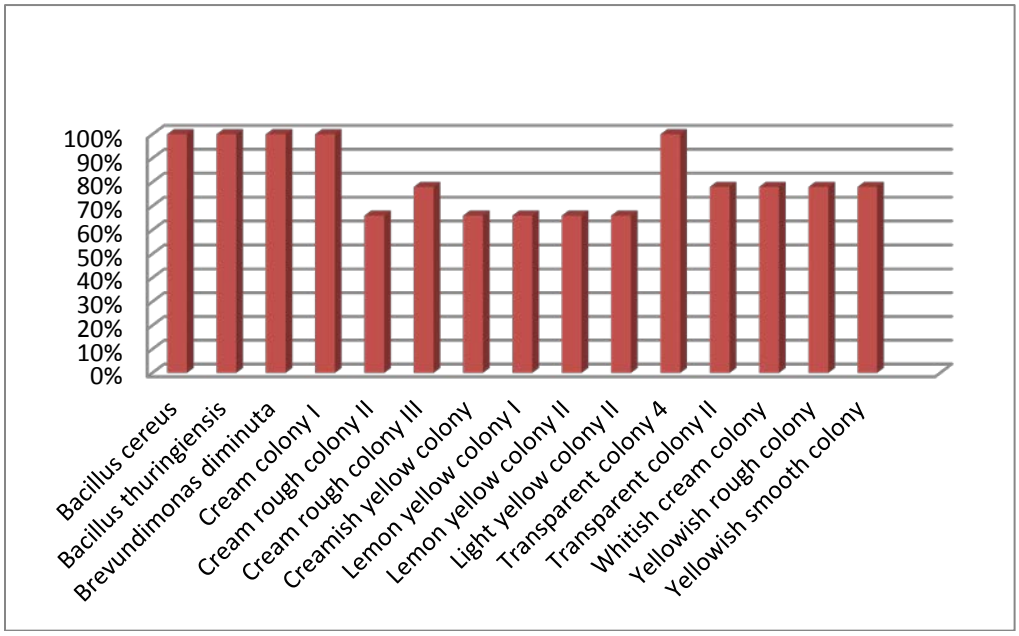

Fig- 5: Percentage Frequency of bacterial species in all three seasons.

During studies it was found that bat guano are mostly preferred by bacteria. During studies we have also noticed that the degradation of log and twig deposits were preferred by only few groups of bacteria, thus indicating that lignin, (as log and twig contains more percentage of lignin) is hard to degrade because of its complex aromatic structure. Similar work has been shown by Rajput *et al.* (2012) and isolated bacterial species from different sediments of Kotomsar cave of Bastar, Chhattisgarh. Engel *et al.* (2004) investigated bacterial and other microbial diversity of Lower Kane Cave, a small system located in the Bighorn Basin, Wyoming. On the other hand Northup *et al.* (2004) *also* studied about the nature of bacterial communities in four window caves in El Malpais National Monument, New Mexico, USA. There study revealed that there is diverse group of bacterial communities. Zhou *et al.* (2007) analyzed the phylogenetic composition of bacterial community in the soil of the earth cave using a culture- independent molecular apporoach.

Conclusion

Results revealed that bat guano is mostly preferred than by log and twig deposits which may be due to partially digested cellulose, lignin etc. leaf litter deposits were also preferred more than log and twig deposits. As log and twig contains more percentage of lignin and lignin as hard to degrade, so preferred less by bacterial species. Even bacterial species isolated were mesophilic thus helpful in decomposition during mesophilic condition also.

Reference

Amann, R.I., Ludwig, W., and Schleifer, K.H., 1995, Phylogenetic identification and in situ detection of individual microbial cells without cultivation: Microbiological

Barton Hazel A. – Introduction to cave microbiology: A review for the non-specialist. *Journal of* Cave and Karst Studies, v. 68, no. 2, p. 43–54.

Barton, H.A. and Northup, D.E., 2006, Geomicrobiology in cave environments: Past, current and future perspectives: Journal of Cave and Karst Studies,
Boletín 7 119-125.
Ehrlich, H.L. 1999. Microbes as geologic agents: Their role in mineral formation. Geomicrobiology Journal **16**(2): 135-153.
Engel, A.S., Stern, L.A., and Bennett, P.C., 2004, Microbial contributions to cave formation: New insights into sulfuric acid speleogenesis: Geology, v. 32, p. 369–372.
Hugenholtz, P., Goebel, B.M., and Pace, N.R., 1998, Impact of culture-independent studies on the emerging phylogenetic view of Bacterial diversity: Journal of Bacteriology, v. 180,
Koilraj A. J, Marimuthu.G, · Natarajan. K, Saravanan. S, Maran.P, and Hsu.M.J. (1999). Fungal diversity inside caves of Southern India. *Curr. Sc.*, **77:** 1081- 1084.
Mohr, C. E. and Poulson, T. L. (1996). *The Life of the Cave*. McGraw-Hill, New York.
Northup .Diana E, Cynthia A. Connolly, Amanda Trent, Vickie M. Peck, Michael N. Spilde, W. Calvin Welbourn, and Donald O. Natvig, 2004, The nature of Bacterial communities in four windows cave, El Malpais National Monument, New Mexico, USA. *AMCS Bulletin 19 / SMES*
Northup, D.E., and Lavoie, K.H., 2001, Geomicrobiology of Caves: A Review: Geomicrobiology Journal, v. 18, p.199–222.
p. 4765–4774
Poulson, T. L. and White, W. B. (1969). The cave environment. *Science.* **165**: 971–981.
Rajput yogita, Rai Vibhuti and Biswas Jayant. (2012). Screening of bacterial isolates from various microhabitat sediments of Kotumsar cave: A cogitation on their respective benefits and expected threats for complete biosphere and tourist. Research Journal of Environmental Toxicology, **6(1):** 13- 24.
Reviews, v. 59, p. 143–169.
Zhou J., Gu Y., Zou C. and Mo M. (2007). Phylogenetic diversity of bacteria in an earth-cave in Guizhou province, southwest of China. *J. Microbiol.*, **45(2):** 105-120.

Table- 4: Bacterial isolates from different sources in different seasons

Isolated Bacteria	Summer season			Post rainy season			Winter season		
	Number of colony			Number of colony			Number of colony		
	Guano deposits	Leaf litter deposits	Log and twig deposits	Guano deposits	Leaf litter deposits	Log and twig deposits	Guano deposits	Leaf litter deposits	Log and twig deposits
Bacillius cereus	++	+	++	++	++	+	++	+	
Bacillus thuringiensis	++	+++		++	++++				
Brevundimonas diminuta	++++	++	+	+	+++	+	++	++	++
Brown smooth colony	+			++			+		+
Cream colony 4				+		+			
Cream colony I	++	++	++	+++	+	+	+	++	+
Cream colony II	+++	+			++	+	+		
Cream colony	+			+++				++	

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III									
Cream rough colony I	++				+		+++	+	
Cream rough colony II	+	+	+		++	+	++		
Cream rough colony III	++	+	++	+++	++			++	+
Creamish yellow colony	+	++	+	++++		+++		+	
Creamish yellow colony II				+	+	++		++	
Lemon yellow colony I		++	++++	+	++	+	+		
Lemon yellow colony II	++	+	++	+			+	+	
Light pink colony				+				+	
Light yellow colony I	+				+				
Light yellow type II			+++	+		+	++	+	+
Milky white colony			++			++	++		
Orange colony	+	+	+	++	++				
Pink colony				+	+		+		
Transparent colony 4	+	++	+	+	+	+	+	+	+
Transparent colony I		++			++		+		+
Transparent colony II	++	+++		++	++	+	++	+	
Transparent colony III				+		++	++		+
White colony I		++		+		+		+	
White colony II	++	+++		++	++				
White colony III	++				+		++	++	+
White rough colony				+	+		++	+	
Whitish cream colony		++	++	+	+	++	++	+	
Yellowish rough colony	++	++		++	+		+	++	+
Yellowish smooth colony	+	+		+	++	+	+	+	

+ = poor, ++ = moderate, +++ = good, ++++ = excellent.

Table – 5: Colony morphology and cell morphology of isolated bacteria

Isolated bacteria	Colony morphology				Cell morphology		
	Colour	Size	Shape	Texture	Cell shape	Cell grouping	Gram reaction

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Bacillus cereus	Dull white	Small	Irregular	Rough	Rod	In chain	Positive
Bacillus thuringiensis	White	Medium	Rounded	Smooth	Rod	Single	Positive
Brevundimonas diminuta	Light brown	Small	Rounded	Smooth	Rod	In chain	Negative
Brown rough colony	Brown	Large	Irregular	Rough	Rod	In chain	Positive
Brown smooth colony	Brown	Small	Rounded	Smooth	Spiral	Irregular colony	Negative
Cream colony I	Cream	Small	Rounded	Smooth	Rod	In chain	Negative
Cream colony III	Cream	Small	Irregular	Rough	Round	Paired	Positive
Cream colour II	Cream	Medium	Irregular	Rough	Rod	Irregular colony	Negative
Cream rough colony I	Cream	Large	Irregular	Rough	Round	Single	Negative
Cream rough II	Cream	Large	Irregular	Rough	Round	Single	Negative
Cream rough III	Cream	Small	Irregular	Rough	Cocci	In chain	Positive
Creamish yellow colony	Creamish yellow	Small	Rounded	Smooth	Round	In chain	Negative
Creamish yellow colony II	Creamish yellow	Small	Rounded	Smooth	Elliptical	Irregular colony	Negative
Lemon yellow colony I	Lemon yellow	Small	Rounded	Smooth	Rod	Irregular colony	Negative
Lemon yellow colony II	Lemon yellow	Medium	Rounded	Rough	Rod	Paired	Negative
Light pink colony	Light pink	Medium	Irregular	Rough	Round	Paired	Positive
Light yellow colony I	Light yellow	Small	Irregular	Smooth	Round	Single	Positive
Light yellow colony II	Light yellow	Medium	Rounded	Smooth	Rod	In chain	Negative
Milky white colony	White	Large	Rounded	Smooth	Rod	Irregular colony	Negative
Orange colony	Orange	Medium	Rounded	Smooth	Round	Tetrad	Positive
Pink colony	Pink	Small	Rounded	Smooth	Rod	In chain	Negative
Transparent 4	Transparent	Medium	Irregular	Smooth	Cocci	Irregular colony	Positive
Transparent colony I	Transparent	Small	Rounded	Smooth	Round	Irregular colony	Negative
Transparent colony II	Transparent	large	Rounded	Smooth	Rod	Single	Negative
Transparent colony III	Transparent	Small	Rounded	Smooth	Rod	In chain	Negative
White colony I	White	Medium	Rounded	Smooth	Rod	Single	Negative
White colony II	White	Large	Rounded	Rough	Rod	Single	Positive
White colony III	White	Small	rounded	Smooth	Round	Irregular colony	Negative
White rough colony	White	Medium	Irregular	Rough	Round	Single	Positive
Whitish cream colony	Whitish yellow	Large	Irregular	Rough	Rod	In chain	Positive
Yellowish rough colony	Yellowish	Medium	Rounded	Rough	Round	Single	Positive
Yellowish smooth colony	Yellowish	Small	Rounded	Smooth	Round	In chain	Negative

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Fig- 1: Brevundimonas diminuta

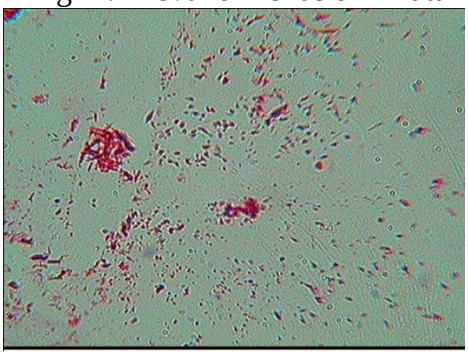

Fig- 2: *Bacillus thuringiensis*

Fig-3: *Bacillus cereus*