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Microhabitat Dependant the Proliferation of Microfungi in Dandak Cave of Kanger Valley National Park, India

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Study Area: Dandak Cave, Bastar, Chhattisgarh, India

Coordinates: 18°51'30"N; 81°57'00"E

Key words: *Bartaliana robillardoides*, *Histoplasma capsulatum*, Cave ecosystem, Seasonal distribution.

Abstract

The possibilities of obtaining biotechnologically potent microbes are always much from the uncommon ecological niches. In the present study, the diversity of microfungi associated with soil sediments of two distinct ecosystems existing inside a cave of Central India "Dandak" were compared. Soil samples were collected from three different seasons; Summer, Rainy, and Winter. Results revealed the Aspergillaceae and Trichomaceae family to be the most dominant over other families. Ecological contributions were also recorded in which *Aspergillus niger* was found in highest percent frequency (66.67%) with 9.57% total percent contribution. *Bartaliana robillardoides* and *Histoplasma capsulatum* are some of the specific types of microfungi were isolated from the soil sediments of the outer chamber of the cave. The possible reason for identifying uncommon microfungi from outer chamber of the Dandak cave has been further discussed.

Introduction:

Caves are usually considered as sterile environment mainly due to the absence of light, maintaining high humidity, low air movement, almost constant temperature and possessing fewer sources of energy (Gunn, 2004; Vandel, 1964; Biswas, 2009; 2010). Thus, the cavernous ecosystem generally also holds a unique biota. Microscopic fungi are an important part of the cave which invade in almost every type of substrate and play a major role in biotic (Boddy, 2008; Stokland, 2012; Biswas *et al.*, 2017) and even abiotic degradation (Sterflinger, 2000; Gu, 2003; Cwalina, 2008; Li *et al.*, 2008), ultimately help in the recycling of nutrients, provides nutrients for other trophic levels. It is usually acting as primary producer of cave food chain (Bastian *et al.*, 2010; Vanderwolf *et al.*, 2013; Biswas & Biswas, 2017).

In the present study, we have tried to investigate the variety, frequency and contribution dynamics of microfungi isolated from the floor sediment samples (mud) of two ecologically variant chambers of Dandak cave during three major seasons of a year. Further, during one season we have also attempted to compare the same parameters with the isolates collected from the sediment sample (mud) outside the cave.

Materials and methods:

Study site: Dandak Cave is a large, spacious, limestone cave

located in Kanger Valley National Park, Jagadalpur. The cave is about 360 m in length (Biswas & Shrotriya, 2011) and horizontally divided into two main chambers or compartments. The outer chamber is exposed directly to the epigeal environmental conditions whereas the inner chamber is connected only with the outer chamber through a narrow convoluted passage. In this study, we referred both the chambers as an outer and an inner chamber. This cave suffers less anthropogenic pressure because the general entry of tourist is restricted for this cave.

Sample collection: Soil samples were collected from both the chambers (outer and inner) in three successive seasons: Summer, Rainy and Winter during the year 2014 to 2015. Samples were taken out with the help of sterile forceps and packed it into sterile plastic bottles. One gram of soil sample was used to isolate the fungal species. For inoculation, selective media, PDA (Potato Dextrose Agar) was used and then after developed inoculated cultures were processed for further studies. Fungal isolation was done by serial dilution method. For observation and identification, standard staining method was done. The lactophenol cotton blue stain was used for fungal staining. Identifications were mainly based on morphological characteristics of the colony and microscopic examinations. It was done mainly on the basis of visual fungal culture characteristics on plates such as topography

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of the colony, mycelial growth, outline of colony, texture of colony, pigmentation of the colony, exudance of substance by the colony, colour of colony both for back and front side of Petri-plate, changes in colour of the colony and medium, diameter of the colony etc. (Gilman, 1957; Barnett & Hunter, 1998). We also took the Professional help for proper identifications of those isolates which we failed to identify in our laboratory.

Dynamical characteristics: Fungi play an important role in the cave environment. Percentage frequency and percentage contribution of the fungal flora were also calculated for further interpretations.

Results :

A total of 38 species belonging to 14 genera and one Mycelia sterilia were isolated from soil sediment collected from both the chambers of Dandak cave during three successive seasons (Summer, Rain, and Winter). From all the collected samples 43 isolates from the outer chamber whereas only 12 isolates from the inner chamber of the Dandak cave were identified. Percent frequency of *Aspergillus niger* shown the highest frequency i.e, 66.67% with 9.57% as total percent contribution. *Penicillium citrinum* and *Rhizopus stolonifer* were the second most dominated species, isolated from the soil sediments collected from both the habitats and their percent frequencies were 50% whereas the percent contribution was 3.19% and 9.57% respectively.

The dominant microfungi families isolated from the mud sediments of both the chambers of the Dandak cave around the three seasons have been presented in Fig.-1. Interestingly from the outer chamber, the members of either Trichomaceae (Rain and Winter) or Aspergillaceae (summer) were found to be dominated, whereas season-wise no fixed pattern was found for inner chamber.

Figure-1: Comparative bar-diagram representing various families of microfungi the members of which have been isolated from floor sediments (mud) collected from both the chambers during three different season of a year.

During the winter season when we achieved maximum numbers of isolates. For comparison, we had also isolated the microfungi from the soil sediment collected from outside the cave. A comparative chart of the results belonged to three distinct habitats; inner, outer chambers of Dandak cave and outside the Dandak cave has

Figure-2: Comparative bar-diagram representing various families of microfungi, the members of which have been isolated from floor sediments (mud) collected from both the chambers and outside the cave during Winter.

been presented of in Table-2. The lowest numbers of fungi and their respective number of colonies was detected from the inner chamber of the Dandak cave whereas the equal numbers of microfungi species were evident from the outer chamber of the cave and from its epigeal (outside) environmental zone, though the total numbers of colonies were isolated more from soil sample collected from outside the cave.

Discussion:

Due to the crises of intrinsic energy sources, the caves are always defined as the starved ecosystem (Vandel, 1965; Gunn, 2004; Biswas, 2009, 2010; Biswas & Biswas, 2017). Thus, the microorganisms which grow on such energy starved conditions are often referred to as extremophiles. The possibilities for obtaining potent enzymes, useful as biotechnological tools are always more from such microorganisms which grow in extremophile conditions. In the present piece of work, we have compared mainly the patterns of microfungi occurrence from two distinct chambers of Dandak cave which differs from each other in various microclimatic conditions (Biswas & Shrotriya, 2011; Biswas *et al.*, 2017). Our result evidenced the growth of very selective types of microfungi in the inner chamber whereas the fungal diversity for the outer chamber was found to be sound. During Summer we found two members of *Cladosporium* from the inner chamber which was not evident from the outer chamber against any other season. *Cladosporium* spores are able to disperse even in a small air current and the species may grow on any such surfaces where moisture is high (Kwon *et al.*, 2001; Ogórek *et al.*, 2012). Possibly, this is the reason of its survival inside the inner chamber. Earlier also species of *Cladosporium* was reported from the cave soil (Bensch *et al.*, 2010). Once the occurrence of *Trichoderma* sp. from the inner chamber is also interesting, though the species was reported earlier also from the subterranean caves (Karkun *et al.*, 2012; Nováková, 2009) and its importance for the cave could not be denied (Zhang *et al.*, 2015). Possibly, the geophysical factors of the inner chamber during summer are favouring

Table-1: Seasons dependent microfungi isolated from the floor sediments (mud) collected from 2 different habitats of Dandak cave

S. No.	Isolated Fungi	Summer		Rainy		Winter		Total 'N' Observation	Percent Contribution	Total 'N' Colonies	Percent Frequency
		Outer	Inner	Outer	Inner	Outer	Inner				
1	<i>Absidia corymbifera</i>			4				1	16.67	4	4.26
2	<i>Absidia spinosa</i>					2		1	16.67	2	2.13
3	<i>Acremonium</i> sp.I					1		1	16.67	1	1.06
4	<i>Alternaria alternata</i>			3	1			2	33.33	4	4.26
5	<i>Alternaria brassicicola</i>			1				1	16.67	1	1.06
6	<i>Alternaria crassa</i>					1		1	16.67	1	1.06
7	<i>Alternaria raphani</i>	1						1	16.67	0	1.06
8	<i>Aspergillus flavus</i>	2				1		2	33.33	1	3.19
9	<i>Aspergillus awamori</i>					1		1	16.67	1	1.06
10	<i>Aspergillus fumigatus</i>					2		1	16.67	2	2.13
11	<i>Aspergillus glaucus</i>	1						1	16.67	0	1.06
12	<i>Aspergillus japonicus</i>			1	1			2	33.33	2	2.13
13	<i>Aspergillus niger</i>	2		3	2	2		4	66.67	7	9.57
14	<i>Aspergillus sulphureus</i>	1						1	16.67	0	1.06
15	<i>Aspergillus sydowii</i>					1		1	16.67	1	1.06
16	<i>Bartaliana robillardoides</i>					2		1	16.67	2	2.13
17	<i>Cladosporium cladosporioides</i>	3	1					2	33.33	1	4.26
18	<i>Cladosporium oxysporum</i>		1					1	16.67	1	1.06
19	<i>Curvularia lunata</i>			1				1	16.67	1	1.06
20	<i>Fusarium moniliforme</i>	1						1	16.67	0	1.06
21	<i>Fusarium oxysporum</i>					1		1	16.67	1	1.06
22	<i>Fusarium</i> sp.I				2	2		2	16.67	4	4.26
23	<i>Histoplasma capsulatum</i>					1		1	16.67	1	1.06
24	<i>Mucor hiemalis</i>			1				1	16.67	1	1.06
25	<i>Paecilomyces lilacinus</i>			2				1	16.67	2	2.13
26	<i>Penicillium rubrum</i>			3				1	16.67	3	3.19
27	<i>Penicillium chrysogenum</i>	2						1	16.67	0	2.13
28	<i>Penicillium citrinum</i>	1		1		1		3	50	3	3.19
29	<i>Penicillium commune</i>			2			2	2	33.33	4	4.26
30	<i>Penicillium glaucum</i>			2	2			2	33.33	4	4.26
31	<i>Penicillium marneffeii</i>					1		1	16.67	1	1.06
32	<i>Penicillium notatum</i>					2	2	2	33.33	4	4.26
33	<i>Penicillium restrictum</i>					1		1	16.67	1	1.06
34	<i>Penicillium</i> sp.II	1						1	16.67	0	1.06
35	<i>Penicillium</i> sp. aff. <i>P.madruti</i> G. Sm.					2		1	16.67	2	2.13
36	<i>Rhizopus oryzae</i>			5		1		2	33.33	6	6.38
37	<i>Rhizopus stolonifer</i>	2		4	3			3	50	7	9.57
38	<i>Trichoderma</i> sp.I						1	1	16.67	1	1.06
	<i>Mycelia sterilia</i>						1	1	16.67	1	1.06
Total number of colonies		17	2	33	11	25	6			94	

its proliferation (Biswas & Shrotriya, 2011).

Though the outer chamber has the direct contact with the outer epigeal environment, the transportation of external spores from the air, animals' visitation and even water stream entering from there overall increases the chances of growing more varieties of microfungi in the outer chamber. The most common fungal species group; *Aspergillus* and *Penicillium* are ubiquitous in worldwide caves. It is well known that *Aspergillus* species are highly aerobic in nature and grows only in oxygen-rich environments (Bolu *et al.*, 2014; Chehri, 2013). Varieties of *Aspergillus* sps. isolated from the samples collected from

the outer chamber. The chamber is highly influenced by the epigeal environmental factors is directly support the same fact. *Aspergillus niger* which was most abundantly found could grow safely in all such environment which is devoid of key nutrients. The species has been well documented from the various caves all over the world (Cunningham *et al.*, 1995; Nováková, 2009; Martin-Sanchez *et al.*, 2014; Taylor *et al.*, 2014; Zhang *et al.*, 2017). But the member of the species was not evident from the inner chamber except for the Rainy season. Perhaps the microclimatic conditions inside the inner chamber do not support its proliferation.

The most interesting finding in this issue was the

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Table-2: Three habitats dependent micro-fungi isolated from the floor Sediments (mud) collected during Winter season

S. No.	Isolated Fungi	Outside Cave	Outer. cham.	Inner. cham.	Percent Contri.	Percent Freq.
1.	<i>Absidia spinosa</i>	2	2		66.67	6.349
2.	<i>Absidia corymbifera</i>	1			33.33	1.587
3.	<i>Acremonium</i> sp.I		1		33.33	1.587
4.	<i>Alternaria alternata</i>	1			33.33	1.587
5.	<i>Alternaria crassa</i>		1		33.33	1.587
6.	<i>Aspergillus albus</i>	1			33.33	1.587
7.	<i>Aspergillus awamori</i>	2	1		66.67	4.762
8.	<i>Aspergillus flavus</i>	2	1		66.67	4.762
9.	<i>Aspergillus fumigatus</i>	2	2		66.67	6.349
10.	<i>Aspergillus niger</i>	5	2		66.67	11.11
11.	<i>Aspergillus sydowii</i>		1		33.33	1.587
12.	<i>Bartaliana robillardoides</i> Tassi		2		33.33	3.175
13.	<i>Curvularia lunata</i>	1			33.33	1.587
14.	<i>Fusarium moniliforme</i>	2			33.33	3.175
15.	<i>Fusarium oxysporum</i>		1		33.33	1.587
16.	<i>Fusarium</i> sp.I	1	2		66.67	4.762
17.	<i>Histoplasma capsulatum</i>		1		33.33	1.587
18.	<i>Mucor</i> sp.I	2			33.33	3.175
19.	<i>Paecilomyces lilacinus</i>	2			33.33	3.175
20.	<i>Penicillium chrysogenum</i>	1			33.33	1.587
21.	<i>Penicillium citrinum</i>	2	1		66.67	4.762
22.	<i>Penicillium commune</i>	1		2	66.67	4.762
23.	<i>Penicillium digitatum</i>	1			33.33	1.587
24.	<i>Penicillium marneffei</i>		1		33.33	1.587
25.	<i>Penicillium notatum</i>		2	2	66.67	6.349
26.	<i>Penicillium restrictum</i>		1		33.33	1.587
27.	<i>Penicillium</i> sp. aff. <i>P.madriti</i> G. Sm.	2	2		66.67	6.349
28.	<i>Penicillium</i> sp.	1			33.33	1.587
29.	<i>Rhizopus oryzae</i>		1		33.33	1.587
30.	<i>Trichoderma</i> sp.			1	33.33	1.587
	<i>Mycelia sterilia</i>			1	33.33	1.587

occurrences of some unique microfungi from soil sediments of the outer chamber which were neither isolated from the soil of its epigeal counterpart nor from the inner chamber. During Winter, *Bartaliana robillardoides* Tassi, had been isolated from the outer chamber of the cave which is a very rare and biotechnologically sound microfungus. Earlier it was reported from the leaves of a medicinal plant *Aegle marmelos* Correa ex Roxb., and it is used as an anticancer drug produced by endophytic fungus (Gangadevi & Muthumary, 2008). During Rain *Paecilomyces lilacinus* (Thom) Samson was also found from the soil sediment of the outer chamber. It is a soil fungus widely used for biological control i.e., efficient as nematocide (Kerry, 1990; Schenck, 2004) and also to check the of greenhouse insects and mite pests (Fiedler & Sosnowska, 2007).

For any ecological niche, the chances for occurrences of more microfungi are always more during Rainy season, as the high humidity, moist media, and other various biotic, as well as abiotic factors, support the same. Nevertheless, our study evidenced the abundance of isolates from the outer chamber of Dandak cave during the Winter season. During Rain, frequent water flushing possibly not allowing the proliferation of microfungi properly in the floor sediments of the outer chamber of the cave. It is to be added here that the location where Dandak cave is situated is a deep jungle, surrounded by big trees. The place is always blessed by winter rain (Biswas, 1992 a,b). Somehow, little winter rain is always promoting the growth of microfungi and its proliferation in the same region.

For further comparison, during the Winter season, we have also collected the mud sample from the epigeal (outside the cave area) environmental condition and isolated the microfungi. It was very interesting see that the members of the Aspergillaceae family (both numbers of colonies and varieties) were found to be maximum from the sample collected from outside the cave area which gradually diminished from the sample collected from the outer chamber of the Dandak cave and finally no member was found from the sample collected from the soil sample collected from the inner chamber.

The species dynamics for the outside open area of the cave and the outer chamber is comparable during the winter season in addition to it some notable species of microfungi were also isolated from the floor sediments (mud) of the outer chamber of the Dandak cave which altogether indicate that the outer chamber of the Dandak cave which enjoys the privileges of epigeal environmental condition, as well as partial sterile condition of cave ecosystem, is more potent for producing rare microfungi which could be useful for various biotechnological tools.

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