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## **MICROBIAL COMMUNITIES OF EARTHWORM (*PERIONYX EXCAVATUS* PERRIER) GUT, CAST AND ADJACENT SOIL IN TWO DIFFERENT FIELDS OF WEST BENGAL**

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### **INTRODUCTION**

The microbial activities in the earthworm gut content have been studied by numerous workers in India and abroad (Parle, 1963; Dash and Cragg, 1972; Dash *et al.*, 1979; Scheu, 1987; Edwards and Fletcher, 1988; Barois, 1992; Daniel and Anderson, 1992; Kristufek *et al.*, 1992; Edwards and Bohlen, 1996; Wolter and Scheu, 1999). Some researchers have studied the activities of fungi flora in different types of soil (Choudhary and Sachar, 1934; Ghatak and Roy, 1939; Gujarati, 1968; Hazra, 1984; Hazra and Choudhuri, 1990 and Bhattacharyya and Hazra, 1997).

However, in India so far no serious attempt have been made to study the microbial association of earthworm inhabiting in single crop mango vegetation and a cultivated field of mixed vegetables which changes with season. In view of this, the present study was conducted to estimate the quantitative and qualitative microbial association of earthworm (*Perionyx excavatus* Perrier) gut content, cast and adjacent soil.

### **MATERIALS AND METHODS**

The collected earthworms were washed thoroughly with fresh tap water, followed by sterile double glass distilled water for several times. The anterior gut, mid gut and hind gut region (each 1 cm.) have been dissected out aseptically from fresh living earthworm. Dissected parts were then kept separately in 10 ml. sterile water and homogenized thoroughly aseptically in homogenizer.

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One ml. of this homogenate was plated in Nutrient agar (Beef extract 3.0 g, Peptone 5.0 g, NaCl 8.5 g, Agar 20.0 g, Distilled water 1000 ml., pH  $7.4 \pm 0.2$ ), Potato Dextrose agar (Potato infusion 200.00 gm, Dextrose 20.00 gm, Agar 15.00 gm, Water 1000.00 ml., pH  $5.1 \pm 0.2$ ), Actinomycetes isolation agar (Sodium Caseinate 2.00 gm, L-asparaginase 0.10 gm, Sodium Propeonate 4.00 gm, Dipotassium Phosphate 0.50 gm, MgSO<sub>4</sub> 0.10 gm, FeSO<sub>4</sub> 0.001 gm, Agar 15.00 gm, Water 1000.00 ml, pH  $8.1 \pm 0.2$ ), Brain heart infusion agar (infusion from Beef Heart 250.00 gm, infusion from Calf Brain 200.00 gm, Proteose Peptone 10.00 gm, NaCl 5.00 gm, Disodium Phosphate 2.50 gm, Dextrose 2.00 gm, Agar 15.00 gm, Water 1000.00 ml, pH  $7.4 \pm 0.2$ ) in triplicates.

Separate soil samples (0–25 cm deep profile) for the analysis of soil factor and for culture of microbial flora were drawn at the same time when collection of earthworm was made. Freshly produced casts were collected by using a sterilized forcep and spatula. One gm each of soil and cast were inoculated in the same media by conventional dilution plate method in triplicates, 30°C and 37°C temperatures were maintained for incubation of fungi and bacteria-actinomycetes respectively. Periodical observations have been made after incubation for a period over seven days. Identification of fungi has been made as per Alexopoulos *et al.*, (1996) and bacteria-actinomycetes genera have identified by series of biochemical tests as per Kanwar *et al.*, (1997) and Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 2000).

Organic carbon content of the soil was determined by "Rapid Titration Method" (Walkley and Black, 1934). Soil thermometer was used to record the temperature of the soil. Soil humidity was estimated by dial hygrometer (Huger-85 mm.—Model-8265).

## CHARACTERISTICS OF THE STUDY SITES

**Site-I :** The site is located at Dhapa, East Kolkata and main constituents elements are garbage material of Kolkata city. Major portion of the field is now converted for cultivation of mixed vegetable crops like cauliflower, cabbage, cucurbita, lettuce, raddish etc. Besides maize are also grown during monsoon. The defoliated vegetable leaves are principal litter elements in the site. The site is devoid of any tree species. The soil is alluvial in nature, sandy loam in texture, blackish brown in colour and mainly comprised of decomposed and semidecomposed litter.

**Site-II :** The site is nearly 40 km. north of Kolkata, in the district of North 24 Parganas, West Bengal. The site is mainly covered with mango trees (*Mangifera indica*). Scattered cowdung in the field were observed may be due to occasional visit of the domestic cattles for grazing. The soil in this site is alluvial in nature, brownish in colour and clay loam in texture.

## OBSERVATIONS

### Microbial communities :

The microorganism *viz.*, bacteria-actinomycetes and fungi obtained from the soil, earthworm gut contents and casts of both the sites belonged to twelve genera of bacteria-actinomycetes and twelve species under eight genera of fungi flora (Table 1). The bacteria-actinomycetes were most dominant group found in surrounding soil of both the sites followed by fungi flora. The bacteria-actinomycetes genera *viz.*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Streptomyces* were most dominant and occurred from adjacent soil and earthworm cast of both sites (Tables 1, 2, 2a) followed by *Arthrobacter* found in the soil of site-I, II and earthworm cast of site-II and third dominant genus *Azotobacter* found only from the soil of both the sites (Table 1). The most dominant and well distributed bacteria-actinomycetes genera occurred from the different regions of guts of the earthworm species obtained from both the sites were *Bacillus*, *Micrococcus*, *Pseudomonas* and *Streptomyces* (Table 3, 3a). The *Perionyx excavatus* Perrier which was occurred from the site I showed maximum genera of bacteria-actinomycetes (8 nos.) each from anterior gut and midgut region and 5 genera from hindgut (Table 3). The earthworm obtained from site-II also showed highest number of bacteria-actinomycetes genera (7 nos.) from anterior gut followed by midgut and hindgut (5 nos. from each) (Table 3a).

The quantitative analysis of composition of bacteria-actinomycetes showed maximum in site-I (54 nos. of CFU  $\times 10^5$  gm) from adjacent soil and 72 nos. of CFU  $\times 10^5$  gm from the earthworm cast and site-II shows minimum (Table 4). The quantitative study of bacteria-actinomycetes in earthworm gut regions shows their highest abundance (384 nos.) in hindgut of *P. excavatus* which was obtained from site-I followed by midgut (279 nos.) and minimum from anterior gut (152 nos.). The earthworm which was obtained from site-II also showed that maximum bacteria-actinomycetes are harbouring in hindgut (276 nos.) and minimum in anterior gut (57 nos.) (Table 4a).

The fungi species obtained from soils and earthworm casts of the sites were represented by 9 species and 8 species respectively from soils of site-I and site-II, the cast of earthworm showed 3 species of fungi each from site-I and from site-II (Tables 1, 2, 2a). The *Penicillium rubrum* was most dominant and widely distributed fungus flora obtained from soils of site-I, II and also from the cast of site-I and II followed by *Mucor luteus* represented in both the sites in soil and cast from site-I, the species *Rhizopus nigricans* also holds the same positions which was found in all the sites mentioned above except from the cast of site-I (Table 1, 2, 2a). It is evident from Table 3, 3a the maximum number of fungi species were also found in anterior gut (8 each in site-I and II) followed by midgut (6, 7 respectively in site-I and II) and minimum in hindgut (4, 5 respectively in site-I, II) of *P. excavatus* occurring in site-I and II (Tables 3, 3a).

The *Penicillium rubrum* also showed their maximum activities in the anterior gut, midgut and hindgut of the earthworm species in both the sites (Table 3, 3a).

The fungal population was abundant in adjacent soil of site-I (22 nos. of CFU  $\times$  10<sup>3</sup>/gm) and minimum in site-II. The earthworm cast in site-I bounds maximum (31 nos. of CFU  $\times$  10<sup>3</sup>/gm) fungi in comparison to the cast obtained from site-II. It is evident from Table 4 that the fungi population showed their maximum abundance in the earthworm cast than the culture made from surrounding soil in both site-I, II.

The analysis of different gut regions of the earthworm species showed higher population of fungi were in the anterior gut followed by midgut and lowest in the hindgut region in both the sites (Table 4a).

#### Soil factors :

In site-I the average organic carbon content, sub soil humidity were high (3.25% and 78% respectively) in comparison to the site-II. But the subsoil temperature (25.5°C) was more in site-II than site-I (21.9°C) (Table 5). The maximum and minimum of the above soil factors are shown in Table 5.

## DISCUSSION

The results presented were based on a sample survey of two types of fields. The earthworm species, bacteria-actinomycetes and fungi belong to one genus, twelve genera and eight genera respectively. The number of genera of bacteria-actinomycetes and species of fungi differed in their abundance from one site to other (Table 1). Moreover, the number of bacteria-actinomycetes genera (11 genera) and fungi species (9 species) were maximum in site-I and the corresponding minimum number were 7 genera and 8 species in site-II. In adjacent soil five genera of bacteria-actinomycetes were restricted to site-I and one genus to site-II, while six genera of bacteria-actinomycetes were found to occur in both the habitats in the present study (Table 6). These genera, therefore, are "ubiquitous" as they were occurring in widely different habitats. In case of fungi four species were restricted to site-I and three species to site-II and five species of fungi viz., *Penicillium rubrum*, *Trichoderma viride*, *Mucor luteus*, *Cladosporium herbarum* and *Rhizopus nigricans* were found to occur in both the sites are also "ubiquitous" for their occurrence from distinctly different habitat (Table 6a). Similar results were obtained by Bodvarson (1961), Gujarati (1968), Hagvar (1982) and Hazra and Sanyal (1996) while studying with the fungi flora in cultivated fields and soil micro arthropod fauna respectively. Tables 6 to 9a clearly exhibit the "stenoecious" and "euryecious" bacteria-actinomycetes and fungi occurred from the cast and different regions of gut of the earthworm species *P. excavatus* of the studied sites-I and II.

The gradual increase of number of bacteria-actinomycetes (Table 4a) from the anterior gut to hind gut in this species of earthworm occurred from both the sites may be due to the epigeic nature

of *P. excavatus*, generally feeds on litter enriched with organic matter. When these passes through the gastrointestinal tract it accumulates in hind gut which provides an optimum microclimatic conditions for the profuse growth of the bacteria-actinomycetes population. It coincides with the findings of Karsten and Drabe (1995, '97) and Ihssen *et al.*, (2003). A distinct reduction of fungi population from the anterior gut to the hind gut has been observed (Table 4a) of this earthworm species, which indicates that the hyphae and spores of most of the fungi species serve as their food. Moreover, numerically lower population of fungi in the mid and hind gut suggests a gradient exists with regard to the digestion of fungi in different regions of this earthworm gut. Similar findings were also made by Dash *et al.*, (1979). Both the bacteria and fungi population in the cast of the studied earthworm were significantly higher in both the sites in comparison to microbial population of the adjacent soil (Table 4). This might be due to reason that the cast usually rich in ammonia and partially digested organic matter and some amount of egested intestinal mucus which provide good substrate for microbial growth (Edwards and Bohlen, 1996).

It has been observed that *Escherichia coli*, *Enterobacter* sp., *Flavobacterium* sp. present in adjacent soil, anterior gut, mid gut but were absent in the hind gut and casts of this earthworm species. This might be due to release of some inhibitory factors secreted from the hind gut of earthworm which perhaps prevents the growth of these organisms (Table 3). Similar result was also obtained in *Metaphire* sp. by Khambata and Bhatt (1957).

It is evident from the Table 3 that bacteria-actinomycetes and fungi *viz.*, *Cytophaga* sp. and *Cephalosporium asperum* were not found in the gut of earthworm, but their occurrence in the adjacent soil suggests that the studied earthworm species do not prefer these microorganisms as their food. The selective feeding of the earthworm was also suggested by Dash *et al.*, (1979) and Satchell (1983).

The average values of soil factors like organic carbon and relative humidity were maximum in the site-I in comparison to site-II. The microbial population also showed their maximum abundance in the site-I (Tables 4, 5). This result coincides with the findings of Gujarati (1968), Hazra (1984), Bhattacharyya and Hazra (1997). The subsoil temperature in this study might have exerted a cumulative effect with other factors (Hazra and Choudhuri, 1990) on soil microorganisms.

It might be concluded from the present study that (i) Mixed cultivated vegetations supports maximum abundance and diversity of microorganisms in the surrounding soil as well as the microorganisms occurred inside the gut contents of *P. excavatus*. (ii) The hindgut of *P. excavatus* bounds appreciably higher bacteria-actinomycetes population and anterior gut harboured maximum fungi flora. The present study showed that the earthworm *P. excavatus* has wide range of tolerance of habitat conditions and their capacity to utilize micrororganisms of surrounding soil as their source of food for survival. However, final conclusion can be made only after detailed study under laboratory condition.

**Table 1** : Characteristics of sampling sites and microbial communities.

		Site-I (mixed vegetation)	Site-II (Mango orchard)
Dominant plant species		No single dominant plant	<i>Mangifera indica</i>
Other vegetations		<i>Lactuca sativa</i> Linn., <i>Zea mays</i> Linn., <i>Brassica oleracea</i> Linn., var. <i>capitata</i> , <i>Brassica oleracea</i> Linn., var. <i>botrytis</i> , <i>Cucurbita pepo</i> Linn., <i>Raphanus sativus</i> Linn., <i>Solanum nigrum</i> Linn., <i>Coccinia cordifolia</i> , <i>Marsilea minuta</i> , <i>Colocasia esculenta</i>	<i>Annona squamosa</i> Linn., <i>Cocos nucifera</i> Linn., <i>Musa paradisiaca</i> , <i>Cynodon dactylon</i> , <i>Chrysopogon aciculatus</i> Trin, <i>Solanum nigrum</i> Linn., <i>Centella asiatica</i> Urb., <i>Coccinia cordifolia</i> , <i>Eclipta rostrata</i> Linn., <i>Marsilea minuta</i> , <i>Colocasia esculenta</i>
Bacteria-actinomycetes and Fungi communities	Bacteria-actinomycetes	<ol style="list-style-type: none"> <li>1. <i>Bacillus</i> sp</li> <li>2. <i>Micrococcus</i> sp</li> <li>3. <i>Arthrobacter</i> sp</li> <li>4. <i>Azotobacter</i> sp</li> <li>5. <i>Pseudomonas</i> sp</li> <li>6. <i>Escherichia coli</i></li> <li>7. <i>Enterobacter</i> sp</li> <li>8. <i>Flavobacterium</i> sp</li> <li>9. <i>Streptomyces</i> sp</li> <li>10. <i>Promicromonospora</i> sp</li> <li>11. <i>Cytophaga</i> sp</li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Bacillus</i> sp</li> <li>2. <i>Micrococcus</i> sp</li> <li>3. <i>Nocardia</i> sp</li> <li>4. <i>Arthrobacter</i> sp</li> <li>5. <i>Azotobacter</i> sp</li> <li>6. <i>Pseudomonas</i> sp</li> <li>7. <i>Streptomyces</i> sp</li> </ol>
	Fungi	<ol style="list-style-type: none"> <li>1. <i>Penicillium rubrum</i></li> <li>2. <i>Penicillium humicola</i></li> <li>3. <i>Aspergillus niger</i></li> <li>4. <i>Fusarium roseum</i></li> <li>5. <i>Trichoderma viride</i></li> <li>6. <i>Rhizopus nigricans</i></li> <li>7. <i>Cephalosporium asperum</i></li> <li>8. <i>Mucor luteus</i></li> <li>9. <i>Cladosporium herbarum</i></li> </ol>	<ol style="list-style-type: none"> <li>1. <i>Penicillium rubrum</i></li> <li>2. <i>Aspergillus flavus</i></li> <li>3. <i>Fusarium nivale</i></li> <li>4. <i>Trichoderma viride</i></li> <li>5. <i>Rhizopus nigricans</i></li> <li>6. <i>Mucor hiemalis</i></li> <li>7. <i>Cladosporium herbarum</i></li> <li>8. <i>Mucor luteus</i></li> </ol>

**Table 2** : List of Bacteria-actinomycetes and Fungi isolated from the casts of *P. excavatus* in site-I.

Bacteria-actinomycetes	Fungi
1. <i>Bacillus</i> sp	1. <i>Penicillium rubrum</i>
2. <i>Micrococcus</i> sp	2. <i>P. humicola</i>
3. <i>Pseudomonas</i> sp	3. <i>Mucor luteus</i>
4. <i>Streptomyces</i> sp	
5. <i>Promicromonospora</i> sp	

**Table 2a** : List of Bacteria-actinomycetes and Fungi isolated from the casts of *P. excavatus* in site-II.

Bacteria-actinomycetes	Fungi
1. <i>Bacillus</i> sp	1. <i>Penicillium rubrum</i>
2. <i>Micrococcus</i> sp	2. <i>Aspergillus flavus</i>
3. <i>Pseudomonas</i> sp	3. <i>Rhizopus nigricans</i>
4. <i>Streptomyces</i> sp	
5. <i>Arthrobacter</i> sp	

**Table 3** : List of Bacteria-actinomycetes and Fungi obtained from different regions of gut of *P. excavatus* in site-I.

Bacteria-actinomycetes	Anterior gut	Mid gut	Hind gut
1. <i>Bacillus</i> sp	+	+	+
2. <i>Micrococcus</i> sp	+	+	+
3. <i>Arthrobacter</i> sp	-	-	-
4. <i>Azotobacter</i> sp	-	-	-
5. <i>Pseudomonas</i> sp	+	+	+
6. <i>Escherichia coli</i>	+	+	-
7. <i>Enterobacter</i> sp	+	+	-
8. <i>Flavobacterium</i> sp	+	+	-
9. <i>Streptomyces</i> sp	+	+	+
10. <i>Promicromonospora</i> sp	+	+	+
11. <i>Cytophaga</i> sp	-	-	-

**Table 3 :** (Cont'd.).

Fungi		Anterior gut	Mid gut	Hind gut
1.	<i>Penicillium rubrum</i>	+	+	+
2.	<i>Penicillium humicola</i>	+	+	+
3.	<i>Aspergillus niger</i>	+	+	+
4.	<i>Fusarium roseum</i>	+	-	-
5.	<i>Trichoderma viride</i>	+	+	-
6.	<i>Rhizopus nigricans</i>	+	-	-
7.	<i>Cephalosporium asperum</i>	-	-	-
8.	<i>Mucor luteus</i>	+	+	+
9.	<i>Cladosporium herbarum</i>	+	+	-

+ isolated; - not isolated

**Table 3a :** List of Bacteria-actinomycetes and Fungi obtained from different regions of gut of *P. excavatus* in site-II.

Bacteria-actinomycetes		Anterior gut	Mid gut	Hind gut
1.	<i>Bacillus</i> sp	+	+	+
2.	<i>Micrococcus</i> sp	+	+	+
3.	<i>Nocardia</i> sp	+	-	-
4.	<i>Arthrobacter</i> sp	+	+	+
5.	<i>Azotobacter</i> sp	+	-	-
6.	<i>Pseudomonas</i> sp	+	+	+
7.	<i>Streptomyces</i> sp	+	+	+
<b>Fungi</b>				
1.	<i>Penicillium rubrum</i>	+	+	+
2.	<i>Aspergillus flavus</i>	+	+	+
3.	<i>Fusarium nivale</i>	+	-	-
4.	<i>Trichoderma viride</i>	+	+	-
5.	<i>Rhizopus nigricans</i>	+	+	+
6.	<i>Mucor hiemalis</i>	+	+	-
7.	<i>Cladosporium herbarum</i>	+	+	+
8.	<i>Mucor luteus</i>	+	+	+

+ isolated; - not isolated

**Table 4** : Showing number of microorganisms in surrounding soil and fresh casts in site-I and site-II.

Soil		
Microorganisms	Site-I	Site-II
Bacteria-actinomycetes (no. of CFU × 10 <sup>5</sup> /gm)	54	44
Fungi (no. of CFU × 10 <sup>3</sup> /gm)	22	13
Cast		
Microorganisms	Site-I	Site-II
Bacteria-actinomycetes (no. of CFU × 10 <sup>5</sup> /gm)	72	68
Fungi (no. of CFU × 10 <sup>3</sup> /gm)	31	19

**Table 4a** : Showing number of microorganisms in different regions of gut of *P. excavatus* from site-I and site-II.

Microorganisms	Site-I			Site-II		
	A. gut	M. gut	H. gut	A. gut	M. gut	H. gut
Bacteria-actinomycetes (no. of CFU × 10 <sup>1</sup> /cm)	152	279	384	57	79	276
Fungi (no. of CFU × 10 <sup>1</sup> /cm)	12	10	4	16	9	2

**Table 5** : Showing average soil temperatures, humidity and organic carbon at site-I and site-II. (from June, '01 to Aug, '03)

	Subsoil Temperature (°C)	Subsoil Humidity (%)	Organic carbon (%)
<b>Site-I</b>	21.90°C	78%	3.25%
Maximum	May (30°C)	Aug (98.5%)	Aug (4.23%)
Minimum	Jan (17°C)	Jan (80%)	Apr (2.21%)
<b>Site-II</b>	25.5°C	73%	1.64%
Maximum	May (31.5°C)	Aug (96%)	Dec (2.21%)
Minimum	Jan (17.5°C)	Apr (72%)	Mar (0.9%)

**Table 6** : “Euryecious” and “Stenoecious” genera of Bacteria-actinomycetes in adjacent soils of studied sites.

Genera restricted to Site-I	Genera restricted to Site-II	Genera present in both Sites
1. <i>Escherichia coli</i>	1. <i>Nocardia</i> sp	1. <i>Bacillus</i> sp
2. <i>Enterobacter</i> sp		2. <i>Micrococcus</i> sp
3. <i>Flavobacterium</i> sp		3. <i>Arthrobacter</i> sp
4. <i>Promicromonospora</i> sp		4. <i>Azotobacter</i> sp
5. <i>Cytophaga</i> sp		5. <i>Pseudomonas</i> sp
		6. <i>Streptomyces</i> sp

**Table 6a** : "Euryecious" and "Stenoecious" species of fungi in adjacent soils of studied sites.

Species restricted to Site-I	Species restricted to Site-II	Species present in both Sites
1. <i>Penicillium humicola</i>	1. <i>Aspergillus flavus</i>	1. <i>Penicillium rubrum</i>
2. <i>Aspergillus niger</i>	2. <i>Fusarium nivale</i>	2. <i>Trichoderma viride</i>
3. <i>Fusarium roseum</i>	3. <i>Mucor hiemalis</i>	3. <i>Rhizopus nigricans</i>
4. <i>Cephalosporium asperum</i>		4. <i>Mucor luteus</i>
		5. <i>Cladosporium herbarum</i>

**Table 7** : "Euryecious" and "Stenoecious" genera of bacteria-actinomycetes isolated from the cast of *P. excavatus* in studied sites.

Genera restricted to Site-I	Genera restricted to Site-II	Genera present in both Sites
1. <i>Promicromonospora</i> sp	1. <i>Arthrobacter</i> sp	1. <i>Bacillus</i> sp
		2. <i>Micrococcus</i> sp
		3. <i>Pseudomonas</i> sp
		4. <i>Streptomyces</i> sp

**Table 7a** : "Euryecious" and "Stenoecious" species of fungi isolated from the cast of *P. excavatus* in studied sites.

Species restricted to Site-I	Species restricted to Site-II	Species present in both Sites
1. <i>Penicillium humicola</i>	1. <i>Aspergillus flavus</i>	1. <i>Penicillium rubrum</i>
2. <i>Mucor luteus</i>	2. <i>Rhizopus nigricans</i>	

**Table 8** : "Euryecious" and "Stenoecious" genera of bacteria-actinomycetes found in different gut regions of *P. excavatus* in site-I.

Genera restricted to Anterior Gut	Genera restricted to Mid Gut	Genera restricted to Hind Gut	Genera present in All Guts
Nil	Nil	Nil	1. <i>Bacillus</i> sp
			2. <i>Micrococcus</i> sp
			3. <i>Pseudomonas</i> sp
			4. <i>Streptomyces</i> sp
			5. <i>Promicromonospora</i> sp

**Table 8a** : “Euryecious” and “Stenoecious” species of fungi found in different gut regions of *P. excavatus* in site-I.

Species restricted to Anterior Gut	Species restricted to Mid Gut	Species restricted to Hind Gut	Species present in All Guts
1. <i>Rhizopus nigricans</i>	Nil	Nil	1. <i>Penicillium rubrum</i>
2. <i>Fusarium roseum</i>			2. <i>Penicillium humicola</i>
			3. <i>Aspergillus niger</i>
			4. <i>Mucor luteus</i>

**Table 9** : “Euryecious” and “Stenoecious” genera of bacteria-actinomycetes found in different gut regions of *P. excavatus* in site-II.

Genera restricted to Anterior Gut	Genera restricted to Mid Gut	Genera restricted to Hind Gut	Genera present in All Guts
1. <i>Nocardia</i> sp	Nil	Nil	1. <i>Bacillus</i> sp
2. <i>Azotobacter</i> sp			2. <i>Micrococcus</i> sp
			3. <i>Pseudomonas</i> sp
			4. <i>Streptomyces</i> sp
			5. <i>Arthrobacter</i> sp

**Table 9a** : “Euryecious” and “Stenoecious” species of fungi found in different gut regions of *P. excavatus* in site-II.

Species restricted to Anterior Gut	Species restricted to Mid Gut	Species restricted to Hind Gut	Species present in All Guts
1. <i>Fusarium nivale</i>	Nil	Nil	1. <i>Penicillium rubrum</i>
			2. <i>Aspergillus flavus</i>
			3. <i>Rhizopus nigricans</i>
			4. <i>Cladosporium herbarum</i>
			5. <i>Mucor luteus</i>

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