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Microorganisms in the Food, Gut Contents and Faeces of *Burmoniscus kempi* (Isopoda; Philosciidae)

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ABSTRACT

Fungal and bacterial growth remained more or less constant in the control chamber, but in the experimental chamber the fungal standing crop was reduced by all levels of isopod feeding. However bacterial standing crop increased with the number of isopods. Numbers of colony forming units of fungi and bacteria were higher in faces than in the litter. While bacterial growth was stimulated in the midgut (12 x 10^6 g^{-1} dry mass), hindgut (14 x 10^6 g^{-1} dry mass) and faeces (17 x 10^6 g^{-1} dry mass) and then increased in the faeces (7.1 x 10^4 g^{-1} dry mass).

INTRODUCTION

Interaction between microorganisms and litter feedling fauna plays an important role in the decomposition process of plant litter (Coleman and Crossley 1996). Soil fauna may graze directly on fungi and bacteria (Binet and Trehen 1992) or indirectly affect them by feeding on litter, producing faecal pellets (Scheu and Wolters 1991). The grazing activities may have other significant interactive effects, for instance in improving the release of nutrients immobilized in microbial tissues (Forster *et al.* 1995), which is equally important to the functioning of the decomposers sub-system.

Influences of soil fauna on soil microorganisms are currently an active research area, judging from the amount of materials being published on this topic. The impact of isopod feeding activities on microorganisms and mineralization in the soil are meager. However there is enough evidence from laboratory studies to suggest that microbial activities are stimulated by interactions with the soil fauna (Corsmann 1990). The complimentary question of how such feeding affects fungi and bacteria in the soils of North Eastern India has so far been neglected and received little attention. These considerations tempted us to design a series of experiments to examine the feeding activities of isopods on fungi and bacteria by analyzing their litter, gut contents and faces

MATERIALS AND METHODS

Preparation of litter samples: Leaf litter of *Alnus nepalensis* D. Don was collected from the natural site situated at 6.5 km up from Shillong (altitude 1500m MSL, latitude 25° 34' N; longitude 91° 56' E). The litter sample was air dried at 25° C and brushed to remove the faecal material and debris. Midribs were removed and the remaining laminae cut into small fragments (2mm x 2mm).

Experimental Setup: The litter fragments were mixed well and aliquots of 2g were placed in experimental chambers similar to the experimental design described by Anderson and Ineson (1982). It consisted of an outer Perspex tube, which was modified to provide a sloping base for draining and leaching port. The litter sample was kept within an inner removable container. The inner container rested upon alkathene beads which acted as an inert supporting medium of low surface area. The sample was held in place by find nylon mesh to support the litter and allow the faeces to fall clear of feeding fauna. Leaching of the microcosms was achieved by flooding the experimental material through the leaching port using a large syringe. The litter was rehydrated by the addition of 100ml distilled water and keeping for 24 h in each experimental chamber. This treatment leached out soluble tannins and readily metabolized materials mobilized by the drying and wetting

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regime. Then the fresh leaf litter was macerated in distilled water to produce a suspension which was used to inoculate the soaking litter and then incubated at 18° C. Isopods were not added to the experimental chambers during an initial three week-period, thus permitting the establishment of microorganisms.

Burmoniscus kempi (Isopoda; Philosciidae) were collected from the field of alder forest and allowed to feed in the laboratory for several days on alder litter collected from the same site. Before being introduced into the experimental chambers, the isopods were allowed to starve for 72 h in order to void their guts of prior contents. The small amount of food that could remain in the gut was egested upon resumption of feeding. Subsequently, 0, 2, 5, 10 and 15 isopods were introduced in experimental chambers separately in replicates, and the experiment was carried out for 40 days. Three replicates were destructively sampled on each occasion. Subsequent experiments were carried out using groups of 25 isopods to determine the distribution of fungi and bacteria in their guts.

Fungal and bacterial standing crop: Alder litter from the sampled experimental set up was examined for fungal and bacterial standing crop, using the membrane filter technique (Hanseen *et al.* 1974). A 1.0 g sample of leaf litter was homogenized in sterile distilled water and 1 ml of the sub-samples of the homogenate were stained with 1 ml (0.1% w/v) aqueous phenylaniline blue. The stained material was flushed through a 25 mm cellulose acetate membrane (pore size 0.22 μm) and mounted for microscopic examination. Bacterial numbers and fungal hyphal length were converted to standing crop estimates (Parkinson *et al.* 1971).

Determination of fungi and bacteria in litter and intestinal tract of B. *kempi:* Litter samples from the experimental chambers were removed and taken for dry mass determination at 105° C. Simultaneously, 0.5 g wet mass of litter was removed, placed in a sterile conical flask containing 4 ml peptone water (0.1%), shaken for 2-5 min to disintegrate the sample materials, and filtered through a sterile 2 mm-pore mesh nylon filter with the filtrate being used to prepare a dilution series for counting of colony forming units (cfu) of fungi and bacteria. The faecal material collected from the bases of the microcosm was also processed in the same way.

Isopods (*B. kempi*) were removed from the experimental chambers and surface sterilized in sodium hypochlorite solution (1.6% available chlorine). They were blotted dry on sterile paper and the body cavity was opened ventrally and flooded with insect Ringer's solution. The gut was partitioned *in situ* by a double placed between the midgut and the hindgut and an additional single ligature was placed around the oesophagus and the rectum. Midgut and hindgut were dissected free and agitated gently in Ringer's solution to remove contaminable. The sections were then transferred to 4ml peptone water in a conical flask and shaken to disintegrate the gut wall and disperse the contents. Then it was filtered through a Millipore membrane (pore size 0.45 μm), gut tissue and ligatures being removed and the oven dried mass of litter fragments in that section of the gut was determined. Dilution series in peptone water were prepared from the gut and faecal samples. Fungi were isolated on Rose Bengal agar containing streptomycin (10mg/l) to suppress bacterial growth. Bacteria were isolated on nutrient agar added with nystatin (10mg ml^{-1}) to suppress fungi. The plates were incubated at 20° C for 5-10 days for fungi and 2-3 days at 25°C for bacteria and cfu were counted. Identification of the fungi was done following Gilman (1957) and Domsch *et al.* (1980). No attempt was made to identify bacteria.

RESULTS AND DISCUSSION

Isopod grazing activity reduced fungal standing crop but increased bacterial standing crop (Table 1) as observed earlier (Kayang *et al.* 1994). The faecal particles contained more cfu of fungi and bacteria than the guts and leaf litter. Extensive bacterial growths were detected in the guts, while fungal growth decreased along the length of the intestinal tract, but then increased in the faeces (Table 2). This phenomenon was regulated by isopod grazing as fungi are sensitive to such feeding and thus render favourable environment for the multiplication of bacteria. (Bouwman *et al.* 1994). The high numbers of cfu count of bacteria in the intestinal tract of the isopod studies suggests a significant contribution to digestive processes (Cazemier *et. al.* 1997). Altogether 29 fungal species were isolated from the litter microcosms, 26 species from the gut content (19 in midgut and 10 in hindgut) and 16 species from the faeces of isopods. Species that subsist in the intestinal tract were *Mucor hiemalis, Aspergillus flavus, A. niger, Cladosporium cladosporioides, Fusarium moniliforme, F. oxysporum, Penicillium chrysogemum P. citrimum, Trichoderma viride*, and *T. harziamum.* All of them were present in the faeces. The faeces were recolonised by some species that had disappeared

during the transit time, such as *Aspergillus nidulans, Fusarium solani, Humicola grisea, Paecelomyces verioti, Geotrichum candidum* and white sterile mycelia (Table 3). The feeding activity in such micro-ecosystems may be species specific because some fungi or the decomposing litter remained unaffected but there was an overall reduction in the total fungal counts. The generally low counts of fungi in intestinal tract suggest that the fauna were destroying fungi during digestion. Furthermore the particularly low count in litter suggests active grazing on the part of isopod. However, the relative contributions of these processes have not been quantified. The faecal particles contain significantly more microorganisms than the ingested litter and may thus form micro-habitats of intense microbial activity (Hassal *et al.* 1987). Lavelle *et al.* (1994) have also reported that the faecal particles of soil fauna are a more favourable habitat for soil microorganisms than whole leaf litter. The high population of bacteria in the guts was related to the favourable micro-environment and nutrients provided for the multiplication of bacteria that were ingested along with the litter (Ullrich *et al.* 1991) or that it contains a high density reservoir of microortanisms which invade the food as it is passing through.

Table 1. Fungal (F) and Bacterial (B) standing crop (mg/g)

Time	Control		Number of Isopods							
	F	B	F	B	F	B	F	B	F	B
Days	(0)		(2)		(5)		(10)		(15)	
5	4.4	1.8	4.1	2.1	3.7	2.4	3.3	2.8	2.8	3.0
10	4.7	1.9	3.6	2.4	3.2	2.6	2.9	3.1	2.2	3.3
15	4.6	2.1	3.1	2.8	2.9	3.1	2.4	3.5	1.9	4.1
20	4.7	2.0	2.7	3.4	2.4	3.6	1.9	3.9	1.6	4.7
25	4.8	2.1	2.2	3.8	2.0	4.2	1.6	4.4	1.1	5.2
30	4.8	2.1	1.9	4.3	1.6	4.5	1.4	4.7	0.7	5.4
35	4.7	1.9	1.7	4.9	1.2	5.2	0.9	5.8	0.4	6.1
40	4.4	1.8	1.5	4.4	1.0	4.4	0.7	4.8	0.2	4.9

Table 2. Total counts of fungi and bacteria in litter, gut and faeces of isopods

Colony forming units	Faeces	Litter	Midgut	Hindgut
Fungi (10^4 x g/dry mass)	4.7	3.5	2.3	7.1
Bacteria (10^6 x g/dry mass)	7.0	12	14	17

A general decrease in the number of fungal species from litter to intestinal tract suggests that the gut of isopods can be selective and the proliferation of a single species can assist both in metabolism and in the elimination of other microbes ingested with the food (Kayang 1998). High spore producing fungi dominated the intestinal tracts rather than either less spore producing species. This indicated that certain species were more resistant to the isopod digestive enzymes as compared to mycelial forms and this largely affected the species composition of microbial communities. Such a selective pressure allowed to dominate *P. chrysogenum, P. citrinum, Trichoderma viride, T. harzianum, F. moniliforme, F. oxysporum, C. cladosporioides, Aspergillus flavus, A. niger* and *mucor hiemalis* with their saprophytic and pathogenic ability (Kayant *et al.* 1996).

Table 3. Distribution of percentage relative abundance of fungi in the litter, gut contents and faeces of *Burmoniscus kempi*

Fungal species	Litter	Gut contents		Faeces
		Midgut	Hindgut	
Absidia cylindrospora	3.55	-	-	-
A. glauca	1.76	-	-	-
Mucor hiemalis	6.71	6.40	11.12	7.80
M. racemosus	1.76	4.00	-	-
Rhizopus oryzae	3.55	-	-	-
R. stolonifer	6.71	6.40	-	-
Pythium sp	1.76	-	-	-
Alternaria alternata	3.55	4.00	-	-
Aspergillus candidus	6.71	6.40	-	-
A. flavus	3.55	6.40	-	-
A. nidulans	1.76	4.00	-	5.80
A. niger	6.71	6.40	11.12	7.80
Cladosporium cladosporioides	3.55	6.40	11.12	7.80
C. herbarum	1.76	4.00	-	-
Fusarium moniliforme	6.71	6.40	11.12	7.80
F. oxysporum	3.55	4.00	8.32	7.80
F. Solani	1.76	-	-	4.10
Penicillium chrysogenum	6.71	6.40	11.12	7.80
P. citrinum	3.55	4.00	8.32	5.80
P. funiculosum	1.76	4.00	-	-
Trichoderma harzianum	3.55	6.40	8.32	5.80
T. viride	6.71	6.40	8.32	7.80
Humicola grisea	1.76	4.00	-	4.10
Paecilomyces verioti	1.76	4.00	-	4.10
Verticillium chlamydosporum	1.76	-	-	-
Geotrichum candidum	1.76	-	-	4.10
Aureobasidium pullulans	1.76	-	-	-
White sterile mycelia	1.76	-	-	5.80
Black sterile mycelia	1.76	-	-	-

By feeding on microorganisms associated with leaf litter the isopod affects the decomposition indirectly as the isopod egestus is a more ideal medium for microbial growth than the uneaten litter (kayang *et al.* 1996). Quantitative and qualitative removal of fungi either through selective grazing or by changing the nutrient availability of the litter may affect microbial growth, species composition and metabolic activities. Further research is being initiated to examine the significance of intestinal microorganisms for the digestion of food by measuring fermentation patterns and enzyme activities.

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