Microscopic fungi isolated from *Fridericia galba* (Oligochaeta, Enchytraeidae)

Mikroskopické houby izolované z roupice *Fridericia galba* (Oligochaeta, Enchytraeidae)

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Microscopic fungi were isolated from the enchytraeid *Fridericia galba* (Hoffmeister, 1843), and from surrounding soil. Isolation was done from aseptically squashed enchytraeid bodies and from enchytraeid excrements prepared by defaecation into sterile conditions. The cultivation media were soil extract agar, cherry decoction agar and beer wort agar. A total of 39 fungal species was found, 29 in the excrements and 19 in the soil. In most cases, fungi isolated from the soil differed from those isolated from the excrements. Feeding preference and seasonal variation were not demonstrated. Spores of fungi were viable after passage through the enchytraeid gut. The biochemical activity of the isolated fungi indicates preferential feeding on plant remains where the degradation of cellulose, pectin and xylose predominates.

Mikroskopické houby byly izolovány z roupic druhu *Fridericia galba* (Hoffmeister, 1843) a z okolní volné půdy. Izolace byla provedena jak z roupic asepticky rozdrcených, tak z exkrementů roupic získaných defekací ve sterilním prostředí. Kultivačními médii byly půdní agar, třešňový agar a sladinový agar. Celkem bylo zjištěno 39 druhů mikromycetů, z toho 29 v exkrementech a 19 v půdě. Mikroskopické houby izolované z půdy se ve většině případů lišily od hub zjištěných v exkrementech. Selektivní příjem hub roupicemi ani sezónní dynamika výskytu hub v exkrementech nebyly pozorovány. Spóry hub byly po průchodu střevem roupic životaschopné. Biochemická aktivita izolovaných mikromycetů naznačuje, že roupice dávají přednost potravě složené z rostlinných zbytků, kde dochází k rozkladu celulózy, pektinu a xylózy.

Introduction

Relations between soil animals and soil microorganisms affect many biochemical processes proceeding in the soil environment but knowledge of the gut microflora of soil animals, its activity and function is yet incomplete. Enchytraeid worms *(Oligochaeta, Enchytraeidae)* are important components of the soil mesofauna. They inhabit the uppermost soil layers in quantities of thousands per square metre (O'Connor 1967) and participate in the formation of the soil microstructure. Information on enchytraeids and their food requirements has been published by O'Connor (1967), Striganova (1980) and Dunger (1983)). They feed mostly on dead or decaying organic matter; some species prefer organic or mineral material and others are more catholic in their feeding. Striganova (1980) classed enchytraeids as detritivores inclined to selective mycophagy. Dash et Cragg (1972), Kozlovskaya (1976) and Dash et al. (1980) studied the occurrence of fungi in enchytraeid intestines and found the gut to contain a high percentage of fungi, however, Dash et Cragg (1972) only cultivated the fungi from the anterior part of the digestive tube.

The aim of this investigation was to study the fungi of the intestine and the excrements of *Fridericia galba* (Hoffmeister, 1843) and to compare them with those in the surrounding soil. This study was a part of a research project on the interactions between soil microflora,

soil fauna and soil processes, undertaken by the Institute of Soil Biology, Czechoslovak Academy of Sciences.

Material and methods

Samples of brown soil for heat extraction of Fridericia galba were collected within the town of České Budějovice. South Bohemia, in November 1986 and in April, May and June 1987. Soil samples for fungal isolation were taken at the same site on each sampling occasion.

Two methods were employed to isolate fungi from enchytraeids: 1, isolation from squashed animals (e.g. Dash et Cragg /1972/) and 2, isolation from enchytraeid excrements. The first method was used in 1986, and the second in 1986 and 1987. In the latter method, the bulked sample of twenty individuals was washed into sterile distilled water and then transferred into a sterile glass chamber with wet filter paper covering the base and placed at 4°C for 24 hours. The enchytraeid excrements obtained were mixed with 50 ml of water. One ml of resulting suspension was added to each of three Petri dishes. Media used were soil extract agar (SEA), cherry decoction agar (CDA) and beer wort agar (BWA) with bengal red (Booth 1971; Fassatiová 1979; Gams 1980). Bacterial growth was suppressed with streptomycin. The plates were incubated at 25°C for 7 days. Fungi from the surrounding soil were isolated by the soil dilution method (Garrett 1963) from 1ml of soil suspension (dilution $1:10^4$).

Results and discussion

The investigation of soil fungi in enchytraeids was carried out in two steps. Firstly, the efficacy of both the squashed enchytraeid body method and enchytraeid excrement method was estimated; secondly, the investigation was completed using the latter method. Isolation of fungi from enchytraeid excrements was more effective as it produced a higher number of fungal isolates and also indicated the viability of fungal propagules passing through the enchytraeid intestine (Table 1). Kozlovskaya (1976) states that enchytraeids can digest some fungal hyphae and since the excrements contain higher numbers of fungi than the surrounding soil, she suggested that the indigestible fungi multiplied in the intestine. Dash et Cragg (1972), using the squash method, isolated fungi only from the anteclitellar part of the intestine and therefore they did not consider the spores to be viable after passing through the intestine. Whether this inactivation of propagules was brought about by the intestinal activity or by the treatment used in the experiment was not distinguished. Dash et al. (1980) reported high numbers of fungal species from the anterior part of intestine. In 1987, fungi were isolated from enchytraeid excrements and from surrounding soil. On five sampling occasions a total of 39 fungal species was isolated; 19 from the soil and 29 from the excrements (Table 2). This table shows that on each sampling occasion the species isolated from the excrements were different from those isolated from the soil. There was no sign of enchytraeid feeding preference or seasonal variation. The wide variety of isolates indicates that fungal spores and hyphae are viable after passage through the intestine. Biochemical activities listed in Domsch et al. (1980) show that most of the fungi isolated from the excrements are able to decompose cellulose, pectin, starch and xylose. These

compounds are chiefly derived from plant remains and it would seem that the enchytraeids search out the microsites where the half decayed remains of plants occur and that they prefer ingesting soil containing products of degradation of structural and storage polysaccharides.

Conclusions

1. The method of isolation of fungi from enchytraeid excrements was shown to be more effective than isolation from squashed enchytraeid body.

2. Higher number of fungal species was isolated from enchytraeid excrements than from the surrounding soil; the soil often yielded fungi not isolated from the excrements.

3. Enchytraeid feeding preference and seasonal variation of fungal isolates were not demonstrated.

4. Assessment of the biochemical activity of the fungal isolates according literature indicates that the enchytraeids preferred to ingest soil particles containing amounts of decaying structural polysaccharides (pectin, xylose and cellulose).

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Table 1 – A list of microscopic fungi isolated from *Fridericia galba* using squash method and method of isolation from enchytraeid excrement (SEA – soil extract agar, CDA – cherry decoction agar, BWA – beer wort agar).

	squash enchytraeid			excrements		
	SEA	CDA	BWA	SEA	CDA	BWA
Acremonium sp. I						+
Acremonium sp. II				+		
Aspergillus oryzae						+
Monocillium sp.				+		
Penicillium janthinellum		+				
Penicillium sp.					+	
sterile dark mycelium III					+	
sterile dark mycelium V				+		
sterile dark mycelium VI						+
Tolypocladium niveum				+	+	+
Trichoderma hamatum	+	+	+			+
Trichophyton sp.						+
undetermined species of *Ascomycetes*						+
undetermined species of *Moniliales*						
Verticillium sp.						+
total isolated species		2			13	

Table 2 – A list of microscopic fungi isolated in 1987 from excrements of *Fridericia galba* (E) and from surrounding soil (S).

	6 Apr	14 May		27 May		1 June		29 Jun	
	E	E	S	E	S	E	S	E	S
Acremonium butyri						+			
Acremonium murorum						+			
Acremonium strictum									+
Beauveria bassiana								+	
Chloridium virescens var. virescens	+							+	
Cladosporium cladosporioides			+	+					
Cladosporium herbarum				+		+	+		+
Cladosporium sphaerospermum					+				
Cylindrocarpon destructans	+	+		+		+	+		
Fusarium sp.									+
Gliocladium roseum		+		+					
Humicola fuscoatra				+					
Humicola grisea					+				
Mortierella sp.					+		+		
Myrothecium verrucaria						+			
Paecilomyces carneus	+								
Paecilomyces farinosus	+								
Paecilomyces fumosoroseus									+
Penicillium martensii	+								
Penicillium simplicissimum	+								
Penicillium verrucosum var. *cyclopium*	+	+							
Penicillium sp.					+				
Phoma fimeti	+								
Phoma sp.									+
sterile light mycelium					+				
sterile dark mycelium I	+	+		+		+		+	+
sterile dark mycelium II									+
sterile dark mycelium III		+							
sterile dark mycelium IV								+	
sterile dark mycelium VII									+
Trichoderma hamatum		+		+		+			
Trichoderma koningii	+	+		+		+			+
Trichoderma polysporum				+					
Trichoderma viride	+				+	+	+		
undetermined species of Dematiaceae								+	
undetermined species of Sphaeropsidales I				+					
undetermined species of Sphaeropsidales II					+				
Aureobasidium pullulans				+					