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Mycoflora in the intestine of Eisenia andrei (Oligochaeta, Lumbricidae) and in vermiculture substrates

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Mycoflora of three commercial vermiculture systems based on cattle manure derived substrates and *Eisenia andrei* earthworms was studied using several isolation methods. A total of 172 taxa of saprotrophic micromycetes were isolated (19 taxa of Zygomycetes, 9 taxa of Ascomycetes and 144 taxa of mitosporic fungi). *Aspergillus fumigatus* was the most frequent microfungal species in the intestine of *Eisenia andrei*. In vermicuture substrates, *Aspergillus flavus* and *Aspergillus fumigatus* were among species isolated very frequently by the soil dilution method, while *Rhizopus stolonifer* was estimated as frequent species using the soil washing isolation technique.

Key words: cattle manure, saprotrophic and cellulolytic microfungi, earthworms

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Mykoflóra tří komerčních vermikultur založených na stájovém hnoji a žížalách *Eisenia andrei* byla studována pomocí několika izolačních metod. Celkem bylo izolováno 172 taxonů saprotrofních mikromycetů (Zygomycetes 19, Ascomycetes 9 a mitosporní houby 144 taxonů). Druhem nejčastěji izolovaným ze střevního traktu žížal byl *Aspergillus fumigatus*, ze substrátů vermikultur byly nejčastěji izolovány *Aspergillus fumigatus, Aspergillus flavus* (zřeďovací metoda) a *Rhizopus stolonifer* (promývací metoda).

INTRODUCTION

Thermophilic composting is used traditionally for organic waste decomposition or a production of natural fertilisers and plant growth substrates. Vermicomposting represents a related technique, in which earthworms are additionally introduced into composted material. Various earthworm species, most often *Eisenia fetida, Eisenia andrei* and *Eudrilus eugeniae* (Frederickson et al. 1997, Edwards 1998, Ndegwa and Thompson 2001, Hirashi 2002), as well as various substrates – e.g. pig, cattle, horse or rabbit manures, crop residues, lignocellulose and household wastes, and sewage sludge (Allievi et al. 1986, Elvira et al. 1998, Kale 1998, Singh and Sharma 2002), are currently used in large scale systems producing vermicomposts and/or vermiproteins. The differences between traditional composting processes and vermicomposting are described in several studies, e.g. Vinceslas-Akpa and Loquet (1997) and Ndegwa and Thompson (2001).

The vermicomposting process is a result of the combined action of earthworms and microorganisms living in both earthworm intestines and vermiculture substrate. However, there is little information on the diversity of microorganisms in earthworm intestines and vermiculture substrates or on earthworm effects on the diversity of microfungal communities. Aira et al. (2002) studied the influence of *Eisenia fetida* on some characteristics of microbial populations (microbial biomass N, microbial respiration, substrate dehydrogenase activity) during the vermicomposting of pig manure. Byzov et al. (1993) examined yeasts associated with soil invertebrates including *E. fetida* using dilution plate method for their isolation. Flack and Hartenstein (1984) measured growth parameters of *E. fetida* fed on substrates with and without various species of microorganisms. More records are available about the effects of earthworms on quantitative parameters of microbial communities and about the feeding of earthworms on microbes. Changes in density of microorganisms during the transit through the earthworm intestine were investigated by Krištůfek et al. (1992), while Trigo and Lavelle (1993) measured changes in respiration rate. Food preferences of earthworm belonging to different ecological groups of microfungi were studied by Bonkowski et al. (2000), and Marfenina and Ishchenko (1997) examined earthworm preference for soil microscopic fungi. Several authors reported records about the occurrence of fungal particles in earthworm guts or about the effect of the passage trough the gut on the viability of spores (Shonholzer et al. 1999, Pedersen and Hendriksen 1993, Judas 1992). Microfungal species associated with the gut content and casts of tropical earthworm *Drawida assamensis* were reported by Tiwari et al. (1990).

Since 2000, the research has been carried out in the Institute of Soil Biology AS CR to obtain a better understanding of the interactions between earthworms and microflora in vermicultures. Preliminary results of this research were presented by Nováková and Pižl (2002a,b). Quantitative data obtained are to be published by Pižl and Nováková (in press). In our study, species composition of fungal communities associated with the intestine of *Eisenia andrei* is described and compared with those of fresh and processed vermiculture substrates.

MATERIAL AND METHODS

Three commercial vermiculture systems based on cattle manure and *Eisenia andrei* earthworms located in Mikulčice (Plant 1), Sokolnice (Plant 2) and Frýdek-Místek (Plant 3), Czech Republic, were examined. They differred in both nutrient and heavy metal contents in substrates (Table 1), as well as in their productivity (Pecl, pers. comm.), Plant 2 having been the least and Plant 3 the most efficient ones (as concerns the production of earthworm biomass) at the start of the study. However, the last plant declined strongly in its productivity due to

Table 1. pH, C_{ox}, N_{total} and contents of extractable nutrients and heavy metals in fresh (A) and processed (B) vermiculture substrates on individual localities

		pH	Extractable nutrients ($g.kg^{-1}$)				C_{ox}	N_{total}	Heavy metals ($mg.kg^{-1}$)			
		(CaCl)	Ca	P	K	Mg	%	%	Cd	Cu	Pb	Zn
Plant 1												
(Mikulčice)	A	7.39	7.32	3.25	9.12	3.06	27.3	3.21	0.46	16.9	2.61	113
	B	7.51	8.76	3.35	7.68	3.02	23.4	2.59	0.58	20.7	7.18	276
Plant 2												
(Sokolnice)	A	8.53	10.82	4.50	47.52	5.40	22.8	2.89	0.39	9.7	8.40	134
	B	8.36	9.23	3.40	16.80	3.00	18.5	1.84	0.38	15.0	5.89	124
Plant 3												
(Frýdek-	A	7.23	6.83	4.10	15.84	5.52	17.7	2.01	1.05	26.9	9.45	209
Místek)	B	7.20	6.15	5.23	23.04	4.05	19.5	2.05	0.97	23.1	11.10	172

the poor management (no input of fresh substrate) during the course of our study, having became almost abandoned in 2002.

Five subsamples (ca. 100 g FW) of fresh and earthworm-processed vermiculture substrates were randomly taken from Plants 1 and 2 in spring and autumn 2000 and 2001, and in spring 2002, and used for preparing a mixture samples. From Plant 3, the earthworm-processed substrates were sampled in the same way as from other plants, the samples of fresh substrate were however taken in 2000 (no fresh substrate was available later on). Additionally, batches of earthworms were collected close to the sampling points of substrates.

Soil dilution plate method (DPM, 10^{-4} dilution prepared from 1 g of mixture material, 1 ml of suspension was transferred to each Petri dish, three replicates) and soil washing technique (SWT, approximately 0,5 g of mixture material was washed by 500 ml of sterile destilled water, ten small particles were placed on the surface of each Petri dish, three replicates) (Garrett 1981, Kreisel and Schauer 1987) were used for the isolation of saprotrophic microfungi from vermiculture substrates. Soil extract agar, beer-wort agar and Sabouraud agar, all with rose Bengal, were the isolation media (Fassatiová 1979, Kreisel and Schauer 1987). Cellulolytic fungi from vermiculture substrates were isolated by the dilution method using filter paper on the surface of water agar (Kreisel and Schauer 1987). Samples of the gut contents of *E. andrei* were taken using a modified method described by Krištůfek et al. (1993). Earthworms were washed in sterile tap water and immobilised by dipping into CO_2 saturated water 2 minutes. Subsequently, earthworms were washed again in sterile tap water and massaged by sterile forceps to obtain fresh gut content material. Three earthworm individuals were used for collecting a mixture sample of gut content. It was weighed and diluted to 10^{-2}. A part of sample was additionally homogenised in an ultrasonic bath (50 kHz) for 4 min. and diluted to 10^{-4}. Three replicated mixture samples per vermiculture system and sampling date were prepared. The fungi were isolated from one ml of 10^{-2} and 10^{-4} dilution, respectively, using three above mentioned media. Chloramphenicol (200 $mg.l^{-1}$) and streptomycin (100 $mg.l^{-1}$) were added to all isolation media for the suppression of bacterial growth. Fungi were cultivated for seven days at 25 °C in the dark.

Results

A total of 172 taxa of saprotrophic micromycetes were identified during the study (19 taxa of Zygomycetes, 9 taxa of Ascomycetes and 144 taxa of mitosporic fungi). Of those 129 taxa were isolated from the intestine of *Eisenia andrei* (Table 2: 60 taxa from the Plant 1, 76 from Plant 2 and 89 from Plant 3). 122, 72 and 65 taxa were respectively isolated from vermiculture substrates by the DPM, SWT and the isolation technique for cellulolytic microfungi (Tables 3, 4, 5). Using the DPM, higher numbers of microfungal taxa were obtained from processed than from fresh substrates of all vermiculture plants. Except for Plant 3, however, the numbers of microfungi taxa isolated by the SWT were higher in fresh than processed substrates.

Aspergillus fumigatus was most frequently isolated from the intestine of *Eisenia andrei* in all vermiculture plants studied (Table 2). Additionally, *Aspergillus flavus* and *Geotrichum candidum* were classified as very frequent species in earthworm intestines in the Plant 1. Frequent species of earthworm intestines were represented by *Penicillium expansum*, *Scopulariopsis brevicaulis* (in all plants), *Mucor dimorphosporus* f. *sphaerosporus*, *Aspergillus niger* (in Plant 1) and *Aspergillus versicolor* (in Plant 2).

Aspergillus fumigatus and *Aspergillus flavus* were very frequent species isolated by the DPM (Table 3) from fresh and processed vermiculture substrates, respectively. *Penicillium expansum* and *Geotrichum candidum* were frequent in both fresh and processed substrates, and *Emericella nidulans* (all plants), *A. parasiticus* (in Plants 1 and 3), *Mucor circinelloides* f. *circinelloides*, *Rhizopus stolonifer* (in Plant 1), *Absidia cylindrospora*, *Mucor hiemalis* f. *hiemalis*, *Chaetomium indicum* (in Plant 2), *Mucor dimorphosporus* f. *sphaerosporus*, *Doratomyces putredinis*, *A. ustus*, *A. sydowii*, *Scopulariopsis brevicaulis*, *Trichoderma atroviride*, *Trichurus spiralis* (in Plant 3) belonged to species frequently isolated from processed substrates.

Table 2. Presence (%) and total numbers of micromycete taxa isolated from the intestine of *Eisenia andrei* on individual localities (100 % = 5 samples; 1, 2, 3 – replicates)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Absidia coerulea Bain.							20	20	20
Absidia glauca Hagem	20	20	20				20		
Acremonium bactrocephalum W. Gams					20				
Acremonium berkeleyanum (P. Karst.) W. Gams									20
Acremonium charticola (Lindau) W. Gams								20	
Acremonium cf. *kiliense* Grütz						20			
Acremonium murorum (Corda) W. Gams								20	
Acremonium strictum W. Gams						20	20	20	20
Acremonium sp.					20			20	
Aspergillus asperescens Stolk					20		20		
Aspergillus caespitosus Raper et Thom				20		40	20		40
Aspergillus candidus Link: Fr.				40	40	20		20	
Aspergillus clavatus Desm.	60	20	20				20	20	
Aspergillus flavus Link: Fr.	100	80	100	40		60	40	40	80
Aspergillus fumigatus Fresen.	80	80	80	80	80	80	100	100	80
Aspergillus niger Tiegh.	40	60	60		20	40			
Aspergillus ochraceus K. Wilh.	20								
Aspergillus oryzae (Ahlburg) Cohn	20								
Aspergillus parasiticus Speare	60	60	80	20	20		20	20	40
Aspergillus puniceus Kwon et Fennell									20
Aspergillus sydowii (Bain. et Sart.) Thom et Church	20						20		20
Aspergillus terreus Thom			20						20
Aspergillus ustus (Bain.) Thom et Church							40	60	60
Aspergillus versicolor (Vuill.) Tirab.		20	20	80	80	60	40	40	40
Aspergillus wentii Wehmer							20		20
Aspergillus sp.					20	20			
Botrytis cinerea Pers.: Fr.						20			
Chaetomium indicum Corda							20	20	
Chrysosporium sp.							20	20	
Cladobotryum sp.						20			
Cladosporium cladosporioides (Fresen.) de Vries									20
Cladosporium sphaerospermum Penz.					20	20			
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers		20							
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*			20	20	20				20

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Coniothyrium fuckelii Sacc.								20	
Cylindrocarpon magnusianum (Sacc.) Wollenw.			20						
Cylindrocarpon sp.					20				20
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.	20			60	20	40	60	20	40
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.				20		20		20	20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.				20	20	20	20	40	60
Emericella nidulans (Eidam) Vuill.	20	40	20	40			40	20	40
Eupenicillium sp.	20						20		
Eurotium amstelodami Mangin	20						20	20	20
Fusarium culmorum (W. G. Sm.) Sacc.							20		
Fusarium oxysporum Schlecht.: Fr.	20	20							
Fusarium solani (Mart.) Appel et Wollenw.			40			20	40	40	60
Fusarium ventricosum Appel et Wollenw.						20	20		40
Fusarium sp.		20	20	20	20		20	20	20
Geomyces pannorum (Link) Sigler et J. W. Carmich.							20		20
Geotrichum candidum Link	60	100	100	40	40	40	40	60	20
Graphium sp.								20	
Humicola fuscoatra Traaen		20		20					
Metarhizium anisopliae (Metchn.) Sorok.									20
Microascus desmosporus (Lechmere) Curzi							40		
Monodictys levis (Wiltshire) S. Hughes				20		20			
Mucor circinelloides Tiegh. f. *circinelloides*	40	40	60	40		20	20	20	20
Mucor circinelloides Tiegh. f. *griseocyanus* (Hagem) Schipper	20								
Mucor hiemalis Wehmer f. *corticolus* (Hagem) Schipper	20	20		20					
Mucor hiemalis Wehmer f. *hiemalis*	20						20	60	40
Mucor hiemalis Wehmer f. *silvaticus* (Hagem) Schipper	20	20	20						
Mucor dimorphosporus Lendn.	40			20	20				
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová	60	60	60	20	20		20	20	20
Mucor mucedo Fresen.	20	20	20	20				20	
Mucor piriformis A. Fischer								20	
Mucor sp.	20	20	20						20
Mycocladus corymbifer (Cohn in Licht.) Váňová	20		20	20	20	20	20	20	40
Myrothecium roridum Tode: Fr.									20
Nodulisporium sp.								20	
Papulaspora sp.	20	20							20

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Penicillium aurantiogriseum Dierckx	40	20	40			20	20		20
Penicillium brevicompactum Dierckx			20				20		
Penicillium canescens Sopp						20			
Penicillium chrysogenum Thom					20	20		20	
Penicillium citrinum Thom	40	20	20		20		60	40	40
Penicillium commune Thom	20	20	20			20	40	20	20
Penicillium decumbens Thom				20			20		
Penicillium expansum Link: Fr.	60	60	80	20	20	20	80	80	80
Penicillium fellutanum Biourge							20		
Penicillium glabrum (Wehmer) Westling				20					
Penicillium griseofulvum Dierckx		20		20	20				
Penicillium inflatum Stolk et Malla						20			
Penicillium cf. *islandicum* Sopp	40	40	40		40	20		20	
Penicillium janthinellum Biourge	20					20			
Penicillium cf. *lividum* Westling				20					
Penicillium pinophilum Hedgc.	20								40
Penicillium piceum Raper et Fennell					40	20			
Penicillium purpurescens (Sopp) Biourge					20				
Penicillium purpurogenum Stoll			20						
Penicillium roqueforti Thom	20	20	40	20	20		20	20	20
Penicillium rugulosum Thom	20		20		20		40	20	20
Penicillium solitum Westling								20	
Penicillium verrucosum Dierckx					20				40
Penicillium sp.	20						40		
Petriella setifera (Schmidt) Curzi									20
Pithoascus shumacheri (Hansen) Arx								20	20
Phoma eupyrena Sacc.					20				
Phoma leveillei Boerema et Bollen				20					
Phoma lingam (Tode: Fr.) Desm.						20	20	20	
Phoma pinodella (L. K. Jones) Morgan-Jones et Burch				20	20	20			
Phoma sp.		20				20			
Rhizopus arrhizus Fischer			20	20					
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.		40	40			60	20	20	
Scopulariopsis brevicaulis (Sacc.) Bain.	20	40	80	60	60	60	60	80	60
Scopulariopsis brumptii Salv.-Duval				20				20	
Scopulariopsis candida (Guéguen) Vuill.							20		

Table 2. (continuation)

Micromycete taxa	Mikulčice			Sokolnice			Frýdek-Místek		
	1	2	3	1	2	3	1	2	3
Scopulariopsis chartarum (G. Sm.) F. J. Morton et G. Sm.				20	20	20	20	40	40
Scopulariopsis state of *Microascus*				20					
Sepedonium niveum Massee et Salmon					20				
Sordaria humana (Fuckel) Wint.				20					
Stachybotrys chartarum (Ehrenb.: Fr.) S. Hughes	20				20				
Talaromyces wortmanii (Klöcker) C. R. Benj.									20
Thysanophora penicillioides (Roum.) W. B. Kendr.					20				
Trichoderma atroviride P. Karst.	20	20	20	20	20	20	20		40
Trichoderma hamatum group		20						20	20
Trichoderma harzianum Rifai	40	60	60		20		20	60	80
Trichoderma koningii Oudem.				20	20		20	20	20
Trichoderma pseudokoningii Rifai							20		
Trichoderma sp. (pink)									20
Trichoderma spp.		20	20			20	40	20	
Tritirachium roseum Vincens							40		
Verticillium lecanii (Zimm.) Viégas				20					
Verticillium luteoalbum (Link: Fr.) Subram.		20				40	40	40	20
Verticillium nigrescens Pethybr.			40		20	40			
sterile dark mycelium		20		20		20	20	40	40
sterile mycelium	20			40	40	40	40	20	20
sterile yellow mycelium							20		
undeterminad species of *Moniliales* 2									20
undetermined species of *Dematiaceae* 2									20
Number of isolated micromycete taxa 129	**41**	**36**	**36**	**40**	**41**	**42**	**56**	**52**	**57**
		60			**76**			**89**	

Table 3. Presence (%) and total numbers of micromycete taxa isolated by DPM from fresh (A) and processed (B) vermiculture substrates on individual localities (100 % = 5 samples, * samples collected only in 2000, 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia cylindrospora Hagem				60		
Absidia glauca Hagem		20		20		
Acremonium bactrocephalum W. Gams	20		20			20
Acremonium charticola (Lindau) W. Gams						20
Acremonium murorum (Corda) W. Gams						20
Acremonium strictum W. Gams	20		40			40
Acremonium sp.						20
Arthrinium arundinis (Corda) Dyko et B. Sutton					50	
Aspergillus asperescens Stolk			20			
Aspergillus caespitosus Raper et Thom	20			20	50	20
Aspergillus candidus Link: Fr.			20			40
Aspergillus clavatus Desm.				20		20
Aspergillus flavus Link: Fr.	20	100	40	100	50	100
Aspergillus fumigatus Fresen.	100	40	100	80	100	80
Aspergillus niger Tiegh.	20	60	20	20	50	100
Aspergillus niger var. *phoenicis* (Corda) Al-Musalam	20	20				
Aspergillus ochraceus K. Wilh.	20			40	50	20
Aspergillus parasiticus Speare	40	80	40	20		60
Aspergillus puniceus Kwon et Fennell						20
Aspergillus sydowii (Bain. et Sart.) Thom et Church	20	20			50	60
Aspergillus ustus (Bain.) Thom et Church				20	50	60
Aspergillus versicolor (Vuill.) Tirab.		20	20	20	50	40
Aspergillus sp. (*A. versicolor* group)		20		20		20
Aspergillus wentii Wehmer					50	20
Beauveria bassiana (Bals.) Vuill.	20		20			
Botryotrichum piluliferum Sacc. et March.						20
Chaetomium indicum Corda	20		40	60		40
Chaetomium spinosum Chivers						20
Cladosporium cladosporioides (Fresen.) de Vries			20			40
Cladosporium herbarum (Pers.: Fr.) Link			40			
Cladosporium sphaerospermum Penz.	20			20		
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*				20		
Cylindrocarpon magnusianum (Sacc.) Wollenw.	20	40	40	40		40
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.			20	40		20

Table 3. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.			40	20		20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.			20			60
Emericella nidulans (Eidam) Vuill.		60	20	60	50	80
Eupenicillium sp.						40
Eurotium amstelodami Mangin	20					
Eurotium chevalieri Mangin	20					20
Fusarium avenaceum (Fr.) Sacc.				20		
Fusarium oxysporum Schlecht.: Fr.	40			40		
Fusarium solani (Mart.) Appel et Wollenw.				20	50	40
Fusarium ventricosum Appel et Wollenw.		20	40	40	50	
Fusarium sp.		40	20			20
Geomyces pannorum (Link) Siegler et J. W. Carmich.				20		
Geotrichum candidum Link	60	80	40	80	100	60
Humicola fuscoatra Traaen var. *fuscoatra*			20			
Hypocrea sp.				20		20
Metarhizium anisopliae (Metchn.) Sorok.			20			
Mortierella spp.	20		20			40
Mucor circinelloides Tiegh. f. *circinelloides*		60	40	40	50	20
Mucor circinelloides Tiegh. f. *griseocyanus* (Hagem) Schipper	20	20				
Mucor dimorphosporus Lendn.	40	20			50	
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová		20	20			60
Mucor hiemalis Wehmer f. *corticola* (Hagem) Schipper		40			50	
Mucor hiemalis Wehmer f. *hiemalis*	20	20	20	60		
Mucor globosus A. Fisher					50	
Mucor mucedo Fresen.	20				50	
Mucor sp.		20				
Mycocladus corymbifer (Cohn in Licht.) Váňová		40		20	50	20
Myrothecium roridum Tode: Fr.					50	20
Paecilomyces lilacinus (Thom) Samson						20
Paecilomyces variotii Bain.		20				20
Papulaspora sp.		20				40
Penicillium atramentosum Thom			20	20		
Penicillium aurantiogriseum Dierckx	40	20	20	20	50	20
Penicillium cf. *aurantiogriseum* Dierckx		20				
Penicillium chrysogenum Thom		20	20	40	50	

Table 3. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Penicillium citrinum Thom	40	20	40	20		
Penicillium commune Thom					50	20
Penicillium corylophilum Dierckx					50	
Penicillium decumbens Thom						20
Penicillium digitatum (Pers.: Fr.) Sacc.				20		
Penicillium expansum Link: Fr.	60	60	20	60	50	60
Penicillium fellutanum Biourge				20		
Penicillium cf. *funiculosum* Thom						20
Penicillium glandicola (Oudem.) Seifert et Samson		20			50	
Penicillium griseofulvum Dierckx			40	20		
Penicillium cf. *islandicum* Sopp	20	40	20	40		20
Penicillium cf. *italicum* Wehmer					50	
Penicillium janthinellum Biourge					50	
Penicillium lanosum Westling			20			40
Penicillium miniolutеum Dierckx					50	
Penicillium olsonii Bain. et Sartory	20					
Penicillium piceum Raper et Fennell	20			20		20
Penicillium purpurogenum Stoll						40
Penicillium roquefortii Thom	40	40	20	40	50	20
Penicillium rugulosum Thom		20			50	40
Penicillium scabrosum Frisvad, Samson et Stolk			20			
Penicillium simplicissimum (Oudem.) Thom				20		20
Penicillium solitum Westling			20	40		20
Penicillium sp.			20			
Phoma leveillei Boerema et Bollen			20	20		
Rhizopus arrhizus Fischer	20	20			100	
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	20	60	20	40		20
Scopulariopsis brevicaulis (Sacc.) Bain.	20	40	100	20		60
Scopulariopsis brumptii Salv.-Duval			20	20		
Scopulariopsis chartarum (G. Sm.) F. J. Morton et G. Sm.			20	20		
Scopulariopsis state of *Microascus*	20			20		20
Sepedonium niveum Massee et Salmon	20			40	50	
Stachybotrys chartarum (Ehrenb.: Fr.) S. Hughes				40		
Tolypocladium cylindrosporum W. Gams				20		
Trichoderma atroviride P. Karst.		40		60	50	60

Table 3. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Trichoderma hamatum group			20	60		
Trichoderma harzianum Rifai		40	20	40	50	80
Trichoderma koningii Oudem.				20		
Trichoderma virens (Miller et al.) Arx						20
Trichoderma viride Pers.: Fr.		20		20		
Trichoderma spp.	40	40	40	40		60
Trichophyton sp.			20			
Trichurus spiralis Hasselbr.				20		60
Tritirachium roseum Vincens						20
Verticillium lecanii (Zimm.) Viégas						20
Verticillium luteoalbum (Link: Fr.) Subram.	20	20	60	40		60
Verticillium nigrescens Pethybr.				20		
Verticillium psalliotae Treschow	20					
sterile mycelium	20	20	40	20		40
sterile dark mycelium	20	20			50	20
undetermined species of *Moniliales* 1			20			
undetermined species of *Moniliales* 2	20					
undetermined species of *Dematiaceae* 1	20			20		20
Number of isolated micromycete taxa 122	**42**	**43**	**51**	**62**	**37**	**69**

Table 4. Presence (%) and numbers of micromycete taxa on individual localities isolated by SWT from fresh (A) and processed (B) vermiculture substrates (100 % = 5 samples, * 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia coerulea Bain.			20			
Absidia cylindrospora Hagem	20		40	20		
Absidia cylindrospora var. *nigra* Hesselt. et J. J. Ellis			20			
Absidia glauca Hagem	20	20	60			
Alternaria alternata (Fr.: Fr.) Keissler			20			
Aspergillus caespitosus Raper et Thom	40					40
Aspergillus clavatus Desm.		20	20	20		
Aspergillus flavus Link: Fr.	40	40	60	40	100	60
Aspergillus fumigatus Fresen.	20		40			
Aspergillus niger Tiegh.	40					20
Aspergillus parasiticus Speare	40	20	40	20	50	20
Aspergillus niger var. *phoenicis* (Corda) Al-Musalam			20			
Aspergillus ochraceus K. Wilh.			20			
Aspergillus ustus (Bain.) Thom et Church	40		40	20		20
Aspergillus versicolor (Vuill.) Tirab.			20			
Aspergillus wentii Wehmer			20			
Beauveria brongniartii (Sacc.) Petch			20			
Chaetomium indicum Corda			20			
Chaetomium spinosum Chivers	20					
Cladosporium cladosporioides (Fresen.) de Vries			20			
Cylindrocarpon magnusianum (Sacc.) Wollenw.	20					
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.	20					
Emericella nidulans (Eidam) Vuill.			40			
Eurotium amstelodami Mangin				20		
Fusarium culmorum (W. G. Sm.) Sacc.			40	60		
Fusarium oxysporum Schlecht.: Fr.	40	20		20		
Fusarium solani (Mart.) Appel et Wollenw.	20		20			
Fusarium ventricosum Appel et Wollenw.			60	20		20
Fusarium sp.	20	20	40	20		20
Geotrichum candidum Link	60		60	40	50	20
Gibberella fujikuroi (Sawada) Wollenw.	20		40			
Hormoconis resinae (Lindau) Arx et de Vries			20			
Mucor circinelloides Tiegh. f. *circinelloides*	40	20	60	40	50	20
Mucor dimorphosporus Lendn.	20		40	20		
Mucor dimorphosporus Lindn. f. *sphaerosporus* (Hagem) Váňová	40	20	60	40	50	80
Mucor hiemalis Wehmer		20	20		50	
Mucor hiemalis Wehmer f. *corticola* (Hagem) Schipper	40	20		20	50	20

Table 4. (continuation)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Mucor hiemalis Wehmer f. *silvaticus* (Hagem) Schipper			20			20
Mucor mucedo Fresen.		20		20	50	20
Mucor sp.	20			40		
Mycocladus corymbifer (Cohn in Licht.) Váňová			20	20		
Papulaspora sp.	20	40		40		
Paecilomyces farinosus (Holm.: Fr.) A. H. S. Br. et G. Sm.			40			
Penicillium aurantiogriseum Dierckx	20		20	20		
Penicillium canescens Sopp	20					
Penicillium chrysogenum Thom			20	20		
Penicillium citrinum Thom			20			
Penicillium commune Thom	20					20
Penicillium corylophilum Dierckx				20		
Penicillium daleae K. M. Zallessky			20			
Penicillium expansum Link: Fr.	60	20	40	40	50	20
Penicillium griseofulvum Dierckx			40	20		
Penicillium cf. *islandicum* Sopp	20		20	20		20
Penicillium janthinellum Biourge	40	40				
Penicillium piceum Raper et Fennell			20			
Penicillium purpurogenum Stoll	20		20	20		
Penicillium roquefortii Thom		20	40	20		20
Penicillium scabrosum Frisvad, Samson et Stolk			20			
Penicillium solitum Westling	20		20			
Penicillium sp.	20	40				
Rhizopus arrhizus A. Fischer	20	20		20		20
Rhizopus stolonifer (Ehrenb.: Fr.) Vuill.	100	100	60	80	100	80
Scopulariopsis brevicaulis (Sacc.) Bain.	20		60	40		
Stachybotrys chartarum (G. Sm.) F. J. Morton et G. Sm.	20					
Trichoderma atroviride P. Karst.	40	40	60	60	50	40
Trichoderma hamatum group		40			50	20
Trichoderma harzianum Rifai	20	60	40		50	60
Trichoderma koningii Oudem.	40		20			
Trichoderma virens (Miller et al.) Arx		20		40		20
Trichoderma spp.	20	40	40	60		40
Trichurus spiralis Hasselbr.	20					
undeterminad species of *Moniliales* 1			20			
Number of isolated micromycete taxa 72	**46**	**23**	**51**	**33**	**13**	**24**

Table 5. Presence (%) and numbers of micromycete taxa isolated using the DPM on filter paper from fresh (A) and processed (B) vermiculture substrates on individual localities (100 % = 5 samples, * 100 % = 2 samples)

Micromycete taxa	Mikulčice		Sokolnice		Frýdek-Místek	
	A	B	A	B	A*	B
Absidia glauca Hagem				20		
Acremonium bactrocephalum W. Gams			20			
Acremonium murorum (Corda) W. Gams						20
Acremonium polychromum (J. F. H. Beyma) W. Gams						20
Acremonium strictum W. Gams			20			
Aspergillus ochraceus K. Wilh.						20
Aspergillus caespitosus Raper et Thom		20				
Aspergillus clavatus Desm.		20				
Aspergillus flavus Link: Fr.	20	60				
Aspergillus fumigatus Fresen.	40	40	20	40		40
Aspergillus niger Tiegh.		20				
Aspergillus sydowii (Bain. et Sart.) Thom et Church						40
Aspergillus ustus (Bain.) Thom et Church					50	40
Aspergillus versicolor (Vuill.) Tirab.		40			50	20
Aspergillus wentii Wehmer					50	20
Chaetomium indicum Corda				40		
Chaetomium spinosum Chivers						20
Cladosporium cladosporioides (Fresen.) de Vries			20			
Cladosporium sphaerospermum Penz.	20				50	
Clonostachys rosea (Link: Fr.) Schroers, Samuels, Seifert et W. Gams f. *rosea*	20					20
Doratomyces microsporus (Sacc.) F. J. Morton et G. Sm.				20	50	60
Doratomyces purpureofuscus (Fr.) F. J. Morton et G. Sm.			20			20
Doratomyces putredinis (Corda) F. J. Morton et G. Sm.			20			40
Emericella nidulans (Eidam) Vuill.		40			50	40
Fusarium culmorum (W. G. Sm.) Sacc.						20
Fusarium oxysporum Schlecht.: Fr.	40	20				
Fusarium solani (Mart.) Appel et Wollenw.	20	40		20	50	40
Fusarium ventricosum Appel et Wollenw.	20	40		40	50	40
Fusarium sp.		20				
Geotrichum candidum Link	20	20	20			
Graphium sp.						40
Monilia sp.			20			
Mortierella spp.	20		20	20		20

Table 5. (continuation)

Micromycete taxa	**Mikulčice**		**Sokolnice**		**Frýdek-Místek**	
	A	**B**	**A**	**B**	**A***	**B**
Mucor circinelloides Tiegh. f. *circinelloides*	20	20				
Mucor dimorphosporus Lendn.		20		20		
Mucor dimorphosporus Lendn. f. *sphaerosporus* (Hagem) Váňová	40	20		20		
Myrothecium roridum Tode: Fr.					50	
Nodulisporium sp.						20
Oidiodendron maius G. L. Barron			20			
Papulaspora sp.		20				
Penicillium aurantiogriseum Dierckx		40			50	20
Penicillium expansum Link: Fr.	20	20	20			60
Penicillium glabrum (Wehmer) Westling	20		20			20
Penicillium griseofulvum Dierckx				20	50	
Penicillium cf. islandicum Sopp	60	20	20			20
Penicillium janczewskii K. M. Zalessky			20			
Penicillium pinophilum Hedgc.						20
Penicillium roquefortii Thom		20				
Penicillium variabile Sopp		20				20
Penicillium sp.			20			
Phoma exigua Desm.	20					
Phoma lingam (Tode: Fr.) Desm.				20		
Phoma sp.			20	20		20
Scopulariopsis brevicaulis (Sacc.) Bain.			20	20		20
Scopulariopsis brumptii Salv.-Duval					50	
Stachybotrys chartarum (G. Sm.) F. J. Morton et G. Sm.		20		20		
sterile mycelium		20	20			
Trichurus spiralis Hasselbr.		20				60
Verticillium albo-atrum Reinke et Berthold	20					
Verticillium luteoalbum (Link: Fr.) Subramanian		20	40	40		20
Verticillium nigrescens Pethybr.			20			
Trichoderma atroviride P. Karst.				20	50	
Trichoderma hamatum group			20	20		
Trichoderma harzianum Rifai		40				20
Trichoderma spp.	20	60	20	60		40
Number of isolated micromycete taxa 65	**17**	**27**	**22**	**18**	**13**	**32**

Among fungi isolated by the SWT (Table 4), *Rhizopus stolonifer* was the most frequent species in substrates of all plants under study. *Absidia glauca*, *Fusarium ventricosum*, *Mucor circinelloides* f. *circinelloides*, *Mucor dimorphosporus* f. *sphaerosporus*, *Scopulariopsis brevicaulis* (in Plant 2), *Geotrichum candidum* (in Plants 1 and 2) were frequent in fresh substrates, while *Trichoderma harzianum* was frequent in processed substrates of Plants 1 and 3. *Trichoderma atroviride* was frequently isolated from both substrates of the Plant 2.

No generally frequent species was classified among fungi isolated using the technique for cellulolytic fungi (Table 5). Nevertheless, *A. flavus,* and *Doratomyces microsporus*, *Penicillium expansum* and *Trichurus spiralis* were respectively frequent in processed substrates of Plant 1 and Plant 3.

DISCUSSION

Dung and/or manure are very complex substrates and in the case of herbivores they consist of comminuted residual vegetable matter, while both cellulose and lignin are often major components and determine the kind of mycoflora that develops on such substrate (Subramanian 1983).

All microfungal taxa isolated in this study are representatives of saprotrophic fungi according Garret's classification (Garrett 1981). They belong to primary or secondary sugar fungi and to cellulolytic fungi, the majority of them being commonly isolated from soils and/or variety of plant substrates. A number of isolated microfungi are classified as fungi utilising hemicelluloses and cellulose (e.g. *Trichurus spiralis, Myrothecium roridum* and representatives of the genera *Doratomyces, Phoma* and *Chaetomium*) or sugars (mainly Zygomycetes) from various sources. One species only, *Sordaria humana*, may be classified as common coprophilous species (Domsch et al. 1980). According to Tubaki's classification of coprophilous fungi (Subramanian 1983), however, all isolated fungi may be considered facultative coprophilous occurring both in dung and other substrates. Nevertheless, Subramanian (1983) reported that many hyphomycetes were recorded invariably and primarily on dung and excreta of animals but rarely on other substrates. In our study, that group of fungi was represented by species from the genera *Graphium, Myrothecium, Papulaspora, Scopulariopsis, Trichoderma, Trichurus*, and *Tritirachium,* which were isolated from both fresh and processed vermiculture substrates. Two microfungi, *A. fumigatus* and *Scopulariopsis brevicaulis,* are considered human-pathogenic and many other species of the genus *Aspergillus* are potential mycotoxin producers (Domsch et al. 1980).

In terms of fungal ecology (Cooke and Rayner 1984, Dix and Webster 1985), species isolated from vermicultures could be classified either as S-selected fungi, e.g. species of the genera *Mucor, Trichoderma, Fusarium, Gliocladium* and *Penicillium,* or as R-selected fungi characterised by a rapid mycelial growth and an ability to colonise fastly the new resources, e.g. many representatives of *Mucorales*, including those of the genera *Mucor* and *Rhizopus*.

Variations in species occurrence obtained by different isolation methods resulted from their different ability to grow from spores or fragments of active mycelium, as well as from a selection based on different nutrient spectra. Using the SWT (isolation from mycelium), a wide spectrum of saprotrophic fungi was isolated from fresh vermiculture substrate (Table 4), which did not correspond with the statement by Cooke and Rayner (1984) that fungi are present mainly as inactive spores in dung. Interestingly, *A. flavus* and *A. parasiticus* were frequently isolated using both the DPM and SWT. It seems that they occur in mycelial form in vermiculture substrates and together with *R. stolonifer* may play an important role in their decomposition.

In comparison with the results of other studies (Tiwari et al. 1990, Krištůfek et al. 1992), high numbers of microfungi were isolated from earthworm gut. Nevertheless, majority of them are probably inactive, as only fungi from spores can be isolated by the DPM (Zak and Rabatin 1997). The highest numbers of microfungal species were found in the earthworm intestines and vermiculture substrates of the Plant 3 which was classified as the best prospering one at the start, but strongly declined in its productivity during the course of our study. Presumably, the changes in vermiculture management (see Material and Methods) were responsible.

Using the DPM, *Aspergillus fumigatus* and *A. flavus* were the most frequently isolated species. *A. fumigatus* is a thermotolerant fungus with world-wide distribution, listed regularly from soils but never as a dominant species. It was previously recorded from various types of composts, composted municipal wastes and from dung of cattle and horses (Domsch et al. 1980). Pugh and Boddy (1988) classified it as characteristic for the self-heating phase of composts. In vermicultures however the temperature is steadily low, and the high frequency of *A. fumigatus* corresponds with the records of Domsch et al. (1980) that this species is able to grow well also at temperature about 20 °C or lower. Similarly to our results, Striganova et al. (1988) recognised *A. fumigatus* as obligate inhabitant of the intestines of soil earthworms. According to Domsch et al. (1980) *A. flavus* is also distributed world-wide, however, it prefers mainly tropical and subtropical regions. This species was also previously recorded in earthworm cultures and casts. Using the SWT, *Rhizopus stolonifer* was frequently isolated from vermiculture substrates of Plants 1 and 2. This species is one of the commonest members of the *Mucorales* and has a world-wide distribution. It was frequently isolated from soils (predominately slightly alkaline ones), but its typical microhabitats include litter, garden compost and composted municipal waste (Domsch et al. 1980). *A. niger* was frequently isolated from intestines of earthworms in Plant 1 and from substrates of all vermiculture plants, and its frequency was higher in processed then in fresh substrates (Plants 1 and 3, DPM). On the contrary, Marfenina and Ishchenko (1997) reported that *A. niger* is not attractive, but rather repellent and toxic for *Eisenia fetida* earthworms.

Correspondingly to Toyota and Kimura (2000), several microfungal species were isolated from earthworm intestines and fresh substrate, but not from processed substrate. The probable explanation is that viability of spores of those fungi changed during their passage through the earthworm gut rather that they were completely digested by worms.

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