2.4 Species composition of fungi in biological soil crusts on natural substrata compared with agricultural habitats

ALENA KUBÁTOVÁ & KAREL PRÁŠIL

INTRODUCTION

While lichens, cyanobacteria or algae frequently form conspicuous macroscopic biological soil crusts on soil surfaces and are intensively studied by lichenologists and phycologists, fungi are somewhat neglected. Although in an early study of biological soil crusts by Fletcher & Martin (1948) the free-living fungi are considered as important components of the crust structure, they were for a long time ignored. At present we have only limited information on species composition of fungi and their function in crusts. A fundamental mycological study on biological soil crusts is found in the work of States & Christensen (2001), dealing with biological soil crusts in desert grasslands in USA. Another recent study is that of Grishkan et al. (2006) dealing with soil crust microfungi in the Negev desert. Knowledge on diversity and function of fungi in the biological soil crusts of Central Europe is very scarce. The only paper which mentioned some fungus in biological soil crusts, is the algological study by Hoppert et al. (2004).

In this chapter, results of our pilot study of microfungal communities in the biological soil crusts or surface soil layer on two natural localities (Střezovská rokle ravine and Borový důl ravine) compared with two agricultural localities (all in the Czech Republic) are given.

MATERIALS AND METHODS

Localities and sampling of biological soil crusts or surface soil layer: Střezovská rokle ravine (June 2005, see Colour plates, Figs. 2.1.2e, f), Borový důl ravine (November 2005), a field near Uhříněves and pasture near Netluky (November 2005). Characteristics of the localities studied are given in the Chapter 2.1. Biological soil crust was developed only at Střezovská rokle ravine. From each locality, one composed sample of biological soil crust or soil layer (of ca 0.5 cm thickness) was taken into a sterile plastic bag. For the mycological analysis the same material of soil crusts was used as for study of algae and cyanobacteria.

Cultivation and identification: Microscopic fungi in biological soil crusts were studied by cultivation methods. For isolation of fungi, both soil suspension plating method and direct inoculation of soil were applied. Samples were inoculated onto four different isolation media: soil agar with rose bengal and glucose (SEGA), wort agar (WA), Sabouraud's agar (SAB), and potato carrot agar (PCA) (Fassatiová 1986). All media contained streptomycin to suppress bacteria (0.1 g/l). For one sample of biological soil crust or surface soil layer, some 16 isolation Petri dishes were used (see Colour plates, Fig. 2.4.1a). Incubation of the Petri dishes was made at 25 °C. After the 7th day of incubation, the visible colonies were transferred to other agar media for identification. These media were malt extract agar (MEA), Czapek yeast extract agar (CYA), soil extract agar (SEA), wort agar (WA), potato carrot agar (PCA), corn meal agar (CMA), potato succrose agar (PSA) and synthetic nutrient agar (SNA) – see in Nirenberg (1976), Fassatiová (1986), Samson et al. (2004).

Identification of soil micromycetes was made on the base of microscopic and macromorphological features according to Gams (1971), Pitt (1979), Ramírez (1982), Burgess et al. (1988), Domsch et al. (1993), Schroers (2001), Samson et al. (2004), Zare & Gams (2004) and other relevant literature. Several fungal strains were deposited at Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, Czech Republic (see in Tab. 2.4.1).

RESULTS AND DISCUSSION

A list of fungal species isolated from biological soil crust or surface soil layer on four localities studied during 2005 is given in the Tab. 2.4.1. Altogether 50 taxa (species, forms, and undetermined isolates) of microscopic fungi belonging to 26 genera were discovered.

The majority of the fungi isolated are anamorphs of *Ascomycota* (40 taxa, 80%); nine taxa (18%) belong to *Zygomycota*. One isolate (cf. *Pythium* sp.) is member of *Peronosporomycota* (fungi-like organisms). The most frequent genera were *Penicillium* (7 species), F*usarium* (6 species) and *Trichoderma* (5 species).

The most frequent fungi were *Cladosporium cladosporioides*, *Clonostachys rosea* f. *rosea*, and *Mucor* sp., which occurred with higher frequency on two localities, and *Alternaria* sp., *Aspergillus flavus*, *Epicoccum nigrum*, *Fusarium crookwellense*, *Penicillium pulvillorum*, *Penicillium spinulosum*, *Trichoderma polysporum*, *T. viride*, and *Umbelopsis angularis*, which were frequently isolated on one of four localities. The majority of isolated microfungi occurred in low frequency, and many species were found only once. All

Table 2.4.1 Microscopic fungi isolated from biological soil crust or surface layer on natural and agricultural localities.

	Locality			
Microscopic fungi	**S**	**B**	**U**	**N**
Absidia spinosa				1
Acremonium sp.			1	
Alternaria alternata			1	
Alternaria sp.	2			
Arthrinium arundinis	1			
Aspergillus flavus	2			
Cladosporium cladosporioides		1	2	2
Cladosporium herbarum	1	1	1	
Clonostachys rosea f. *catenulata*				1
Clonostachys rosea f. *rosea*			2	2
Curvularia sp.	1			
Epicoccum nigrum	2			
Fusarium crookwellense ***CCF 3619***			2	
Fusarium culmorum			1	1
Fusarium tricinctum				1
Fusarium spp. *(3)*	1		1	1
Geomyces pannorum		1		
Metarhizium anisopliae		1		
Mortierella spp. *(2)*	1			1
Mucor hiemalis f. *hiemalis*	1			
Mucor sp. *(2)*			2	2
Mycocladus sp.		1		
Myrothecium roridum			1	
Paecilomyces lilacinus	1			
Penicillium cf. *coalescens*		1		
Penicillium daleae	1			
Penicillium pulvillorum ***CCF 3720***	2			
Penicillium simplicissimum	1			
Penicillium spinulosum	1	2		
Penicillium spp. *(2)*	1			
Phoma cf. *eupyrena*				1
Phoma spp. *(2)*	1	1		
Pochonia bulbilosa		1		
cf. *Pythium* sp.			1	
Rhizopus arrhizus			1	1
Torulomyces lagena	1			
Trichoderma polysporum		2		
Trichoderma viride		2		
Trichoderma spp. *(4)*	1	1	1	1
Umbelopsis angularis	1	2		
undetermined pycnidial fungus			1	
Total No. of taxa: 50	**20**	**13**	**14**	**12**

Notes: CCF = Culture Collection of Fungi, Prague, CZ; Localities: **S** = Střezovská rokle ravine, **B** = Borový důl ravine, **U** = field near Uhříněves, **N** = pasture near Netluky; Occurrence: **1** = rarely isolated species, **2** = frequent species.

the fungi isolated are known as saprotrophic soil and litter fungi equipped by many enzymes useful for destruction of substrates (Domsch et al. 1993). Some of them are also phytopathogenic (*Acremonium*, *Clonostachys*, *Fusarium*), foodborne (*Aspergillus flavus*), coprophilous (*Mucor*), or entomogenous (*Metarhizium anisopliae*).

In the Tab. 2.4.1, comparison of fungal species richness of localities and data on the structure of fungal communities are given. Comparing the four localities, the numbers of species isolated are: 20 for Střezovská rokle ravine, 14 for field near Uhříněves, 13 for Borový důl ravine, and 12 for pasture near Netluky.

The spectrum of discovered fungi on these localities differs (see also Colour plates, Figs. 2.4.1 b–d). On the locality Střezovská rokle dominated *Alternaria* sp., *Aspergillus flavus*, *Epicoccum nigrum* and *Penicillium pulvillorum*. Nevertheless, only *Alternaria* sp. and *Epicoccum nigrum* belong to typical soilborne and litter fungi. *Aspergillus flavus* is a foodborne toxigenic fungus occurring in our country mainly on imported foods and feeds. *Penicillium pulvillorum* in the Czech Republic was recorded mainly on anthropogenic localities, e.g., in substrate of an abandoned ore-washery settling pit in Chvaletice (Kubátová et al. 2002). On the locality Borový důl were the most frequent *Penicillium spinulosum*, *Trichoderma polysporum*, *T. viride* and *Umbelopsis angularis*. All four fungi are typical in our country for forested localities (e.g., Šumava Mts., see Kubátová et al. 1998). On agricultural localities (field near Unříněves and pasture near Netluky) *Cladosporium cladosporioides*, *Clonostachys rosea* f. *rosea*, *Fusarium* spp., and *Mucor* spp. dominated. *Fusarium* species are commonly associated with cereals and their remains (e.g., straw), *Mucor* species are soil and coprophilous fungi, therefore their high occurrence on these localities is not surprising. While the structure of fungal communities of biological soil crusts on these agricultural localities is somewhat similar, the structure of fungal communities on Střezovská rokle and Borový důl is very different (see Fig. 2.4.2).

Fungal species composition in biological soil crusts is little known. In early studies only rare mentions are present. For example, Fletcher & Martin (1948) discovered on desert soil in Tucson, USA, a distinct biological soil crust composition of cyanobacteria *Oscillatoria*, *Microcoleus*, and *Nos-*

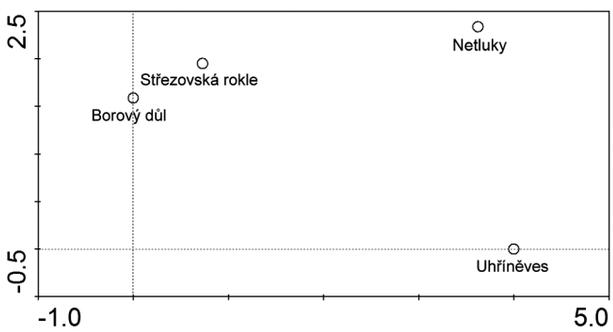

Fig. 2.4.2 Testing of differences in fungal species richness and composition of biological soil crusts on four localities. DCA ordination diagram showing the position of samples in the range of the first two ordination axes.

toc and fungi *Rhizopus*, *Mucor*, and *Botrytis pyramidalis* related fungus (*Botryosporium pulchrum* in present sense). The most comprehensive recent paper is by States & Christensen (2001). They found in biological soil crusts in desert grasslands of Utah and Wyoming, USA, numerous crust associated fungi not previously reported from soil (e.g., basidiomycete *Cyphellostereum* sp., loculoascomycetes *Kalmusia utahensis*, *Macroventuria wentii* etc.). Among other fungi they discovered also members of *Cladosporium*, *Trichoderma*, *Alternaria*, *Epicoccum*, *Fusarium*, *Phoma* etc., as in our study. Hoppert et al. (2004), who studied algae in biological soil crust on sandy soil in Germany, also found *Fusarium*. It parasited on the conjugate alga *Zygogonium*, which dominated the biological soil crust studied. Grishkan et al. (2006) in their detailed study of microfungi diversity and structure of biological soil crust in the Negev desert discovered 87 species, dominant among them being melanin containing fungi (*Alternaria*, *Ulocladium*, *Embellisia* etc.). In our study, due to the climate of our temperate region, the proportion of dark pigmented fungi was relatively lower.

Results of all inventorial surveys strongly depend on the methods used and intensity of sampling (see also Gams 2007). In our study, we were restricted by several factors. (1) Unrepeated sampling severely limits the number of fungal isolates obtained. Undoubtedly, the species accumulation curve would grow with repeated samplings. (2) We discovered only culturable microscopic fungi that were able to grow on the agar media used. Other recently used molecular methods could reveal other organisms living in and forming biological soil crusts. (3) We could not effectively differentiate fungi actively growing in biological soil crust and forming mycelium from fungi grown on agar media from inactive spores only. Better results could be gained by particle filtration techniques (Bills et al. 2004). However, this method is not used as widely as the suspension plating method, mainly because it is more time-consuming.

Thus, the present study could be considered as only a partial contribution to knowledge of the fungal diversity of biological soil crusts.

CONCLUSIONS

Microfungi of biological soil crusts recorded during this survey belong mostly to the known soil and litter fungi. Fungal species composition of biological soil crusts or surface soil layer on the localities studied was very different reflecting the different abiotic and biotic factors. CCA analysis, comparing species richness and composition, differentiated natural localities from agricultural localities. The sampling was unrepeated, which strongly limits the number of fungal isolates obtained. Thus, the results present only partial contribution to the fungal diversity of biological soil crusts on studied localities.