CZECH MYCOL. 54 (3–4), 2003

The occurrence of yeasts in grass-grown soils

ELENA SLÁVIKOVÁ and RENATA VADKERTIOVÁ

Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava, Slovakia

Sláviková E. and Vadkertiová R. (2003): The occurrence of yeasts in the grass-grown soils – Czech Mycol. 54: 239–247

One hundred and fifty six yeast strains were isolated from 160 grass-grown soil samples collected in four different localities in Bratislava, Slovakia. The collection of soil took place in March, May, August, and October. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in the unpolluted localities Rusovce and Dúbravka. These species represented 92.1 % of total yeast counts found in these soil samples. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii*, and *Rhodotorula glutinis* were the most frequently isolated species from the samples taken in the polluted localities Polianky and Mlynská Dolina. These species represented 93.3 % of total yeast counts there.

Yeast densities ranged from 400 to 80.000 CFU/g soil. We found that yeasts occurred unevenly in soils during the year. The lowest average number of yeasts was found in August and the highest one in May.

Key words: yeast community, total yeast counts, Slovakia, grass-grown soil

Sláviková E. and Vadkertiová R. (2003): Výskyt kvasiniek v zatrávnených pôdach. – Czech Mycol. 54: 239–247

Zo 160 vzoriek zatrávnenej zeme, ktoré boli odobraté v štyroch rôznych oblastiach Bratislavy, bolo izolovaných 156 kvasinkových kmeňov. Zber vzoriek sa robil v marci, máji, auguste a v októbri. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor* a *Trichosporon cutaneum* boli najčastejšie izolovanými druhmi zo vzoriek zeme odobratej v neznečistených oblastiach Rusoviec a Dúbravky a reprezentovali 92,1 % z celkového počtu kvasiniek zistených v odobratých vzorkách zeme. *C. laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii* a *Rhodotorula glutinis* boli najčastejšie izolovanými druhmi zo vzoriek zeme odobratej v znečistených oblastiach Polianky a Mlynskej doliny a tvorili 93,3 % z celkového počtu kvasiniek z týchto lokalít.

Počet kvasiniek tvoriacich kolónie (CFU) v 1 g vzorky sa pohyboval v rozmedzí od 400 do 80 000. Výskyt kvasiniek v pôde bol v priebehu roka nerovnomerný, pričom najnižší priemerný počet kvasiniek bol zistený v auguste a najvyšší v máji.

INTRODUCTION

Microorganisms form the basis of the ecological balance of the biosphere. The composition of the microbial communities influences the transformation of plant residues into soil organic matter and plant available nutrients, and also stabilises soil aggregates, reduces erosion and maintains the water-holding capacity (Beare et al. 1993, Kennedy & Gewin 1997). Yeasts are important organisms in many ecosystems and form a significant contribution to biodiversity (Fleet 1998). The soil is the ultimate repository for storage and an even development of certain species of yeasts (Phaff & Starmer 1987).

The occurrence of yeasts in soil has been studied in various parts of the world (Jensen 1963, Vishniac 1996, Dmitriev et al. 1997). Yeasts were found in tropical (Mok et al. 1984) as well as antarctic soils (Baublis et al. 1991). A number of interesting species of yeasts have been isolated from soil (Kurtzman & Fell 1998). Soil biodiversity is generally high in forests, which may represent "biodiversity hot spots" in agricultural landscapes. Forest soils tend to be species-rich and represent stable and often old environments (Hägvar 1998).

The presence of certain species in soil depends on many factors, e.g. type of soil, rainfall, climate, the presence of plants or animals. It is evident that the ability of yeasts to survive in this habitat plays a fundamental role. The yeast density may range from none or a few to several thousand cells per gram of soil (Phaff & Starmer 1987).

Only little information on yeasts associated with soil on the territory of Slovakia and neighbouring countries is available. This work is part of a broader survey of the occurrence of yeasts in various types of soil in the Záhorská nížina lowlands, Slovakia. In our previous investigations we studied the occurrence of yeasts and yeast-like species in forest soil (Sláviková & Vadkertiová 2000). The aim of this work was to study yeast populations occurring in grass-grown soil with reduced plant species richness.

MATERIAL AND METHODS

Study sites

The soil samples came from four different localities: Rusovce, Dúbravka, Mlynská Dolina, and Polianky. The localities Mlynské Dolina and Polianky are in the city centre of Bratislava and the soil samples were taken from zones adjacent to the main road (in the distance about 3 m), where air pollution by exhaust fumes is very high. The grass on these places was mowed very irregularly and before our sampling it was not mowed for a long time. The localities Rusovce and Dúbravka are located in the vicinity of Bratislava (about 20 km from the city centre) and we considered them not or less polluted in comparison with the two foregoing localities. The grass on these places was never mowed. The collection of soil was carried out in March, May, August, and October. In each locality, ten samples were collected from grass-grown soil. In total, this resulted in 160 samples from which yeasts and yeast-like organisms were isolated. The samples were taken from a depth of 5 cm and put into the sterile bottles, transported to the laboratory, and processed within 2 hours after collection.

Methods

The soil pH was measured by the method described by Rump & Krist (1992). The average pH values of the soil samples from the localities Mlynská Dolina and Polianky were equal, namely 7.6, from the localities Rusovce and Dúbravka 7.5 and 6.5, respectively.

Five grammes of the soil sample were suspended in 50 ml of sterile tap water and shaken on a rotary shaker for 1 h. The suspension was diluted to a 1 % solution. An inoculum of 0.5 ml was put on each 10 cm plate with agar and spread with a glass spreader. For cultivation, malt-extract agar and glucose agar (2 % glucose, 0.3 % yeast extract, and 0.1 % $(NH_4)_2SO_4$, 0.02 % K_2HPO_4, 0.01 % KH_2PO_4, 0.02 % $MgSO_4.7H_2O$, 0.02 % NaCl, 0.01 % K_2SO_4, 2 % agar, water) were used. Both media contained 80 $\mu g.ml^{-1}$ streptomycin. The plates were incubated at 25 °C (2–3 d) and 7 °C (14 d). Sodium propionate (0.25 %) reduces the growth of hyphal fungi and was added to the part of medium used for cultivation at 25 °C. Colonies of different appearance were counted in both medium variants, and their representatives were purified according to Sláviková et al. (1992). Yeast counts were calculated as the number of colony-forming units (CFU) per dry gramme of soil sample. Dry weights for soil samples were determined gravimetrically by subsampling c. 1 g of soil and drying in a drying oven at 105 °C to constant weight.

The morphological and physiological characteristics of isolates were examined by the methods described by Van der Walt & Yarrow (1984). Strains were identified to species according to Kurtzman & Fell (1998) and Kocková-Kratochvílová (1990).

RESULTS AND DISCUSSION

One hundred and fifty six yeast strains belonging to 8 genera and 11 species were isolated from the 160 soil samples. Table 1 provides a list of the species isolated. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in the localities Rusovce and Dúbravka. These species represented 92 % of total yeast counts found in the soil samples there. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii*, and *Rhodotorula glutinis* were the most frequently isolated species from the samples taken in the polluted localities Polianky and Mlynská Dolina and represented 93 % of total yeast counts obtained there.

The dominant species *Cryptococcus laurentii*, as well as *C. albidus*, occurred in soil samples from all four localities, and formed up to 26.4–45.1 % and 10.2–28.2 % of total yeast counts found in unpolluted and polluted zones, respectively (Table 1). Investigations of various soils indicate that these species were the most frequently

Table 1: The occurrence of individual species isolated from soil samples collected from studied areas

Species	Occurrence of individual species related to the total yeast counts (%) at each locality			
	Rusovce	Dúbravka	Ml. Dolina	Polianky
Aureobasidium pullulans	0.4	0	0	3.0
Candida maltosa	3.0	0	0	0
Cryptococcus albidus	26.4	35.4	10.2	25.9
Cryptococcus laurentii	45.1	34.5	28.2	18.9
Cystofilobasidium capitatum	8.5	9.7	23.9	12.2
Debaryomyces castellii	1.1	3.7	30.8	24.9
Debaryomyces hansenii	0	1.3	0	0
Rhodotorula glutinis	2.9	0	0.5	11.1
Sporobolomyces salmonicolor	5.0	9.7	4.6	2.3
Trichosporon cutaneum	7.2	2.5	1.5	0.2
Trichosporon pullulans	0.4	3.2	0.3	1.5

encountered in tundra ass on these places 1980), Antarctic (Vishniac 1996), and prairie (Spencer & Spencer 1997) soils. *C. laurentii* constituted on average 15 % of total yeast counts found in forest soil (Sláviková & Vadkertiová 2000). On the other hand, *C. laurentii* and *C. albidus* formed just 6.0 % of the total yeast population occurring in eutrophised Morava river water (Sláviková & Vadkertiová 1997). Species of *Cryptococcus* belong to the group of capsulated yeasts, which survive better in habitats poor in nutrients and during periods of desiccation. Capsules may serve to protect cells from physical and biological stresses encountered in their natural habitat and may influence the ability of the cells to survive low moisture conditions (Spencer & Spencer 1997, Golubev 1991).

The proportion of carotenoids producing species varied seasonally. They were widely distributed in October and May (Fig. 1). The majority belonged to the species *Cystofilobasidium capitatum,* which formed 8.5–9.7 % and 12.2–23.9 % of total yeast counts found in unpolluted and polluted zones, respectively. The occurrence of this species could be affected by its ability to grow at lower temperatures. Nearly all strains of this species grew well at 5 °C. *C. capitatum* was very frequently found also in forest soils (Sláviková & Vadkertiová 2000). *Sporobolomyces salmonicolor* occurred in smaller proportions. Its mean percentage was 2.3–9.7 %. This species forms ballistoconidia and is often associated with the phyllosphere of plants. During periods of bright sunlight, carotenoids protect

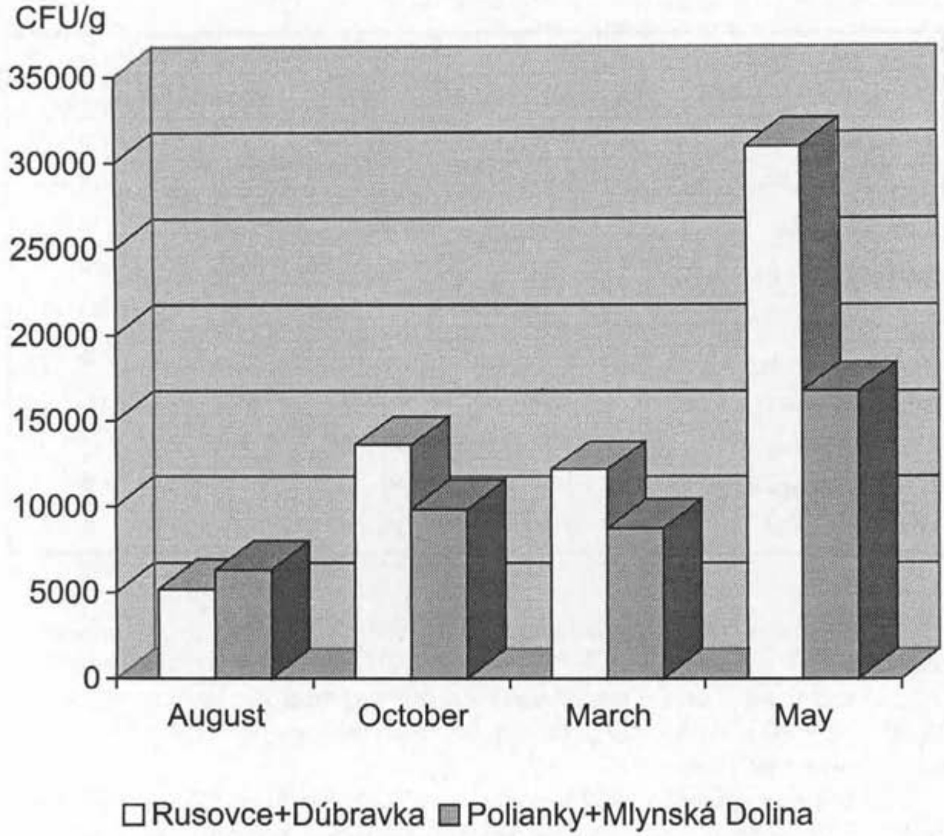

Fig. 1 Changes in counts of the most frequently isolated yeasts through the year

the photosynthetic apparatus of plants against photodestruction (Goodwin 1981). Similarly, they may also protect the vital structures and processes of red yeasts in upper layers of soil. *Rhodotorula glutinis* constituted at most 2.9 % of the total yeast population in three localities, only in the (polluted) locality of Polianky it constituted up to 11.1 %. The high proportion of red yeasts in soil is in an agreement with results obtained by other authors (Golubev 1986, Babyeva & Belianin 1966, Dmitriev et al. 1997).

The other yeast-like species, *Trichosporon cutaneum* and *T. pullulans,* characterised by the formation of true hyphae and arthrospores, were present in lower quantity; they formed only 0.2–7.2 % of the total yeast population, contrary to forest soils, where the species *T. cutaneum* formed up to 30 % and *T. pullulans* 9.8 % of the population. The frequent occurrence of the above yeast species in soil was also observed by other authors. Spencer & Spencer (1997) isolated numerous cultures of the species *T. cutaneum* from Californian and Florida soils.

Table 2: Survey of some features of yeast population

Feature	% of the occurrence of individual feature			
	Rusovce	Dúbravka	Ml. Dolina	Polianky
Presence of urease	96	95	69	75
Fermentation of saccharides	4	5	31	25
Assimilation of nitrate	44	58	39	56
Assimilation of D-xylose	100	100	100	100
Assimilation of L-arabinose	97	100	100	100
Assimilation of cellobiose	95	90	96	98
Assimilation of trehalose	100	100	100	100
Assimilation of lactose	89	90	95	87
Assimilation of soluble starch	92	90	96	98
Assimilation of inositol	88	86	64	61

Debaryomyces castellii appeared to be the only common ascosporogenous species in the grass-grown soils. We recorded its presence particularly in May (Fig. 1) and in soil samples taken from both polluted localities, making up around 25–30 % of yeast counts found in the soil. *D. hansenii* occurred only in one locality and in a small proportion.

The last two species, *Candida maltosa* and the "black yeast" *Aureobasidium pullulans,* were found only in one and two localities, respectively. Their proportion in the total yeast counts did not exceed 3 % but they were the most frequently isolated species from river water (Sláviková & Vadkertiová 1997, Sláviková & Vadkertiová 1997a).

The yeast communities isolated from the grass-grown soils consisted predominantly of species with an ability to assimilate not only glucose, saccharose and maltose but also xylose, arabinose, cellobiose, trehalose, lactose, and soluble starch (Table 2). A substantial part of the species was able to use also inositol as a sole carbon source. Approximately one half of yeasts was capable to utilise nitrate as the sole nitrogen source. The marked prevalence of basidiomycetous yeasts in the populations is interesting; they represented 95–96 % in unpolluted zones. These yeasts lacked fermentative abilities and thus depend on aerobic metabolism for their growth. The occurrence of basidiomycetous yeasts in polluted zones is lower (69–75 %), because up to 25–31 % of the yeast population fermented saccharides and belonged to ascomycetous species or anamorphs. In comparison, the presence of ascomycetous and basidiomycetous yeasts in water taken from lakes situated in this area was approximately equal (Sláviková et al. 1992).

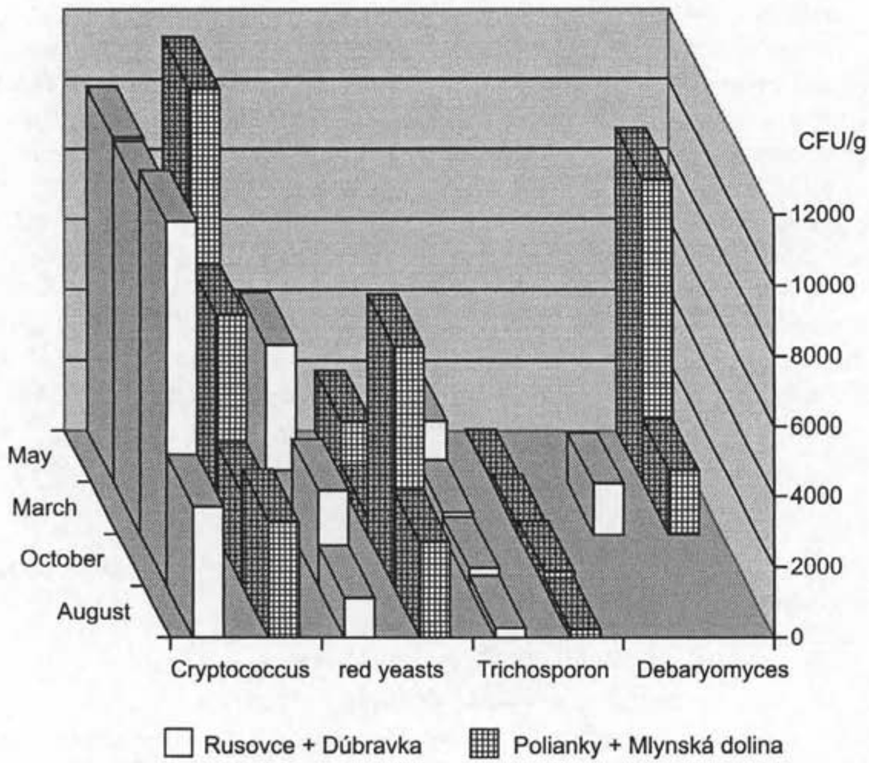

Fig. 2 The average number of yeasts (CFU/g) isolated from soil samples

The number of yeasts in the grass-grown soils ranged from 4×10^2 to 8×10^4 CFU/g soil and the average number reached approximately 1.4×10^4 CFU/g soil. The samples were gathered in March, May, August, and October. We found that the yeasts occurred unevenly in the soil from a quantitative point of view during the year (Fig. 2). The samples collected in May contained the highest number of yeasts, presumably due to the very suitable temperature and moisture in this season. The importance of abiotic environmental factors, especially soil moisture and temperature, on the abundance and biomass of microflora was supported also by Berg et al. (1998). The lowest number of yeasts was found in August, when probably sunshine (temperatures were many times higher than 30 °C) and drought had an inhibitory effect on yeasts. We have found also quantitative differences between yeast counts occurring in polluted and unpolluted localities. A noticeable difference was observed in the May collection (Fig. 2).

We can conclude that the species composition was practically the same in both types of grass-grown soils. The differences were found in species frequency. In soil samples taken from zones adjacent to a main road (where air pollution by exhaust fumes is very high), *Debaryomyces castellii* and *Cystofilobasidium capitatum* were much more frequent than in samples taken from unpolluted soil (Table 1). On the other hand, the lower occurrence of *Cryptococcus* sp. in these zones was evident. However, on the whole it can be stated that capsule-forming organisms formed the majority. These capsules may act as an extracellular buffer system preventing rapid loss of water and cause efficient rehydration of the cells (Golubev 1991).

Yeast species abundance in the grass-grown soil was lower than in forest soil (Sláviková & Vadkertiová 2000). This may be influenced by the fact that grassland soil contains a higher percentage of less decomposed material (Tate et al. 1988) and by a reduced plant species richness, because it has been shown that the plant community may have some effect on the organism community colonising soil (Spehn et al. 2000, Wardle & Giller 1996).

ACKNOWLEDGEMENTS

This work was supported by grant no. 2/1054/21 from the VEGA grant agency for biological and ecological sciences.

REFERENCES

BABYEVA I. P. and AZIEVA E. E. (1980): The taxonomic composition and ecological characters of yeasts occurring in tundra soils in west Taimir. – Mikol. Fitopatol. 14: 99–103.

BABYEVA I. P. and BELIANIN A. I. (1966): Yeasts in rhizosphere. – Mikrobiologija 35: 712–720.

BAUBLIS J. A., WHARTON JR R. A. and VOLZ P. A. (1991): Diversity of microfungi in an Antarctic dry valley. – J. Basic Microbiol. 31: 3–12.

BEARE M. H., POHLAD B. R., WRIGHT D. H. and COLEMAN D. C. (1993): Residue placement and fungicide effects on fungal communities in conventional and no-tillage systems. – Soil Sci. Soc. Am. J. 57: 392–399.

BERG M. P., KNIESE J. P. and VERHOEF H. A. (1998): Dynamics and stratification of bacteria and fungi in the organic layers of a scots pine forest soil. – Biol. Fertil. Soils 26: 313–322.

DMITRIEV V. V., GILICHINSKII D. A., FAIZUTDINOVA R. N., SHERSHUNOV I. N., GOLUBEV V. I. and DUDA V. I. (1997): Detection of viable yeast in 3-million-year-old permafrost soils of Siberia. – Microbiology 66: 546–550.

FLEET G. H. (1998): Yeasts in natural habitats. – Food Technol. Biotechnol. 36: 285–289.

GOLUBEV W. I. (1986): Yeasts from arctic East-Siberian tundra. – Izvestija AN, seria biol. No 4, 609–612.

GOLUBEV W. I. (1991): Capsules. – In: Rose A. H. and Harrison J. S. (eds.), The yeasts, Vol. 4. – p. 175–198, London.

GOODWIN T. W. (1981): The Biochemistry of the Carotenoids. Vol. 1. – 360 p. London.

HÄGVAR S. (1998): The relevance of the Rio-Convention on biodiversity to conserving the biodiversity of soils. – Appl. Soil Ecol. 9: 1–7.

JENSEN V. (1963): Studies on the microflora of Danish beech forest soils. IV. Yeasts and yeast-like fungi. – Zentralbl. Bakteriol., Parasitenkunde, Infektionskrankheiten u. Hygiene, II. Abt. 117: 41–65.

KENNEDY A. C. and GEWIN V. L. (1997): Soil microbial diversity: present and future considerations. – Soil Science 162: 607–617.

KOCKOVÁ-KRATOCHVÍLOVÁ A. (1990): Taxonomy of yeasts and yeast-like organisms. – 699 p. Bratislava (in Slovak).

KURTZMAN C. P. and FELL J. W. (1998): The yeasts, a taxonomic study. Fourth revised and enlarged edition. – 1055 p. Amsterdam.

MOK W. I., LUIZÁO R. C. C., DO SOCORRO BARRETO DA SILVA M., TEIXEIRA M. F. S. and MUNITZ E. G. (1984): Ecology of pathogenic yeasts in Amazonian soil. – Appl. Environ. Microbiol. 47: 390–394.

PHAFF H. J. and STARMER W. T. (1987): Yeasts associated with plants, insects and soil. – In: Rose A. H. and Harrison J. S. (eds.), The yeasts, Vol. 1 p. 123–180, London.

RUMP H. H. and KRIST H. (1992): Manual for the examination laboratory of water, waste water and soil. – 190 p. Weinheim.

SLÁVIKOVÁ E. and VADKERTIOVÁ R. (1997): Yeasts and yeast-like organisms occurring in the river Morava. – Food Technol. Biotechnol. 35: 293–297.

SLÁVIKOVÁ E. and VADKERTIOVÁ R. (1997a): Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. – Antonie van Leeuwenhoek 72: 77–80.

SLÁVIKOVÁ E., VADKERTIOVÁ R. and KOCKOVÁ-KRATOCHVÍLOVÁ A. (1992). Yeasts isolated from artificial lake waters. – Can. J. Microbiol. 38: 1206–1209.

SLÁVIKOVÁ E. and VADKERTIOVÁ R. (2000): The occurrence of yeasts in the forest soils. – J. Basic Microbiol. 40: 207–212.

SPEHN E. M., JOSHI J., SCHMID B., ALPHEI J. and KÖRNER CH. (2000): Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. – Plant and Soil 224: 217–230.

SPENCER J. F. T. and SPENCER D. M. (1997): Ecology: where yeasts live. – In: Spencer J. F. T. and Spencer D. M. (eds.), Yeasts in natural and artificial habitats, p. 33–58, Heidelberg.

TATE K. R., ROSS D. J. and FELTHAM C. W. (1988): A direct extraction method to estimate soil microbial C: effects of experimental variables and some different calibration procedures. – Soil Biol. Biochem. 20: 329–335.

VAN DER WALT J. P. and YARROW D. (1984): Methods for the isolation, maintenance, classification and identification of yeasts. – In: Kreger-van Rij N. J. W. (ed.), The yeasts, a taxonomic study, p. 130–145, Amsterdam.

WARDLE D. A. and GILLER K. E. (1996): The quest for a contemporary ecological dimension to soil biology. – Soil Biol. Biochem. 28: 1549–1554.

VISHNIAC H. S. (1996): Biodiversity of yeasts and filamentous microfungi in terrestrial Antarctic ecosystems. – Biodiversity Conservation 5: 1365–1378.