The occurrence of yeasts in grass-grown soils

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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

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 (Hágvar 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 & Vádkertiová 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.

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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$_4$)$_2$SO$_4$, 0.02 % K$_2$HPO$_4$, 0.01 % KH$_2$PO$_4$, 0.02 % MgSO$_4$.7H$_2$O, 0.02 % NaCl, 0.01 % K$_2$SO$_4$, 2 % agar, water) were used. Both media contained 80 µg.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. albidos, 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. albidos, 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. albidos, 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Occurrence of individual species related to the total yeast counts (%) at each locality</th>
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<tbody>
<tr>
<td></td>
<td>Rusovce</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>0.4</td>
</tr>
<tr>
<td>Candida maltosa</td>
<td>3.0</td>
</tr>
<tr>
<td>Cryptococcus albidus</td>
<td>26.4</td>
</tr>
<tr>
<td>Cryptococcus laurantii</td>
<td>45.1</td>
</tr>
<tr>
<td>Cystofilobasidium capitatum</td>
<td>8.5</td>
</tr>
<tr>
<td>Debaryomyces castellii</td>
<td>1.1</td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td>0</td>
</tr>
<tr>
<td>Rhodotorula glutinis</td>
<td>2.9</td>
</tr>
<tr>
<td>Sporobolomyces salmonicolor</td>
<td>5.0</td>
</tr>
<tr>
<td>Trichosporon cutaneum</td>
<td>7.2</td>
</tr>
<tr>
<td>Trichosporon pullulans</td>
<td>0.4</td>
</tr>
</tbody>
</table>

encountered in tundra ass on these places 1980), Antarctic (Vishniac 1996), and prairie (Spencer & Spencer 1997) soils. *C. laurantii* constituted on average 15 % of total yeast counts found in forest soil (Sláviková & Vadkertiová 2000). On the other hand, *C. laurantii* 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...
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.
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).

<table>
<thead>
<tr>
<th>Feature</th>
<th>% of the occurrence of individual feature</th>
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<tbody>
<tr>
<td></td>
<td>Rusovce</td>
</tr>
<tr>
<td>Presence of urease</td>
<td>96</td>
</tr>
<tr>
<td>Fermentation of saccharides</td>
<td>4</td>
</tr>
<tr>
<td>Assimilation of nitrate</td>
<td>44</td>
</tr>
<tr>
<td>Assimilation of D-xylose</td>
<td>100</td>
</tr>
<tr>
<td>Assimilation of L-arabinose</td>
<td>97</td>
</tr>
<tr>
<td>Assimilation of cellobiose</td>
<td>95</td>
</tr>
<tr>
<td>Assimilation of trehalose</td>
<td>100</td>
</tr>
<tr>
<td>Assimilation of lactose</td>
<td>89</td>
</tr>
<tr>
<td>Assimilation of soluble starch</td>
<td>92</td>
</tr>
<tr>
<td>Assimilation of inositol</td>
<td>88</td>
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</table>
The number of yeasts in the grass-grown soils ranged from $4 \times 10^2$ to $8 \times 10^4$ CFU/g soil and the average number reached approximately $1.4 \times 10^4$ 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).

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**References**


SLÁVIKOVÁ E. and VADKERTIOVÁ R.: The occurrence of yeasts in the grass-grown soils


