ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 3, pp. 385–389. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © W.I. Golubev, M.A. Tomashevskaya, 2010, published in Mikrobiologiya, 2010, Vol. 79, No. 3, pp. 405–409.

EXPERIMENTAL ARTICLES

Yeast Fungi in *Picea abies* (L.) Karst. Needle Litter

W. I. Golubev[1] and M. A. Tomashevskaya

All-Russian Collection of Microorganisms, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia

Received August 15, 2009

Abstract—The amount of yeasts in spruce needle litter determined by plating onto needle infusion agar with penicillin can reach about 3 million cells/g, making up over a quarter of the whole micromycete population. During a 3-year survey, over 20 species belonging to nine genera were revealed, among which representatives of the genera *Cryptococcus, Fellomyces, Rhodotorula*, and *Trichosporon* were typical—specifically, *Cr. carnescens, F. penicillatus, Rh. laryngis,* and *Tr. moniliiforme*. The isolates showed lipase activity and were able to utilize hemicelluloses and phenolic compounds and secrete antifungal substances.

Key words: forest litter, spruce needle litter, basidiomycetous yeasts.

DOI: 10.1134/S002626171003015X

Spruce is the main forest constituent in Russia, occupying over 70 million ha in the European region of the country [1]. The bulk of annual needle litter in spruce forests reaches 600–800 kg/ha [2]. Since fungi play a dominant role in decomposition of the needle litter, the diversity and activity of mycelial macro- and micromycetes in forest litter have been thoroughly studied [3, 4]. However, there have been only a few studies on yeast fungi, no differentiation having been made between deciduous and coniferous litters [5]. The specificity of fungi to particular litters and plant species is well known, and the leveling approach to the study of the composition and structure of mycocommunities in forest biocenoses is unacceptable. The physicochemical characteristics of litters are without question of great importance.

In this work, the results of a 3-year study of the number and species composition of yeast fungi in the needle litter of common (European) spruce are presented. To imitate natural ecotopes, needle agar was used for the isolation and enumeration of yeasts.

MATERIALS AND METHODS

Samples of the *Picea abies* (L.) Karst. needle litter were collected in a mixed forest 1.5 km south of Pushchino (Serpukhov region, Moscow oblast) at a distance not exceeding 10–20 m from each other. In the zone below the spruce crown (0.5–1.0 m from the stem), which contained no vegetation, the top layer was removed; samples were collected at a depth of 2–5 cm in sterile parchment parcels. At least two samples per year have been examined.

The dilutions of needle washing were plated in triplicate onto needle infusion agar (NA) the day after sampling. To prepare NA, the suspension of needle litter in water (200 g/l) was boiled for 10 min and filtered through several layers of gauze; the filtrate was diluted with water to 1 l, supplemented with 20 g agar, and sterilized (0.5 atm, 20 min); it was then supplemented with penicillin (1 million U/l) and poured into petri dishes. The inoculated dishes were incubated at room temperature for 10 days. After enumeration of colonies, several representatives of each morphotype were transferred to malt agar (MA) slants. The culture purity was examined both microscopically and by plating.

The isolates were tested according to described methods on recommended media [6] by comparison with the type strains and identified using manuals [6, 7]. The descriptions of new species and the results of mycocinotyping performed by the "culture against culture" method [8] were taken into account. The antagonistic activity of the isolates was studied by using phylogenetically related organisms as test cultures

Lipase activity was determined using yeast nitrogen base agar (Difco, United States) supplemented with 20% emulsion of olive oil in 5% gum arabic (12.5 g/l). Pectinase activity was assayed on yeast nitrogen base agar supplemented with 10 g/l of pectin (Sigma, United States) by formation of the clear zones around colonies after treatment with 1% solution of cetavlon for 20 min at room temperature.

RESULTS

Yeasts were revealed in all samples of the needle litter within the range from 6 to 28% of the total micro-

[1] Corresponding author; e-mail: wig@ibpm.pushchino.ru

Table 1. The number and species composition of yeasts in spruce needle litter

Date of sampling	Number of yeasts		Yeast species
	$\times 10^3$ cells/g	% of the total micromycete number	
September 12, 2006	1190	28	*Debaryomyces hansenii* *Leucosporidium scottii* *Tremella encephala* *Trichosporon cutaneum* *Tr. jirovecii* ***Tr. moniliiforme*** *Tr. porosum*
	840	21	***Cryptococcus albidus* var. *ovalis*** *Cr. carnescens* *Deb. castellii* *T. encephala* *Tr. lignicola* *Tr. moniliiforme* *Tr. porosum*
May 14, 2007	1000	6	*Cr. laurentii* *Cr. carnescens* *Cr. terreus* *Cr. taiwanensis* ***Rhodotorula* spp.** *Rh. yarrowii* *Sporobolomyces inositophilus*
October 18, 2007	2900	21	*Cr. chernovii* *Cr. perniciosus* *Cr. taiwanensis* *Rh. aurantiaca* *Rh. colostri* *Rh. laryngis* *Rh. nothofagi* ***Rh. pilati*** *Rhodotorula* spp. *Tilletiopsis flava* *T. species*
	2300	16	***Cr. taiwanensis*** *Rh. laryngis* *Rh. nothofagi* *Rh. pilati* *Rhodotorula* spp. *Rh. yarrowii*
September 22, 2008	315	8	***Fellomyces penicillatus*** *Cr. taiwanensis*
	520	17	*Cr. taiwanensis* *F. penicillatus* *Rh. glutinis* *Rh. hordea* *Rh. laryngis* *Rh. nothofagi* ***Rhodotorula* sp.** *Rh. sonckii* *Sp. roseus*

Note: Predominant yeast species are shown in bold.

Table 2. Assimilation of aromatic compounds by yeasts from needle litter

Species	Compounds						
	1	2	3	4	5	6	7
Cr. taiwanensis	–	–	s	w	–	–	–
Cr. terreus	w	–	s	+	–	–	–
Deb. hansenii	–	+	w	+	–	+	s
Leu. scottii	w	–	+	s	w	–	–
Rh. spp.	–	–	s	s	w	–	–
Tr. cutaneum	+	–	+	-	–	–	+
Tr. jirovecii	+	–	+	s	–	–	±
Tr. lignicola	+	–	–	–	–	–	s
Tr. moniliiforme	+	–	+	+	–	–	+
Tr. porosum	+	s	+	s	–	w/–	±

Designations: +, s, w, and – stand for good growth, slow growth, weak growth, and no growth, respectively. 1 phenol, 2 *m*-hydroxybenzoic acid, 3 *p*-hydroxybenzoic acid, 4 2,5-dihydroxybenzoic acid, 5 3,4-dihydroxycinnamic acid, 6 3-hydroxyquinone, 7 4-hydroxyquinone.

mycete population; their CFU number varied from 315×10^3 to 2900×10^3 cells/g (Table 1). Throughout the whole period of investigation, yeast fungi from needle litter grown on NA were represented almost exclusively by basidiomycetes. Single colonies of ascomycetous yeasts (*Debaryomyces castellii* and *Deb. hansenii*) were revealed only once. Some species of basidiomycetous yeasts were also not numerous and were isolated only once. Dominant or frequently occurring species included *Cryptococcus albidus* var. *ovalis*, *Cr. carnescens, Cr. taiwanensis* nov. comb., *Fellomyces penicillatus, Rhodotorula laryngis, Rh. nothofagi, Rh. pilati, Rhodotorula* spp., *Rh. yarrowii, Tremella encephala*, and *Trichosporon moniliiforme*. Both the number and diversity of yeasts on vegetative spruce needles were considerably lower than those in the needle litter. Parallel plating from the green needle samples (May 14, 2007) revealed only two species (*Pseudozyma* sp. and *Rh. aurantiaca*); the total number of yeasts did not exceed 30×10^3 cells/g.

Different results were obtained when the same samples (from September 22, 2008) were plated onto NA (pH 6.5) and MA (pH 4.5) acidified with lactic acid (4 ml/l). The number of yeasts grown on MA was six to eight times lower (53 and 65×10^3 cells/g) than that revealed on NA. In both samples plated onto acidified MA, only one yeast species was found (*Candida insectorum*), whereas, in the same samples plated onto NA, up to nine species were revealed (Table 1).

Species of yeast fungi from needle litter were able to utilize a wide range of substances, including polysaccharides. Most of the isolates possessed amylolytic activity. Species *Tr. jirovecii, Tr. moniliiforme*, and *Tr. porosum* possessed xylanase and pectinase activities; these species, as well as *Tr. cutaneum* and *Tr. lignicola*, exhibited also polygalacturonase activity. Inulinase activity was revealed only in *Deb. castellii* and *Tilletiopsis flava*. Low activity of xylanase was found in some cryptococci (*Cr. albidus* var. *ovalis*, *Cr. carnescens*, and *Cr. perniciosus*) and in *T. encephala*. The latter three species together with *Leucosporidium scottii* and all of the revealed *Trichosporon* species possessed lipase activity, which was rather high in *Tr. jirovecii* and *Tr. porosum* as indicated by measuring the width of clear zones around their colonies.

Many of the yeast species revealed in needle litter can utilize some aromatic compounds (Table 2). The ability to assimilate hydroxybenzoic acids is widespread among yeasts in the case of the absence of methoxy groups in the acids (Table 3). The presence of methoxy and other groups also prevented utilization of other phenol derivatives. Phenol was assimilated by *Cr. terreus, Leu. scottii*, and especially strongly by *Tri-*

Table 3. Compounds not utilized by yeasts from needle litter

2-Aminophenol
1,2-Dihydroxybenzene (pyrocatechol)
3,4-Dimethoxybenzaldehyde
3,4-Dimethoxybenzyl (veratrole) alcohol
3,4-Dimethoxybenzoic acid
3,5-Dimethoxy-4-hydroxybenzoic acid
3,5-Dimethoxy-4-oxybenzoic (lilac) acid
2,6-Dimethoxyphenol
Methanol
3-Methylsalicylic acid
3-Methoxy-4-oxybenzoic (vanillic) acid
4-Hydroxy-3-methoxybenzaldehyde (vanillin)
1,2,3-Trihydroxybenzene
4-Ethylphenol

chosporon species, which were able to grow at phenol concentration of 0.4 g/l and sometimes up to 1.0 g/l.

Antifungal activity at low pH values was revealed in the strains of *Rh. colostri* and *Tr. porosum*.

DISCUSSION

Unlike pine forests, no vegetation occurred in the sampling sites under spruces, where the soil was covered by a layer of needle litter only. Contamination of the microbial community by organisms inhabiting other plants and their debris was therefore minimized.

The average total number of yeasts from spruce litter revealed on NA (1700×10^3 CFU/g) exceeded by five to eight times the average values obtained on acidified MA, which is in agreement with the published data. According to Bab'eva and coworkers [9], variations in the yeast number in singly taken samples were lower than those in our samples withdrawn from the same areas for 3 years; when acidified (pH 4.5) NA ("litter agar") was used, the yeast number was lower than that obtained on MA and growth was even inhibited. Acidification of a variety of media (glucose–peptone, inositol, and glucuronate agar) showed a negative effect on yeast growth [10]. It should be noted that yeasts are usually rather acid-resistant but do not belong to acidophiles. Negative effect of acidification of the medium on yeast growth may also be associated with secretion of mycocins and cellobiose lipids since their antifungal activity increases at low pH.

Our results confirm rare occurrence of yeasts on vegetative spruce needles [11]. The yeast number on green needles was 30 times lower than that in the needle litter, possibly because of the presence of antimicrobial substances, including terpenes, in fresh needles [12].

In spruce litter, over 20 yeast species belonging to nine genera were revealed during the 3-year survey (Table 1). Anamorphic basidiomycetes of the genera *Cryptococcus, Rhodotorula*, and *Trichosporon* were responsible for most of the species diversity.

Most of cryptococcal isolates fit to the species *Bullera taiwanensis* according to their physicochemical characteristics. The similarity of these isolates to the type strain VKM Y-2952 of this species was confirmed by comparative studies including the mycocinotyping. However, unlike the original description, we did not observe formation of ballistospores either in the type strain or in our cultures, although fresh isolates usually actively sporulated. No information about ballistospore production was provided for other isolates of *B. taiwanensis*, which were identified by molecular biological methods [13, 14]. *B. taiwanensis* is distant phylogenetically from the other *Bullera* species and, unlike them, utilizes nitrates. Considering the aforesaid, a novel combination is suggested:

Cryptococcus taiwanensis (Nakase, Tsuzuki et Takashima) Golubev (Basionymum: *Bullera taiwanensis* Nakase, Tsuzuki et Takashima, *J. Gen. Appl. Microbiol.*, 48, 2002, pp. 345–355).

It should be noted that reports of ballistospore formation are often erroneous. For example, it was not confirmed for the species *Sporobolomyces albidus* and *Sp. antarcticus*, which were subsequently reidentified as *Cr. humicola* and *Pseudozyma antarctica*, respectively [6, 7].

Many uncolored isolates designated in Table 1 as *Rhodotorula* spp. represent probably novel species, which differ in their morphological, physiological, and biochemical properties from the known species. They often have curved cells and assimilate *i*-inositol and D-glucuronate as the sole carbon sources. At present, only one inositol-positive species, *Rh. yarrowii*, is known, which is assigned phylogenetically to Microbotryomycetes.

Our results on the composition of the yeast community in spruce needle litter are difficult to compare with other information concerning forest biogeocenoses [5], since the literature data contain no information on the individual components of cenoses. Nevertheless, the comparison of our results for spruce needle litter (Table 1) and the yeast composition revealed by Bab'eva and coworkers in a spruce wood [15] made it possible to establish a number of common species, such as *Cr. albidus, Cr. laurentii, Deb. hansenii, Leu. scottii, Rh. aurantiaca, Rh. glutinis, Rh. pilati, Sp. roseus, T. encephala*, and *Tr. cutaneum*. Many of these taxa were shown to be heterogeneous, being a complex of phenotypically similar species. For example, the species *Cr. carnescens, Rh. colostri, Rh. laryngis*, and *Tr. moniliiforme* were restored from *Cr. laurentii, Rh. aurantiaca, Rh. minuta*, and *Tr. cutaneum*, respectively. Taking into account these relatively recent changes, it can be stated that the number of common species in the mentioned lists of yeasts associated with spruce needle litter may be increased considerably.

The overwhelming majority of the yeast fungi isolated from spruce litter were able to utilize a wide range of carbon and nitrogen compounds. Species of the genera *Cryptococcus* and *Trichosporon* are particularly notable, since they can utilize not only almost all low-molecular carbon compounds (mono-, di-, oligo-, and amino sugars, polyols, and organic acids), which are included in standard species descriptions [7], but also certain hemicelluloses, as stated above. The presence of lipase activity in yeasts is indicative of their involvement in the first stage of needle decay, viz., the degradation of cuticle waxes, which are mainly represented by esters of fatty acids and alcohols [16]. Many yeast species isolated from litter assimilated a number of phenolic compounds (Table 2), which are widespread in plants as biogenetic precursors or the products of degradation [17]; specifically, lignin is known to prevail in spruce needle litter [18]. This may explain why yeasts, while unable to decompose lignin and hav-

ing almost no cellulase activity, stimulated the wood-degrading activity of mycelial basidiomycetes [19].

The relatively high density of the yeast population in needle litter (up to 3×10^6 cells/g) amounting to about 20% of the total number of micromycetes (Table 1) evokes strong competition and antagonistic interrelations in mycocenoses. Litter yeasts secrete such fungicidal agents as mycocins [20] and glycolipids [21].

ACKNOWLEDGMENTS

This work was supported by the "Molecular and Cell Biology" program of the Presidium of the Russian Academy of Sciences.

REFERENCES

1. Rysin, L.P. and Savel'eva, L.I., *Elovye lesa Rossii* (Spruce Forests of Russia), Moscow: Nauka, 2002.
2. Zaboeva, I.V., Rusanova, G.V., and Sloboda, A.V., Biological Productivity of Green Moss Spruce Forests of the Komi ASSR Medium and Northern Taiga, *Rastit, Resursy*, 1973, vol. 9, no. 1, pp. 100–106.
3. Burova, L.G., *Ekologiya gribov makromitsetov* (Ecology of Macromycete Fungi), Moscow: Nauka, 1986.
4. Chigineva, N.I., Aleksandrova, A.V., Sidorova, I.I., and Tiunov, A.V., Taxonomic Structure of Micromycete Communities on Tree Waste at Different Levels of Carbon Availability, *Mikol. Fitopatol.*, 2008, vol. 42, no. 6, pp. 540–550.
5. Maksimova, I.A. and Chernov, I.Yu., Community Structure of Yeast Fungi in Forest Biogeocenoses, *Mikrobiologiya*, 2004, vol. 73, no. 4, pp. 558–566 [*Microbiology* (Engl. Transl.), vol. 73, no. 4, pp. 474–481.
6. *The Yeasts: A Taxonomic Study*, Kurtzman, C.P. and Fell, J.W., Eds., Amsterdam: Elsevier Sci. Publ. B.V, 1998.
7. Barnett, J.A., Payne, R.W., and Yarrow, D., *Yeasts: Characteristics and Identification*, Cambrige Univ. Press, 2000.
8. Golubev, W.I. and Golubeva, E.W., Mycocin Typing of Filobasidial Fungi of the Genus *Cryptococcus, Mikol. Fitopatol.*, 2006, vol. 40, no. 6, pp. 480–486.
9. Bab'eva, I.P., Azieva, E.E., Abramenko, N.I., Zaitsev, S.A., and Evtushenko, L.I., On the Method for Quantitative Estimation of Yeasts in Forest Litter, *Vestn. Mosk. Univ., Ser. 17 (Soil Sci.)*, 1980, no. 1, pp. 53–59.
10. Golubev, W.I. and Golubeva, E.W., Yeast Fungi of the Steppe and Forest Phytocenoses of the Prioksko-Terrasnyi Biospheric Preserve, *Mikol. Fitopatol.*, 2004, vol. 38, no. 6, pp. 20–27.
11. Bab'eva, I.P., Golubev, W.I., Kartintsev, A.V., Gorin, S.E., and Zaslavskaya, P.A., Yeasts in the Structure of Forest and Meadow Biogeocenoses, *Vestn. MGU, Ser. 6 (Biology, Soil Csi.),* 1973, no. 6, pp. 67–73.
12. Holubová, V., Hrdlićą P., and Kubán, V., Age and Space Distributions of Monoterpenes in Fresh Needles of *Picea abies* (L.) Karst. Determined by Gas Chromatography-Mass Spectrometry, *Phytochem. Anal.*, 2001, vol. 12, no. 4, pp. 243–249.
13. Baas, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L., Sanders, H., Watkinson, S.C., Willcock, S., and Richards, T.A., Yeast Forms Dominate Fungal Diversity in the Deep Oceans, *Proc. Royal Soc. B*, 2007, vol. 274, no. 1629, pp. 3069–3077.
14. Brad, T., Braster, M., van Breukelen, B.M., van Straalen, N.M., and Röling, W.F.M., Eukaryotic Diversity in an Anaerobic Aquifer Polluted with Landfill Leachate, *Appl. Environ. Microbiol.*, 2008, vol. 74, no. 13, pp. 3959–3968.
15. Bab'eva, I.P., Kartintseva, A.A., Maksimova, I.A., and Chernov, I.Yu., Yeast Fungi in Spruce Forests of the Central State Biospheric Forest Preserve, *Vestn. Mosk. Univ., Ser. 17 (Soil Sci.)*, 1999, no. 4, pp. 45–49.
16. Deineko, I.P., Pranovich, A.V., Rubanova, V.F., and Belov, L.P., Research on Chemical Composition of Conifer Wax, *Khimiya Rast. Syr'ya*, 2005, no. 1, pp. 13–18.
17. Zaprometov, M.N., *Fenol'nye soedineniya* (Phenolic Compounds), Moscow: Nauka, 1993.
18. Johansson, M.-B., The Chemical Composition of Needle and Leaf Litter from Scots Pine, Norway Spruce and White Birch in Scandinavian Forests, *Forestry*, 1995, vol. 68, no. 1, pp. 49–62.
19. Blanchette, R.A. and Shaw, C.G., Associations among Bacteria, Yeasts, and Basidiomycetes During Wood Decay, *Phytopathology*, 1978, vol. 68, no. 4, pp. 631–637.
20. Golubev, W.I. and Tomashevskaya, M.A., Characterization of Mycocin Secreted by *Rhodotorula colostri* (Castelli) Lodder, *Izvestiya RAN, Ser. Biol.*, 2009, vol. 36, no. 3, pp. 373–378 [*Biol. Bull.* (Engl. Transl.), vol. 36, no. 3, pp. 311–317].
21. Golubev, W.I., Shcherbinina, N.R., Tomashevskaya, M.A., and Golubeva, E.W., Antifungal Activity and Distribution of *Trichosporon porosum, Mikol. Fitopatol.*, 2008, vol. 42, no. 3, pp. 257–263.