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EXPERIMENTAL ARTICLES

Yeast Communities of *Formica aquilonia* Colonies

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Abstract—Yeast abundance and species diversity in the colonies of *Formica aquilonia* ants in birch–pine grass forest near Novosibirsk, Russia, were studied. The average yeast number in the anthill material was 10^3–10^4 CFU/g, reaching 10^5 CFU/g in the hatching chambers. Typical litter species (*Trichosporon moniliiforme* and *Cystofilobasidium capitatum*) were predominant in soil and litter around the anthills. Apart from these species, ascomycete species of the family *Debaryomycetaceae, Debaryomyces hansenii*, and *Schwanniomyces vanrijiae* were predominant in the anthill material. Yeast population of the ant' bodies consisted exclusively of the members of the last two species. Thus, highly specific yeast communities formed in the colonies of *Formica aquilonia* ants differ from the communities of surrounding soil. These differences are caused by environment-forming activity of the ants.

Keywords: yeasts, ants, anthills, soil, *Formica aquilonia*, *Debaryomyces hansenii*, *Schwanniomyces vanrijiae*

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Ants are known to play an important role in terrestrial ecosystems. Their trophic and building activities change the physical and chemical properties of soil and the herbage structure in their ecotopes (Bugrova and Pshenitsina, 2003). The vegetation growing near the ant mounds differs from the surrounding plants by larger size and higher yield of a better quality. Firs and birches growing near anthills are stronger and form more developed rootage than the distantly growing trees (Dlusskii, 1967).

Ants also fulfill special soil-reformative functions, which includes soil mixing and changes in mechanical and chemical composition of soil (Frouz and Jilkova, 2008). Inside the ant mounds, at the expense of decayed and transformed organic material, concentrations of available forms of many elements (Ca, Mg, Na, K, and P) are higher than in surrounding soil (Frouz et al., 2005). Anthills accumulate important chemical elements (carbon, phosphorus, and nitrogen) and thereby increase spatial heterogeneity in the distribution of these elements in the ecosystem (Kilpeläinen, 2008). Accumulation of available forms of chemical elements occurs not only in the mounds of red wood ants but also in the immediate vicinity of them (Frouz et al., 2008). According to numerous studies, red wood ants affect the soil acidity: acidic and neutral soils are alkalized, whereas strongly alkaline ones are acidified. Soil acidity is also changed due to high glucose concentration in the ant-brought aphids honeydew (Jilkova et al., 2012). It is known that anthills (nests) of red wood ants are attractive for the great diversity of soil and litter organisms. The number of non-spore-forming bacteria and actinomycetes in the mounds of red wood ants is an order of magnitude higher than that in the surrounding soil (Stebaeva et al., 1977; Pokarzhevskii and Pimenov, 1981). Anthills are favorable habitats for such important soil-forming groups of invertebrates as collembola and moss mites (Stebaeva and Grishina, 1983). For instance, the fluctuating type of the structure of collembola communities, which is typical of insular ecotopes, was revealed within anthills; their number and species diversity varied even in closely situated anthills and endured considerable seasonal variations (Reznikova and Sleptsova, 2003). Different parts of the ant dome are occupied by different ecological groups of *Collembola*. It is important that the number of collembola within anthills is two orders of magnitude higher than that in surrounding soil (Sleptsova and Reznikova, 2006). Ants also have a considerable effect on formation of soil microbial populations (Stebaeva et al., 1977; Pokarzhevskii and Pimenov, 1981; Grishkan, 1989). It was found that fungal and bacterial biomass on the surface of the *F. aquilonia* mound was three times higher than that in the litter of surrounding forest (Laakso and Setälä, 1998). In Russia, the first studies of yeast population in anthills were carried out at the early 1970s (Golubev and Bab'eva, 1972a, 1972b). It was shown that in ground mounds of the

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Lasius ants, yeasts were practically absent, whereas within anthills of the ants belonging to subgenus *Coptoformica*, yeast population was represented by widespread epiphytic species. It is of special interest that the yeast population within *Formica rufa* anthills was highly specific and contained only species of the genus *Debaryomyces: D. cantarellii* and *D. formicarius,* which were later renamed as *Schwanniomyces polymorphus* and *Sch. vanrijiae*, respectively (Kurtzman and Suzuki, 2010); these species were not found in any of the surrounding substrates. The number of these yeasts sometimes reached 10^6 CFU/g of sample, which considerably exceeded the average yeast number in soil and plant debris.

Extensive studies of the ant-associated yeast communities have been recently carried out; however, most of them are limited to investigation of yeast associations with leaf-cutting ants, harvester ants, fire ants, etc., which are typical of regions with a tropical or arid climate and of the New World countries (Ganter, 2006). Until now, yeasts forming associations with the ant groups typical of the temperate latitudes of Russia have not been studied.

The goal of the present work was to study yeast population of the anthills of *Formica aquilonia,* a typical representative of red wood ants belonging to *Formica rufa* group (Dlusskii, 1967).

MATERIALS AND METHODS

The samples of the *F. aquilonia* anthills, as well as of soil and litter as the controls, were collected in four periods (May, August, and November 2011 and March 2012) in a birch–pine forest, Novosibirsk oblast, Russia (55°00′31″ N 83°18′20″ E). The anthill samples were taken from two mounds in each of them from the ground bunk (north and south expositions), upper dome layer (north and south expositions), and from the dome at depths of 25 and 35–40 cm (north and south expositions). Besides, we analyzed samples of the worker ants from inside the nests, which were collected in the same periods, except for March 2012, because at this time, ants in this region hibernate in the underground part of the anthill (Reznikova and Sleptsova, 2003). Soil samples were taken at a depth of 0–5 cm within 2–3 m from the anthills and from the vicinity of anthills used as the control. In total, 107 samples were analyzed.

The samples were transported to Moscow within 3 to 4 days and stored at 4–6°C before plating onto petri dishes with glucose–peptone-yeast extract agar (GPYA) containing the following (g/L): glucose, 20; peptone, 10; yeast extract, 5; and agar, 20. To prevent bacterial growth, the medium was supplemented with levomycetin (1 g/L). Before plating, the samples of anthills and ants, litter, and soil were diluted in ratios of 1 : 50, 1 : 25, and 1 : 10, respectively. Three weighed portions from each sample were diluted with sterile water, shaken on a Multi Reax vortex (Heidolph, Germany) at 1700 rpm for 15 min, and used for inoculation in two replicates. The ant samples were at first inoculated as mentioned above and then the test tube content was thoroughly ground with a sterile glass rod, treated on a vortex, and reinoculated. The plates were incubated in a refrigerator for two to four weeks (Azieva, 1982). The grown colonies were grouped into macromorphological types and calculated; two to three strains from each colony type were isolated in pure culture and then identified to the species level according to their morphological and physiological characteristics (Kurtzman and Fell, 1998; Kurtzman et al., 2011). All tests were carried out according to the standard methods (Kurtzman and Fell, 1998) using the special Difco media. Identification was confirmed by analysis of nucleotide sequences of the ITS1-5.8S-ITS2 region and D1/D2 domain of the 26S (LSU) rDNA by the previously described method (Glushakova et al., 2011). Amplification of the rDNA regions was carried out using the primers ITS1f (5'-CTTGGTCATTTAGAGGAAGTA) and NL4 (5'-GGTCCGTGTTTCAAGACGG). The same primers were used for sequencing. The amplified regions were sequenced on an Applied Biosystems 3130xl Genetic Analyzer at the Research and Production Company Syntol (Moscow, Russia). For yeast identification, the data of the GenBank NCBI (ncbi.nlm.nih.gov) and CBS databases (www.cbs.knaw.nl) were used.

For each sample, the total yeast number of the colony-forming units per gram of the substrate (CFU/g) and the list of species and their relative abundance were determined.

RESULTS AND DISCUSSION

Yeasts were revealed in anthills in all the studied periods and their average number was 10^4 CFU/g. In the breeding chambers and in the ant samples, the yeast number sometimes considerably exceeded 10^5 CFU/g. The lowest number of yeasts was found in the samples of litter and soil. The yeast number in anthills was independent of the season, exposition, and depth of sampling.

In total, in the anthill samples, 22 species were found, which belonged to five genera of ascomycetous and eight genera of basidiomycetous yeasts (table). The species composition of yeasts differed considerably in various substrate types. Yeasts revealed in the ant samples were represented mainly by two species of the family *Debaryomycetaceae* (Kurtzman and Suzuki, 2010): *Schwanniomyces vanrijiae* and *Debaryomyces hansenii;* their relative abundance exceeded 90%. This finding is in agreement with the earlier data on predominance of yeasts of the genus *Debaryomyces* s.l. in the *Formica rufa* anthills (Golubev and Bab'eva, 1972; Bab'eva et al., 1975). As mentioned above, these authors described an independent species

Revealed yeast species and their relative abundance (%)

Species	Soil	Anthill	Ants
Candida galacta (Golubev et Bab'eva) Lee et al.	2.6	0.1	0
Candida saitoana Nakase et Suzuki	0.8	0.1	0
Candida sake (Saito et Oda) van Uden et Buckley ex Meyer et Ahearn	0	0.1	0
Candida santamariae Montrocher	2.6	0	0
Cryptococcus terreus di Menna	0.5	3.2	0
Cryptococcus terricola Pedersen	8.5	2.1	2.5
Cryptococcus victoriae Montes et al.	0.5	1.7	0
Cystobasidium pallidum (Lodder) Yurkov et al.	0.7	1.0	0
Cystofilobasidium capitatum (Fell et al.) Oberwinkler et al.	37.6	11.7	0
Cystofilobasidium infirmominiatum (Fell et al.) Hamamoto et al.	0.2	0.1	0
Debaryomyces hansenii (Zopf) Lodder et Kreger-van Rij	3.5	16.4	40.3
Guehomyces pullulans (Lindner) Fell et Scorzetti	0.3	1.6	0
Holtermanniella wattica (Guffogg et al.) Libkind et al.	0	0.2	0
Mrakia curviuscula Bab'eva et al.	0.1	0	0
Nadsonia fulvescens (Nadson et Konokotina) Sydow	0.8	0.1	0
Ogataea nitratoaversa Péter et al.	0	0.1	0
Rhodotorula mucilaginosa (Jörgensen) Harrison	1.1	0.5	0
Rhodotorula psychrophenolica Margesin et Sampaio	0.5	0	0
Schwanniomyces vanrijiae (van der Walt et Tscheuschner) Suzuki et Kurtzman	1.4	8.9	48.2
Trichosporon dulcitum (Berkhout) Weijman	3.0	0.1	0
Trichosporon guehoae Middelhoven et al.	0	0	0.1
Trichosporon moniliiforme (Weigmann et Wolff) Guého et Smith	34.8	49.5	8.8

Debaryomyces formicarius, which was suggested to form close associations with wood ants. However, we found no yeast species with the phenotypic characteristics of *D. formicarius* in any of the studied samples of ants and anthills. It should be noted that *D. formicarius* is morphologically and physiologically closely related to the species *Sch. vanrijiae*; its only, albeit essential, distinguishing feature is its ability to ferment sugars. Based on this feature, *D. formicarius* was described as an independent species; later, on the basis of DNA–DNA homology, it was synonymized with *D. vanrijiae* by Price et al. (1978) and finally assigned to the genus *Schwanniomyces*. No capability for sugar fermentation was revealed for the *Sch. vanrijiae* strains isolated in this study. The relative abundance of *Trichosporon moniliiforme* and *Cryptococcus terricola*, which are typical inhabitants of litter and soil, was 8.8 and 2.5%, respectively.

The anthill samples were characterized by higher species diversity; they contained the same species as the ant samples, but the ratio between species was different: *Debaryomyces* s.l. became subdominant, whereas the typical litter species *T. moniliiforme* prevailed. These samples also contained the species *Cystofilobasidium capitatum,* which is widespread in various plant substrates (Chernov, 2013). The maximum species diversity was revealed in litter and soil, which contained almost all the yeast species isolated in this study.

The species structure of yeast communities was somewhat different in various parts of anthills, which was apparently due to the ant activity. Apart from *T. moniliiforme*, the share of *D. hansenii* and *Sch. vanrijiae* increased in the deeper parts of the nest, especially in the samples taken at a depth of 20–35 cm, where the breeding chambers were located (Fig. 1).

The study of dynamics of the yeast species diversity revealed that more or less marked changes in proportions of yeast species occurred during a year; however, the taxonomic structure of yeast communities remained almost constant (Fig. 2). Abundance of the dominant species depended on a season and substrate type (Fig. 3). These species were revealed in all the studied periods; however, *D. hansenii* was the most abundant species in the ant samples at the end of the vegetation period. The abundance of *T. moniliiforme* in the anthill samples increased during the vegetation period, whereas the number of *C. capitatum* yeasts increased during the vegetation period in the control soil samples; this finding is in agreement with the data concerning the abundance of this species obtained in the studies of seasonal dynamics of the epiphytic yeast

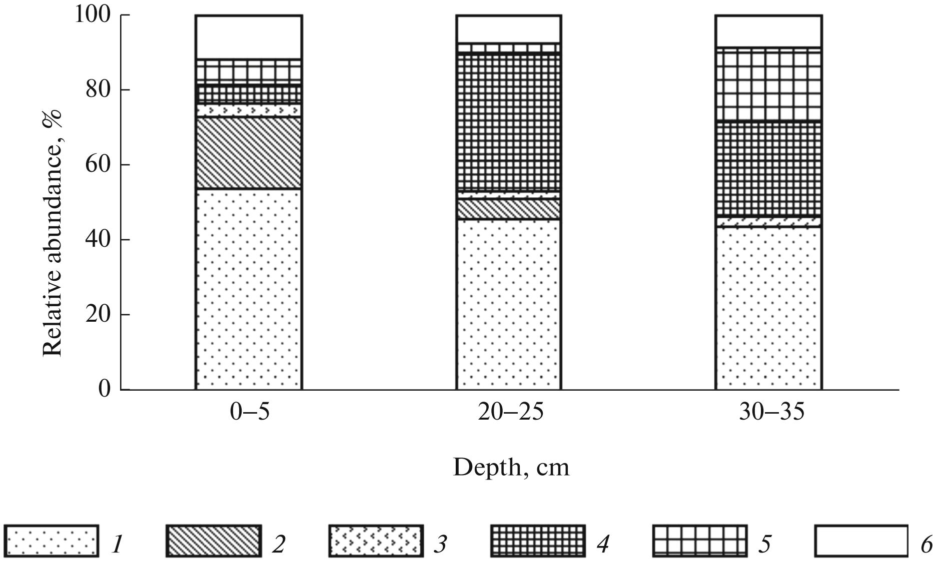

Fig. 1. The species structure of yeast communities in the anthill dome at different depths; yeast species: *Trichosporon moniliiforme* (*1*), *Cystofilobasidium capitatum* (*2*), *Cryptococcus terreus* (*3*), *Debaryomyces hansenii* (*4*), *Schwanniomyces vanrijiae* (*5*), and others (*6*).

communities (Glushakova and Chernov, 2010). Thus, seasonal changes in the species structure of yeast communities occurred in anthills and the background substrates, although they showed no considerable effect on a ratio between the dominating species in the studied ecotopes.

The obtained results indicate that wood ants have a considerable effect on the composition of microbial communities. Although the anthill material and the surrounding litter are composed of the same components, they differ considerably in the composition of yeast inhabitants. Discriminant analysis revealed that litter, soil, and the ant samples were considerably different in the species structure of yeast communities, whereas species composition of the anthill samples occupied the intermediate position. The most peculiar feature of yeast communities in anthills is high relative abundance of *Debaryomyces* s.l.: *Sch. vanrijiae* and *D. hansenii*, possibly because of the high concentration of easily available sugars, including glucose (Jilkova et al., 2012). Yeasts of the genus *Debaryomyces* are known to form associations not only with the *Formica* ants, but also with the borer ants *Camponotus* (Mankowski and Morrell, 2004) and with various xylophage insects (Gusteleva and Isaev, 1982). It had been suggested earlier that these yeasts serve as a

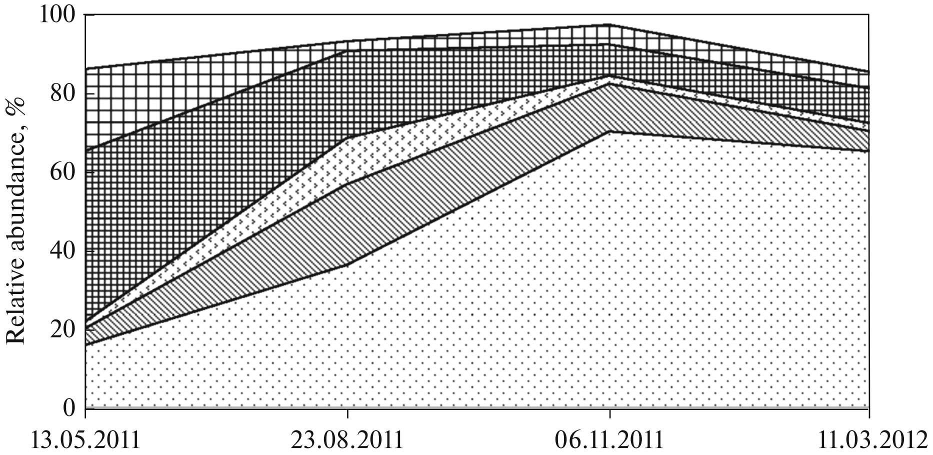

Fig. 2. Seasonal changes in the species structure of the yeast communities in anthills (designations are the same as in Fig. 1).

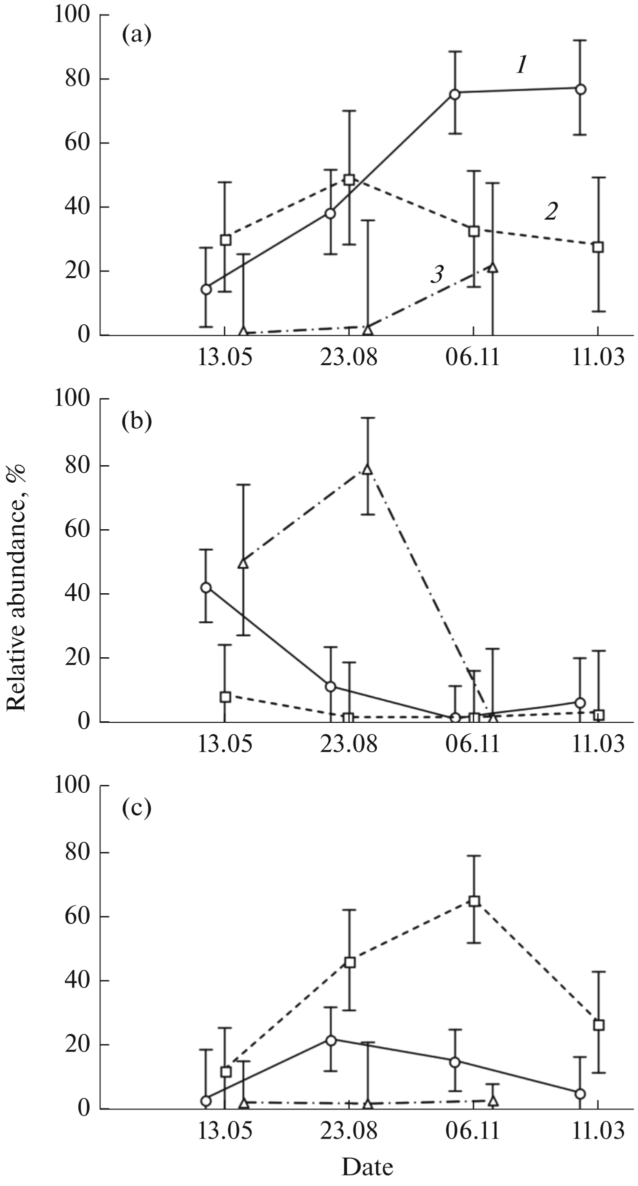

Fig. 3. Dynamics of abundance of the predominant yeast species in anthills (*1*), control soils (*2*), ants (*3*): *Trichosporon moniliiforme* (a), *Debaryomyces hansenii* (b), and *Cystofilobasidium capitatum* (c).

source of food for ants, since they actively produce various proteins and vitamins of the B group (Gusteleva and Isaev, 1982). Using yeast as an additive to the fodder resulted in an increase in the ant weight (Mankowski and Morrell, 2004). Since in this study the highest yeast population was revealed in the breeding chambers, it may be suggested that they served as a foodstuff for the *Formica aquilonia* larvae.

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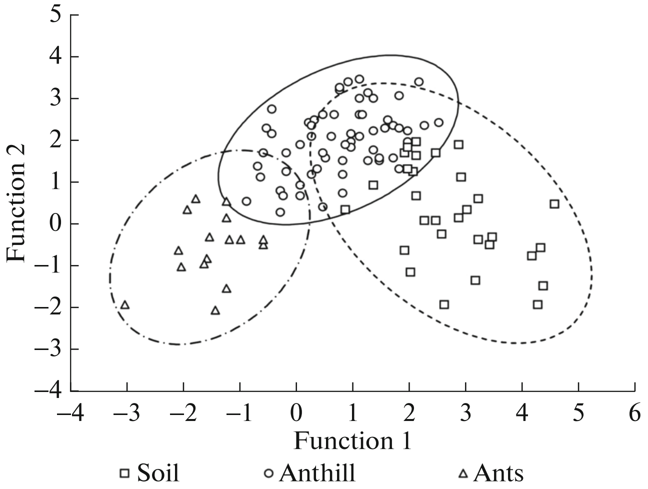

Fig. 4. Ordination of the studied substrates in accordance with the species structure of the yeast communities obtained by discriminant analysis.

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