ISSN 0026-2617, Microbiology, 2015, Vol. 84, No. 5, pp. 717–721. © Pleiades Publishing, Ltd., 2015.
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EXPERIMENTAL ARTICLES

Effect of Invasive Herb Species on the Structure of Soil Yeast Complexes in Mixed Forests Exemplified by *Impatiens parviflora* DC

A. M. Glushakova[a], A. V. Kachalkin[a], and I. Yu. Chernov[a, b, 1]

[a] *Lomonosov Moscow State University, Moscow, Russia*
[b] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia*

Received February 5, 2015

Abstract—Yeast abundance and diversity in a mixed forest sod–podzol soil under *Impatiens parviflora* DC plants was studied in comparison with unimpaired aboriginal herbaceous plants typical of the Central Russian secondary, after-forest meadow. The study was carried out throughout the vegetation period. Standard microbiological plating techniques revealed 36 yeast species. Typical pedobiotic (*Cryptococcus podzolicus, Wickerhamomyces anomalus*) and eurybiotic yeast species (*Rhodotorula mucilaginosa*) predominated in both biotopes. The relative abundance of the autochthonous soil yeast species *Cryptococcus podzolicus* was higher in the soil under aboriginal herbs than under *Impatiens parviflora*. Sites with aboriginal vegetation were also characterized by high abundance of the pedogamous species *Schwanniomyces castelli* and *Torulaspora delbrueckii*. The share of yeastlike *Trichosporon* fungi with high hydrolytic activity was considerably higher under adventitious plants *Impatiens parviflora*, as well as in the previously studied soil under *Heracleum sosnowskyi*.

Keywords: yeasts, soil, invasion, *Impatiens parviflora*, dynamics

DOI: 10.1134/S0026261715050094

The phenomenon of infiltration of living organisms into novel regions and ecosystems has attracted attention for more than 50 years. Intensity of invasive processes rose sharply in many countries at the late 1900s—early 2000s. Global climate changes, as well as anthropogenic disturbances of natural biogeocenoses and increase in transportation, contributed to it. Cases of expansion of species areal became more frequent. They were named biological invasions of alien species. A special branch of biology was formed to study such species. In Russia the problem of alien species has been discussed and studied from the late 1990s. Research on invasive process in aquatic and terrestrial ecosystems was primarily concerned with fauna and flora (compilation and maintaining of the Black books, creating the "black-lists" of the 100 most dangerous adventitious species indicating their invasion status, including detailed description of the biological and ecological properties of the species, history of their appearance, spreading and influence on natural and anthropogenic communities) (Vinogradova et al., 2010; Dgebuadze, 2014). However, no monitoring of changes in microbiocenoses affected by aggressive adventitious flora penetrating into meadow and forest biogeocenoses is conducted. Meanwhile, it was convincingly shown that changes in the producers' community remarkably influence the structure of yeast microbiocenoses. Previously we studied the dynamics of yeasts in the thicket of *Heracleum sosnowskyi* (Glushakova et al., 2015) and *Impatiens glandulifera* (Glushakova et al., 2011). In both cases we observed remarkable transformation of the soil yeast community. In case of *Heracleum sosnowskyi*, total reduction of the taxonomic diversity of allochthonous yeasts in soil, as well as increase of the share of yeastlike fungi (the hydrolytic component—species of the genus *Trichosporon*), was shown. The situation with *Impatiens glandulifera* was completely different: in soil under the plant we discovered yeast groups, absolutely atypical for soil. Ascospore-producing species of *Saccharomyces paradoxus, Kazachstania barnettii,* etc. dominated the community. In both cases the effect of adventitious flora on yeast communities of more labile meadow ecosystems was studied. The data concerning the effect of herbaceous introducents on the structure of yeast complexes of ecologically more inert forest ecosystems are completely absent.

Expansion of *Impatiens parviflora* areal started in the 1970s. Nowadays *I. parviflora* grows in all regions of Central Russia not only on the banks of the rivers and in forest parks, but in forest tracts as well. Its massive introduction into broadleaf and mixed forests started with displacement of aboriginal *Impatiens noli-tangere* (Csontos, 1986), and then of other aboriginal plants of the herb layer (*Asarum europaeum, Carex pilosa, Mercurialis perennis, Galeobdolon luteum, Dryopteris filix-mas, Athyrium filix-femina*, etc.). *Impatiens* demonstrates extremely high flexibility to shading. It is a cold-resistant species. Thus, due to its wide

[1] Corresponding author; e-mail: soilyeast@mail.ru

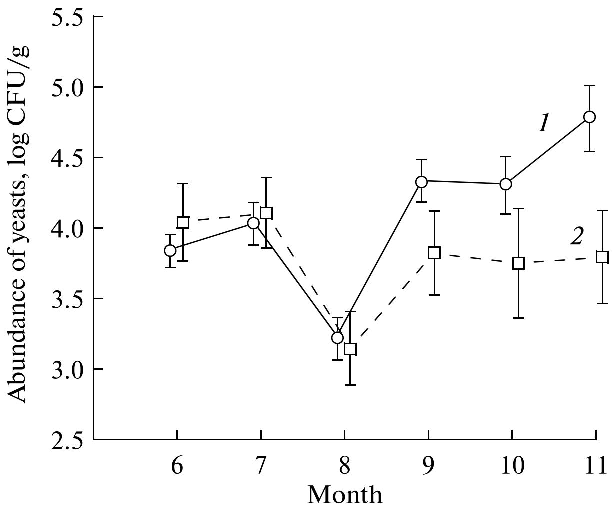

Fig.1 Dynamics of the total abundance of yeasts in studied soils: under *Impatiens* (*1*) and under meadow herbs (*2*).

ecological amplitude, it appears to be more competitive than many of the aboriginal species which are successfully displaced by it (Csontos, 1986; Goderfoid, Koedam, 2010; Markov, 1991). Studies conducted at Moscow Botanical Garden of the Russian Academy of Sciences demonstrated that *I. parviflora* was not damaged by disease or insects (Karpisonova, 1985). The species is known to be one of the intermediate hosts of parasite rust fungi *Puccinia komarovii* (Piskorz, Klimko, 2006).

The goal of the present work was to reveal the features of yeast communities in conditions of multi-year aggressive invasion of *I. parviflora* in forest biogeocenoses in comparison with unimpaired aboriginal herbaceous plants of the after-forest meadow on the territory of protected area in National Park Losiniy Ostrov (Moscow).

MATERIALS AND METHODS

The research was carried out in 2012 at the territory of protected area in the National Park Losiniy Ostrov (Moscow). The sod horizon (0–5 cm) of sod–podzol soil was analyzed in the areas under the thicket of *I. parviflora* and in the areas with unimpaired aboriginal herbaceous plants typical of a secondary, after-forest meadow (meadow formed at the area previously occupied by a broadleaf forest as a result of succession) in the Central Russian region. Approximate age of invasive growth of *I. parviflora* is about 40 years, since it is a well-known fact that active expansion of the areal of *I. parviflora* started in the 1970s (Vinogradova et al., 2010).

Soil samples were collected from June to November throughout the vegetative period of *I. parviflora*. Material was collected twice a week. Total amount of analyzed soil samples was 170.

Directly after sampling, 3–5 subsamples of ~1 g from each sample were transferred to test tubes and supplemented with sterile water to obtain 1:10 dilutions. The mixture was vortexed (MultiReax, Heidolph, Germany) for 15 min. The suspension was then plated on GPYa (glucose–peptone–yeast extract agar) in two replicates. The medium contained the following (g/L): glucose, 20; peptone, 10; yeast extract, 5; and agar, 10; levomycetin (500 mg/L) was added to suppress bacterial growth. The plates were incubated at room temperature for 5–7 days. Obtained yeast colonies were grouped into morphological types using a binocular magnifier. The number of colonies of each type was determined. Pure cultures of each colony type were isolated. The total number of isolated yeast strains was about 1000.

Identification of the yeast species was based on analysis of the nucleotide sequences of the ITS1–5.8S–ITS2 region and D1/D2 domains of 26S (LSU) region of rDNA. DNA isolation and PCR were carried out according to the previously described method (Glushakova et al., 2015; Kachalkin et al., 2015). Primers ITS1f (5'-CTTGGTCATTTAGAGGAAGTA) and NL4 (5'-GGTCCGTGTTTCAAGACGG) were used for amplification. Sequencing of the amplified region was carried out by Syntol Co. (Moscow, Russia). The strains were identified to the species level based on comparison of obtained nucleotide sequences to the NCBI GenBank database (ncbi.nlm.nih.gov) and the CBS database (cbs.knaw.nl).

For each sample, total number of yeasts on GPYa and relative abundance of each species were determined. Statistical analysis of the data was carried out to compare yeast communities of two phytocenoses (F-test and Shannon's diversity index).

RESULTS AND DISCUSSION

Total abundance of yeasts isolated on GPYa from soil under *I. parviflora* and under aboriginal herbs of a secondary, after-forest meadow varied from 10^3 to 10^5 CFU/g and was maximal under the introducent at the period of its massive transient dying out after the early frosts in November (Fig. 1). Total increase of the number of yeasts in soil in autumn might be associated with the introduction of epiphytic yeasts into the soil with the leaf fall during this period. Such type of annual dynamics of yeast abundance in soils is typical of all kinds of plant communities of Central Russia (Glushakova and Chernov, 2007; Chernov, 2013).

A total of 36 species of yeast fungi were isolated during the study (table). Diversity of yeast species in soil under *I. parviflora* and under herbs of the after-forest meadow changed synchronously, reaching the maximum in September. Shannon's index during all the period from June to November (time of *I. parviflora* coming into the senile stage of ontogenesis) was always slightly lower in soil samples under the introducent (Fig. 2).

Typical pedobiont (*Cr. podzolicus, W. anomalus*) and euribiont (*Rh. mucilaginosa*) yeast species predominated in both types of biotopes. High abundance of the pedogamous species *Sch. castellii* and *T. delbrueckii* was also typical of the meadow with aboriginal herbaceous plants. The share of the autochthonous soil species *Cr. podzolicus* in soil under aboriginal herbs was higher than under *I. parviflora*. Under the growth of the adventitious species *I. parviflora*, the share of yeastlike fungi of the genus *Trichosporon* with high hydrolytic activity was remarkably higher.

Similar changes of the soil yeast complex towards increased relative abundance and diversity of yeastlike species was observed under conditions of aggressive invasion of *Heracleum sosnowskyi* in the meadows (Glushakova et al., 2015). In the soil under *I. parviflora*, four members of the genus *Trichosporon* were discovered (*Tr. aquatile, Tr. dulcitum, Tr. laibachii*, and *Tr. porosum*). *Tr. aquatile* was not present in the meadow soil. At the same time, relative abundance of *Tr. porosum* alone was approximately equal for two studied soils. The numbers of *Tr. dulcitum* and *Tr. laibachii* were noticeably higher in soils under the introducent.

It was repeatedly shown previously that the same types of epiphytic yeasts predominate on various types of plants regardless of their taxonomic position and ecological features. However, different species of yeasts demonstrate different types of seasonal dynamics of relative abundance. Thus, combination of dynamics of yeast species with ontogenesis cycles of plants creates a pattern of epiphytic yeast population dynamics, which is unique for each plant species (Glushakova and Chernov, 2010). Different pictures were observed while comparing the dynamics of relative abundance of yeasts in soils under *I. parviflora* and on the aboriginal meadow (Fig. 3). For instance, the features of seasonal dynamics of abundance of *Rh. mucilaginosa* and *W. anomalus* were similar in both types of biotopes: the high share of species in summer–early autumn and then a decrease by the middle and the end of autumn. The dynamics observed for abundance of the soil species *Cr. podzolicus* in soils of both biotopes was also similar: its share gradually decreased by the end of the summer with subsequent recovery to the level of the early vegetation period. Quantitative dynamics of other species exhibited certain differences. Thus, relative abundance of *Tr. porosum* increased regularly by the end of the autumn under *Impatiens* and remained practically unchanged during the whole period of studies under the aboriginal herbs.

Thus, in spite of certain similarity in the structure of the yeast community in soils of the secondary after-forest meadow and invasion thicket of *Impatiens*, considerable differences were observed as well. These differences are connected mostly with an increase in the share of members of the genus *Trichosporon* in soils under *Impatiens* and of members of the pedogamous ascomycete yeast species *Torulaspora* and *Schwanniomyces* under aboriginal herbs of the secondary after-forest meadow.

Average (for all studied period) relative abundance of yeasts in soils under the meadow and under the growth of *Impatiens* (%); species with reliable abundance variations at $p < 0.05$ are shown in bold

Species	Under the meadow	Under *Impatiens*
Asterotremella albida	0	0.03
Babjeviella inositivora	0	0.06
Barnettozyma californica	0	0.09
Candida catenulata	2.84	1.45
Candida pseudolambica	0	0.66
Candida saitoana	1.65	2.34
Cryptococcus himalaensis	2.22	0
Cryptococcus magnus	3.34	0.56
Cryptococcus oeirensis	0	1.49
Cryptococcus podzolicus	16.58	8.67
Cryptococcus saitoi	0.01	0.59
Cryptococcus terreus	0	3.28
Cryptococcus terricola	1.3	1.03
Cryptococcus victoriae	0	3.18
Cystofilobasidium capitatum	0	0.66
Cystofilobasidium macerans	0.43	0
Debaryomyces hansenii	0	0.3
Debaryomyces maramus	1.17	0
Lindnera vartiovaarae	1.96	1.78
Metschnikowia pulcherrima	1.36	0.01
Meyerozyma guilliermondii	0.16	1.36
Mrakia curviuscula	0	0.89
Pichia kudriavzevii	0	0.09
Rhodotorula glutinis	0.21	0
Rhodotorula mucilaginosa	5.99	18.06
Saturnispora zaruensis	0	0.56
Schizoblastosporion starkyi-henricii	0.35	0
Schwanniomyces castellii	17.25	0
Schwanniomyces polymorphys	0	0.58
Torulaspora delbrueckii	12.24	0.63
Trichosporon aquatile	0	0.25
Trichosporon dulcitum	1.2	7.14
Trichosporon laibachii	0.79	13.42
Trichosporon porosum	20.56	18.22
Wickerhamomyces anomalus	8.26	12.62
Yarrowia lipolytica	0.13	0

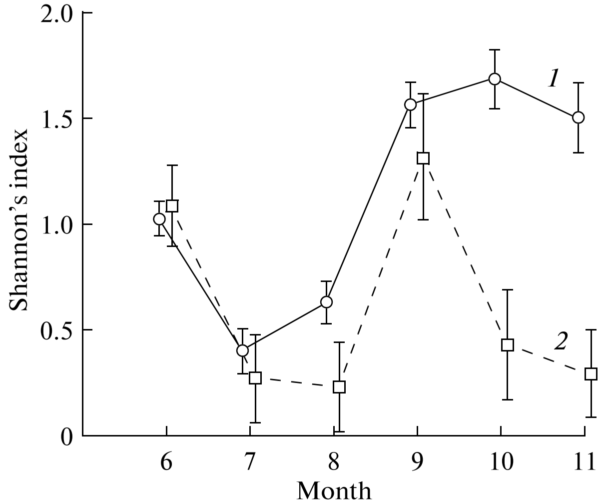

Fig. 2. Dynamics of yeasts diversity in studied soils: under *Impatiens* (*1*) and under meadow herbs (*2*).

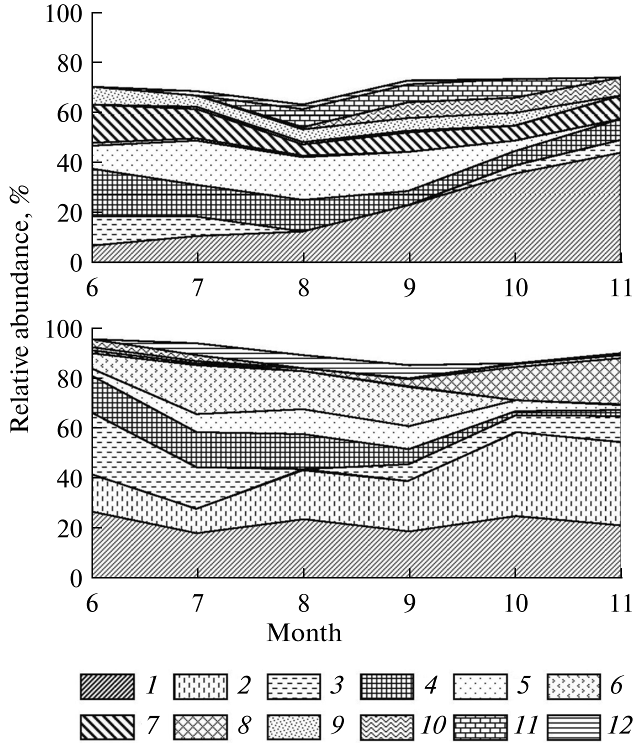

Fig.3. Dynamics of average monthly value of relative abundance of the dominant yeast species in soils under the growth of *Impatiens parviflora* (top) and under the meadow herbs (down); yeast species: *Trichosporon porosum* (*1*), *Schwanniomyces castellii* (*2*), *Cryptococcus podzolicus* (*3*), *Wickerhamomyces anomalus* (*4*), *Rhodotorula mucilaginosa* (*5*), *Torulaspora delbrueckii* (*6*), *Trichosporon laibachii* (*7*), *Cryptococcus magnus* (*8*), *Trichosporon dulcitum* (*9*), *Lindnera vartiovaarae* (*10*), *Cryptococcus victoriae* (*11*), *Candida catenulata* (*12*).

It is interesting to compare the obtained pattern of yeast population in soils under the growth of *I. parviflora* and other previously studied aggressive introducents. As was mentioned, the dynamics of yeast populations was studied previously in soils under *Heracleum sosnowskyi* (Glushakova et al., 2011) and in the thicket of *Impatiens glandulifera.* In case of Sosnowsky's Hogweed, the situation was similar to that revealed for *I. parviflora*: we observed the features of transformation of the yeasts complex resulting in increased abundance of the hydrolytic component (species of the genus *Trichosporon*). Unlike Sosnowsky's Hogweed, the taxonomic diversity of yeast species under *I. parviflora* did not decrease. Due to allelopathic influence of *H. sosnovskyi* seeds can be preserved in soil for a long period of time (Bochkarev et al., 2011), and because this species does not form forest litter, the main share of yeast species from the soil–litter horizon decreases in number and stops to develop. The hydrolytic component of the community (species of the genus *Trichosporon*) was not affected so negatively by *H. sosnowskyi.* Since the seeds of *I. parviflora* do not possess such features, taxonomic diversity of yeasts is not decreased. The situation with *I. glandulifera* was absolutely different: in soil under this species were discovered yeast groups atypical for soils with dominance of ascosporous species (*Saccharomyces paradoxus, Kazachstania barnettii*, etc.). The annual hygrophyte plant *I. glandulifera* forms dense growth. Overground parts of *Impatiens* develop very rapidly, in several days after the frosts. As a result, soil is rapidly enriched with monosaccharides. They stimulate activity of the most copiotrophic species of yeasts, related to saccharomycetes. All species of saccharomycete yeasts revealed in our study demonstrated active sporulation. This fact indicates their ability to survive in soils as spores after exhaustion of the source of nutrients. At the same time, the absence of the forest litter under *Impatiens* results in rapid death of typical epiphytic yeasts being introduced to the soil. Thus, in the absence of typical forest litter under the growth of annual introduced hygrophytes, absolutely specific yeast complexes may be formed, remarkably different in taxonomic structure from both epiphytic and typical soil communities of forest and meadow biocenoses of the Central Russia. *I. parviflora*, as well as *H. sosnowskyi* and *I. glandulifera*, does not form the forest litter. However, it does not matter in the case of forest biocenoses, as the forest litter is formed due to tree leaf fall, and its ecological role can be compared to that of the meadow where the litter is formed of herbal remains. Dying out of *I. parviflora* at the first frosts occurs not so rapidly as in case of *I. glandulifera*, although faster than in case of many other herbs. Fast enrichment with monosaccharides that stimulate the activity of the most copiotrophic species of yeasts, related to saccharomycetes, does not occur. As a result, no sharp transformation of the soil yeast complex is observed. Thus, in the case of *I. parviflora*, aggressive influence of the introducent on the structure of the soil yeast community is minimal compared to the previously studied introducents. Taxonomic diversity is not decreased, while, similar to the case of *H. sosnowskyi*, the share of *Trichosporon* species is increased. The decreased effect of the introducent in case of *I. parviflora* is likely to be associated with the compensatory effect of the forest ecosystem, which is more stable and inert to invasions than the meadow ecosystem.

Revealed specific features of transformation of soil yeast communities in the thicket of *I. parviflora* indicate that introduced organisms cause transformations in the structure of soil microbocenoses in forest ecosystems. The determinative role of traditionally dominant species in the community is decreased, and the number of the minor components is increased. Currently, naturalization of *I. parviflora* occurs very rapidly. In Central Russia, forest ecosystems appeared to be very sensitive to invasion of this species. Unlike the meadow introducents *I. glandulifera*, which transforms the soil yeast complex fundamentally, and *H. sosnowskyi*, which causes a reductive effect on the taxonomic diversity of yeast species of the soil fungal communities, *I. parviflora* has a smaller effect, associated with the decreased abundance of a typical pedobiont *Cr. podzolicus* and increased share of the hydrolytic component in the community.

Thus, our results demonstrate that invasion of the forest phytocenoses by *I. parviflora* leads, first of all, to a dramatic decrease in biodiversity of the plant species and alteration of the allochthonous copiotrophic soil yeast complex, resulting in an increase of the share of yeastlike species functionally similar to mycelial soil fungi. It is important to note the importance of further studies of the effect of euneophytes on the structure of microbial communities to determine the changes and disorders in their structure in order to correct and possibly prevent the negative consequences.

ACKNOWLEDGMENTS

This research was partially supported by the Russian Foundation for Basic Research, project no. 13-04-00822. Yeast strains identification was sponsored by grant Russian Science Foundation, project no. 14-50-00029.

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Translated by E. Botchkova