Cocultivation of *Pleurotus ostreatus* (Jacq.) P. Kumm. with Yeasts

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**Abstract**—Cocultivation of *Pleurotus ostreatus* with eight yeast species were investigated on water agar. Special mycelial structures contacting with yeast cells were found in such cultures: nipple-like appendages and coraloid hyphae. Three out of eight species, *Hanseniaspora uvarum*, *Rhodotorula minuta*, and *Saccharomyces cerevisiae* were identified as trophic preferendum for *P. ostreatus*. These three yeast species were used for mushroom cultivation on sunflower seed peel. The biomass of fruiting bodies increased by 52.8–75.7% with the *H. uvarum* and *S. cerevisiae* suspension presence in the substrate.

**Keywords:** *Pleurotus ostreatus*, cocultivation, trophic preferendum, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*.

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**INTRODUCTION**

Oyster mushroom, *Pleurotus ostreatus* (Jacq.) P. Kumm., is one of the most widely distributed mushroom of *Pleurotus* genus intensively cultivated in various countries of the world. In mushroom culturing, the oyster mushroom is appreciated due to its gustatory properties and expressed “mushroom” odor as well as its indiscriminateness to conditions of cultivation at various substrates of plant origin [1].

The oyster mushroom belongs to the ecological-trophic group of xylotrophic mushrooms. For all tree-destroying mushrooms, the main growth-limiting factor is nitrogen since C : N ratio in wood substance varies from 300 : 1 to 1000 : 1 and even more, while 30 : 1 ratio is optimal for growth of a majority of mushrooms [2]. For many xylotrophic mushrooms, an ability to compensate nitrogen deficiency owing to parasitism on bacteria, yeasts or algae, persisting on wood substrate, was declared [3, 4]. Epiphytic yeasts are widely distributed in nature at surfaces of footstalks, leaves, fruits and berries of grassy and woody plants as well as at the cortex of trees. Yeasts do not directly participate in the process of wood destruction since they do not have the necessary enzymes; however, yeasts serve as nitrogen source for xylotrophic yeasts thereby influencing processes of wood destruction [5].

The feature of the oyster mushroom to use microorganisms as nutrient source may be successfully used in biotechnology and mushroom culturing for increasing of eatable mycelium biomass: for example, cocultivation of the oyster mushroom with *Azospirillum brasilense* bacteria in the liquid medium allows us to increase the output of mycelium biomass by 30% and to reduce the time for culturing of the growth mycelium [6]. To stimulate growth and fruiting of mushrooms, from the viewpoint of safety for health of consumers, it is preferable to use biological objects (bacteria, yeasts) nonpathogenic for humans instead of the addition of chemical compounds—regulators. Moreover, addition of microorganisms to substrate of live culture allows mushroom mycelium to use it as nutrition during the whole period of cultivation.

The aim of the present work is to investigate interactions between oyster mushroom and yeasts in the process of vegetative growth of mycelium in culture and the influence of yeasts on the process of mushroom fruiting.

**MATERIAL AND METHODS**

**Objects of investigation.** Xylotrophic basidiomycete *Pleurotus ostreatus* (Jacq.) P. Kumm. HK-35. Yeasts of ascomycete affinitet are: *Debaryomyces hansenii* (Zopf)Lodder & Kreger-van Rij, *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff, *Kluyveromyces marxianus* (E.C. Hansen) Van der Walt, *Metschnikowia pucherrima* Pitt. & M.W. Mill., *Saccharomyces cerevisiae* Meyen ex E.C. Hansen 3785, 3809, Moment. Yeasts of basidiomycete affinitet are: *Cryptococcus albidos* (Saito) C. E. Skinner, *Cystofilobasidium capitatum* (Fell, I.L. Hunter & Tallman) Oberw. & Bandoni, *Rhodotorula minuta* (Saito) F.C. Harrison.

**Cultivation on agar medium.** Mycelium of the oyster mushroom was cultured in Petri dishes along with yeasts on 1.5% water agar at 25 ± 1°C. Cocultures were
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created via addition of water suspension of yeast (concentration $10^5$–$10^7$ cells per ml) growth on slant wort agar (2.2%) through dropping (50 µl) at a distance of 1.5–2 cm from the edge of mushroom colony by the seventh day of mycelium growth. Microscope investigation was performed by the third to fourth day of coculturing using Axioskop 40 FL device with AxioCam MRc. All experiments were done in triplicate.

Obtaining of fruiting bodies. Fruiting bodies of the oyster mushroom were obtained on sunflower seed peel in conditions of climatic camera. Seed mycelium was grown on wheat grains with 3% chalk-stone for 14 days in the thermostat at 25 ± 1°C. Cultivation substrate was prepared according to a modified technique of Stamets [7]. Humidity (moisture content) was detected using a well-known method: humidity (%) = (mass of wet substrate − mass of dried substrate) / mass of wet substrate ×100% [8]. Forcing of fruiting bodies was performed at a temperature of 20 ± 1°C and air relative humidity of 95%. After inoculation of substrate with mycelium of the oyster mushroom, water suspension of yeasts (concentration $10^5$–$10^7$ cells per ml), 10 ml per each flask, was added to substrate. Two control variants were used: substrate without any supplements (control no. 1) and substrate with addition of sterile water (10 ml per each flask) (control no. 2). All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Cultivation on agar medium. Mycelium of the oyster mushroom was totally overgrown with microcolonies of yeasts at the surface of water agar. In cocultures with C. albicus, H. uvarum, M. pulcherrima, and S. cerevisiae, there is 4–8-fold increase of frequency of branching of mycelium hyphae. Significantly branching mycelium hyphae form coralioid structures [3]; these structures were observed in cocultures with H. uvarum, R. minuta, and S. cerevisiae. In cocultures with C. albicus, D. hansenii, K. marxianus, and S. cerevisiae, there were mycelial rings (Fig. 1a); it presents a part of twirly twisted vegetative hypha and phenotypically similar to chasseur loops that form mycelium of ravenous fungi as well as to P. ostreatus in the presence of nematodes in culture [2]. Functions of mycelial rings of the oyster mushroom are not fully clear: probably, they are formed not for binding of yeast cells but act as physiological response of mycelium to the presence of yeasts in the culture.

Both in the cases of monoculture of the oyster mushroom and cocultures with all yeasts species, except for R. minuta, there were capitate outgrowths (Fig. 1b). In the apical part of the outgrowth, there are drops of secrete. This secrete is active against nematodes: these outgrowths are formed in the oyster mushroom and in ravenous fungi and serve for physi-

Fig. 1. Micromorphology of mycelium of P. ostreatus in combined culture (CC) with yeast cells: (a) mycelial loops in CC with C. albicus; (b) capitate outgrowths in CC with C. albicus (indicated by arrows); (c) contacts with mycelial hyphae with yeast cells in CC with H. uvarum; (d) contacts between of mycelial hypha with yeast cell in CC with R. minuta (nipple-like appendages are indicated by arrows).
Fruiting of *P. ostreatus* on sunflower seed peel in the presence of yeast cells and without them

| Variants          | Time of substrate overgrowth (days) | Duration of incubation (days) | Maximal height of fruiting bodies ± 5 mm | Mass of fruiting bodies from 1 flask (g) |
|-------------------|-------------------------------------|-----------------------------|------------------------------------------|---------------------------------------|
| Control no. 1     | 18                                  | 11                          | 70.5                                     | 7.0 ± 1.0                             |
| Control no. 2     | 18                                  | 13                          | 60                                       | 8.0 ± 0.8                             |
| + *H. uvarum*     | 14                                  | 11–13                       | 55.5                                     | 12.3 ± 1.8                            |
| + *R. minuta*     | 14                                  | 13                          | 70                                       | 9.0 ± 1.0                             |
| + *S. cerevisiae* | 14                                  | 11–13                       | 55                                       | 10.7 ± 0.6                            |

Cal inactivity of nematodes [2]. However, functions of secrete produced by capitate outgrowths of the oyster mushroom are not totally known. Probably, mycelial cells produce not only toxin for nematodes but also some other compound.

In all cocultures, formation of special nipple-like short appendages that connect mycelium and yeast cells was detected (Fig. 1c, 1d) [9]. Nipple-like appendages were rarely seen in cocultures with *C. albidus, C. capitatum, D. hansenii, K. marxianus, S. cerevisiae* 3785; from one to three in the visual field are in cocultures of *M. pulcherrima, R. minuta, S. cerevisiae* 3809, *S. cerevisiae* Moment. It formed especially intensively in coculture of the oyster mushroom with yeasts *H. uvarum* (more than three in one visual field). Contacts between hyphae with yeast cells were detected in cocultures with *C. albidus, C. capitatum, H. uvarum, R. minuta, M. pulcherrima, S. cerevisiae* 3785, 3809 and Moment, especially intensively (more than three in one visual field) with *H. uvarum*. Contacts were formed only in those cocultures where suspension of live yeast cells was introduced. After introduction of boiled cells, contacts with yeast cells and nipple-like appendages were not formed.

Trophic preferendum for *P. ostreatus* was detected on basis of the following features: 4-fold increase in the frequency of hyphae branching, intensive formation of nipple-like appendages (two or more in visual field), coralloid structures, and numerous contacts of mycelium with yeast cells (two or more in visual field). Thus, three of eight yeast species, *H. uvarum, R. minuta* and *S. cerevisiae*, were chosen as trophic preferendum. These yeast species were used in the process of cultivation of the oyster mushroom on sunflower seed peel.

Obtaining of fruiting bodies. In all samples supplemented with yeasts (*H. uvarum, R. minuta, S. cerevisiae*), there was a total overgrowth of a substrate with mycelium by the 14th day (in control nos. 1 and 2, it was by the 18th day); thus, forcing of fruiting bodies in samples with yeasts was detected 4 days earlier. Incubation duration (time since start of forcing to period of collection of fruiting bodies) was almost the same while culturing of the oyster mushroom without and with yeasts: fruiting bodies were cut off by the 11th–13th day in all samples.

Humidity of the substrate after addition of sterile water and suspension of yeast cells was changed minimally inside of a norm stated for cultivation of the oyster mushroom [10]. In control no. 1, substrate humidity was 61.2 ± 2.0%; in control no. 2 and in experimental samples, it was 60.5 ± 1.5%. Thus, influence of substrate humidity on the rate of its overgrowth with mycelium may be ignored: increase in mycelium growth is due to addition of yeast cells to suspension substrate.

Fruiting bodies of *P. ostreatus* were phenotypically similar at cultivation with yeasts or without them. Mushrooms were formed individually or in groups on 2–3 fruiting bodies. It was noted that, in control nos. 1 and 2 and in the presence of *R. minuta*, yeasts fruiting bodies are higher (to 75 mm) and thin, while in the presence of *H. uvarum* and *S. cerevisiae* they are short (to 60 mm) and more thick. Significant differences in biomass in control nos. 1 and 2 and in the presence of *R. minuta* yeasts were not detected. Biomass of fruiting bodies was higher than in control: by 75.7% in the presence of *H. uvarum* and by 52.8% in the presence of *S. cerevisiae* (table).

Maximal increase in biomass of harvest was detected in the presence of *H. uvarum* yeasts, which correlates with maximal amount of nipple-like appendages and contacts between mycelium and *H. uvarum* yeast cells on agar medium. Viable yeast cells were found in the substrate by the 14th and 28th days of cultivation during the whole period of mycelial growth and fruiting. Probably, the oyster mushroom uses yeast cells as additional nutrient source during its growth at the substrate.

The obtained data demonstrate the ability of the oyster mushroom, *P. ostreatus*, transform to parasitism and to use yeast cells as nutrient source in laboratory conditions on water agar, i.e., on medium without sources of carbon and nitrogen needed for the mushroom. Furthermore, different level of parasitic activity toward various species of mushrooms is observed. The introduction of suspension of live yeast cells results in increase of growth rate for the mushroom at the substrate and increase in biomass of fruiting bodies.
REFERENCES

1. Stamets, P., Growing Gourmet and Medicinal Mushrooms, Third ed., Berkeley: Ten Speed Press, 2000.
2. Barron, G.L., Predatory Fungi, Wood Decay, and the Carbon Cycle, Biodiversity, 2003, vol. 4, pp. 3–9.
3. Hutchison, L.J. and Barron, G.L., Parasitism of Yeasts by Lignicolous Basidiomycota and Other Fungi, Can. J. Bot., 1996, vol. 74, pp. 735–742.
4. Hutchison, L.J. and Barron, G.L., Parasitism of Algae by Lignicolous Basidiomycota and Other Fungi, Can. J. Bot., 1997, vol. 75, pp. 1006–1111.
5. Blanchette, R.A. and Shaw, C.G., Associations among Bacteria, Yeasts, and Basidiomycetes during Wood Decay, Phytopathology, 1978, vol. 68, pp. 631–637.
6. Nikitina, V.E., RF Patent 2249614, 2003.
7. Stamets, P., Growing Gourmet and Medicinal Mushrooms, Berkeley: Ten Speed Press, 1993.
8. http://ecocentr.ru/index.php?option=com_content&view=article&id=6:2008–03–24–10–10–48&catid=3:technologies&Itemid=4 22.02.2011.
9. Kamzolkina, O.V., Grishanina, A.N., Pancheva, E.V., Volkova, V.N., and Kozlova, M.V., Micromorphological Features of Pleurotus pulmonarius (Fr.) Quel. and P. ostreatus (Jacq.) P. Kumm. Strains in Pure and Binary Culture with Yeasts, Tsitologiya, 2006, vol. 48, no. 2, pp. 153–160.
10. Zaikina, N.A., Kovalenko, A.E., Galynkin, V.A., D’yakov, Yu.T., and Tishenkov, A.D., Osnovy biotekhnologii vysshikh gribov (Fundamentals of Biotechnology of Higher Fungi), St. Petersburg: Prospekt Nauki, 2007.