Properties of micromycetes – phenoloxidase producers

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Twelve producers of phenoloxidases – the fungi *Galactomyces geotrichum*, *Myrothecium verrucaria*, *Mortierella verticillata*, *Dipodascus armillariae*, *Dipodascus albidus*, *Geotrichum candidum*, *Oedocephalum albidum*, *Mortierella hyalina*, *Hormonema prunorum*, *Papularia sphaerosperma*, *Aspergillus repens*, *Sporotrichum pruinosum* were studied. Their peroxidase, lacase and tyrosinase activity was defined when micromycetes were cultivated in n-Czapek medium (control variant) and in Czapek medium with 5 mM $K_2Cr_2O_7$.

A metal concentration of 5 μM $K_2Cr_2O_7$ was found to reduce the biomass of all micromycetes studied. This decrease in *Myrothecium verrucaria* was 3.3, *Aspergillus repens* 4.14; *Dipodascus armillariae* 3.7 and *Papularia sphaerosperma* 3.13 times larger than in the control.

The concentration of 5 μM Cr^{6+} stimulated the peroxidase of all micromycetes, and its maximum was reached on day 21 of cultivation. *Galactomyces geotrichum*, *Myrothecium verrucaria*, *Mortierella verticillata*, *Mortierella hyalina* and *Hormonema prunorum* showed the greatest peroxidase activity (33.15, 32.19, 29.69, 25.31 and 23.75 u/ml, respectively).

The influence of Cr^{6+}on the lacase activity of various micromycetes was different. The activity of *Dipodascus albidus*, *Dipodascus armillariae*, *Motierella verticillata* (0.098, 0.092, 0.068 D, respectively) was highest after 8 days and of other micromycetes after 13 days of cultivation.

The peak of tyrosinase activity of all micromycetes studied was noted after 21 days of cultivation. The highest tyrosinase activity was shown by *Myrothecium verrucaria* (34.15), *Aspergillus repens* (28.44) and *Mortierella hyalina* (28.31 cu/g).

Key words: micromycetes, peroxidase, lacase and tyrosinase activity

INTRODUCTION

The problem of bioconversion of plant polymers is not new, but it is still urgent. A substratum of plants still remains the main raw material for obtaining fodder and physiologically active substances.

Bioconversion of a plant substratum, which depends on microorganisms, is a complex biochemical and physiological process in which many ferment systems take part.

The main enzymes produced by micromycetes, which take part in the degradation of lignin, are phenoloxidases: peroxidase of lignine, Mn-peroxidase and lacase [1, 3, 6]. An important role in this process falls to tyrosinase, catalase and glucooxidase.

Various ecological factors have a great influence on the activity of phenoloxidases and on the decay course of plant waste. Micromycetes react not only to the amount of nutrition elements in soil, but also to temperature, acidity of soil (pH), moisture and pollution by various chemical substances.

Large concentrations of heavy metals sometimes are over 1 g/l^{-1} and often harmful for microorganisms. Some microorganisms can modify chemical elements and remove them from a medium, if the concentration of these elements is low and they are not toxic.

A certain group of microorganisms are able to use some combination of heavy metals (if their concentration is not toxic) as an energy source and as a final acceptor of electron. Among other elements, chromium is an essential micronutrient in some biological systems, especially in insulin action, possibly glucose utilization [2] and stimulation of enzyme systems [5]. Little is known of microbial requirements; it is more frequently regarded as a toxic element to which some organisms have evolved resistance mechanisms.

The object of the work was to select micromycetes – producers of phenoloxidases and to determine their peroxidase, lacase and tyrosinase activities and to show changes of ferments' activity under the influence of Cr^{6+}.

MATERIALS AND METHODS

The substrates of peroxidases, lacases and tyrosinases were determined by the method of Lyro [4]. The influence of Cr^{6+} on the activity of peroxidase, lacase and tyrosinase was defined when micromycetes – producers of phenoloxidases were cultivated in n-Czapek medium (control variant) and in Czapek medium with 5 μM $K_2Cr_2O_7$. Micromycetes were cultivated under such conditions for 21 days at a temperature of 28 °C. Later on an increase in the biomass and in the activity of peroxidases, lacases and tirozinases were determined.

Peroxidase activity (PA) was determined with o-dianisidin reagent [10], lacase – with p-phenylendiamin chloride [7], and tirozinase – with tyrosine [9].

RESULTS

The following micromycetes – peroxidase and lacase producers were screened by oxydase tests (0.005% α-naphthole): *Galactomyces geotrichum* (Butl. et Petersen) Redhead et Malloch, *Myrothecium verrucaria* (Alb. et Schweinitz) Ditmar ex Fr., *Mortierella verticillata* Linnem, *Dipodascus armillariae* W. Gams, *Dipodascus albidus* Lagerh, *Geotrichum candidum* Link:Fr., *Oedocephalum albidum* (Preuss) Sacc., *Mortierella hyalina* Hair W. Gams, *Hormonema prunorum* (Dennis et Buhagiar) Hermanides-Nijhof, *Papularia sphaerosperma* (Pers.) Höhn, *Aspergillus repens* de Bary, *Sporotrichum pruinosum* Gilman et Abbott.

The influence of Cr^{6+} on increase of biomass of micromycetes. In these studies, the largest amount of biomass (Fig. 1) was determined after cultivation of *Myrothecium verrucaria*, *Aspergillus repens*, *Dipodascus armillariae* and *Papularia sphaerosperma* (9.82; 7.33; 7.24 and 7.28 mg/l respectively).

The metal concentration of 5 μM $K_2Cr_2O_7$ reduced the biomass of all micromycetes studied. This concentration of chromium reduced the amount of biomass of *Myrothecium verrucaria* to 2.95, *Aspergillus repens* to 1.77, *Dipodascus armillariae* to 1.95 and *Papularia sphaerosperma* to 2.32 mg/l. This decrease was 3.3, 4.14, 3.7 and 3.13 times higher in comparison with the control.

Fungi are most resistant to concentrations of toxic substances and to ions of heavy metals [11]. Fungi have two reaction groups which protect them from high concentrations of heavy metals: 1) when the ions of metals are bound outside the cell (exometabolites of the cell which make insoluble combinations with metals are accumulated) or are absorbed on the surface of the cell wall; 2) protective reactions, which are going on inside the cell and whose aim is detoxication of heavy metal ions. The essence of these reactions is induction of substances that convert ions into non-toxic forms. Some microorganisms oxidize the reduced forms of heavy metals, others reduce the oxidized forms of such elements.

The influence of chromium additions on the activity of peroxidases, lacases and tyrosinases of micromycetes. A low peroxidasic activity of all cultivated micromycetes was observed in n-Czapek medium (Fig. 2). More active were *Dipodascus albidus*, *Hormonema prunorum* and *Mortierella verticillata* (1.63; 1.44 and 1.28 units of peroxidasic activity/ml (u/ml), respectively).

Studies of peroxidase activity changes in the dynamic growth of micromycetes indicated that this activity manifested in the later period of the growth.

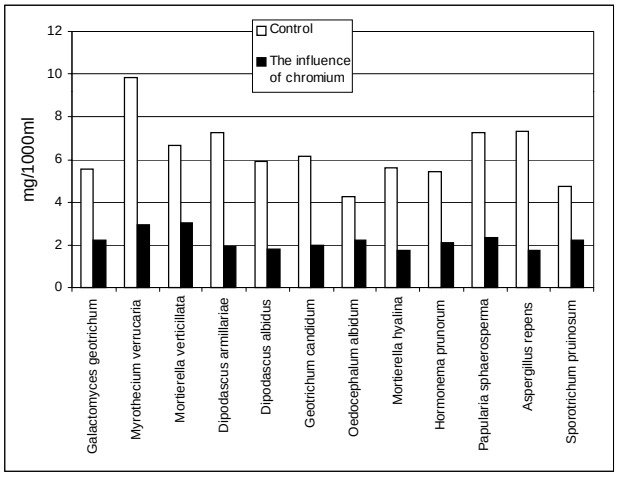

Fig. 1. The influence of chromium on increase of biomass of micromycetes

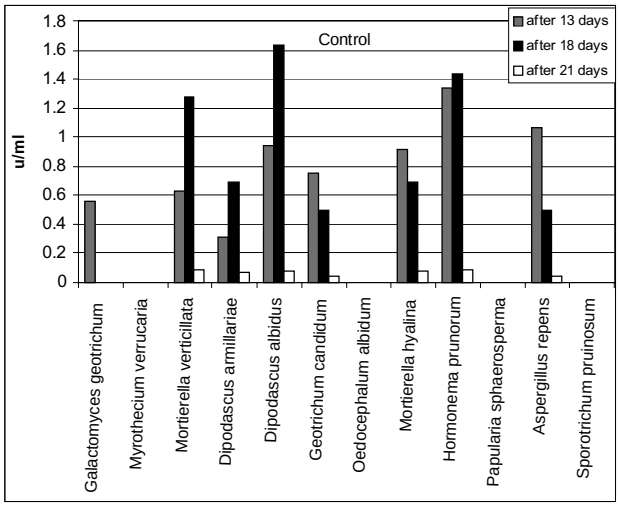

Fig. 2. Peroxidase activity of micromycetes in n-Czapek medium

It was noted that the activity of some species of micromycetes appeared on the fourth day of cultivation, of in others on the 7th day and in some of them in the later period of growth. According to literature sources [8], a large amount of auxin inhibits peroxidase activity in organs of *Elodea densa* which grows fast. The oxidation process begins in later stages of growth when auxin doesn't act any more. Auxins are synthesized not only by plants, but also by fungi [12].

The concentration of 5 μM $K_2Cr_2O_7$ was found to stimulate the peroxidase of all the investigated micromycetes (Fig. 3), and its maximum was reached on day 21 of cultivation. *Galactomyces geotrichum*, *Myrothecium verrucaria*, *Mortierella verticillata*, *Mortierella hyalina* and *Hormonema prunorum* showed the highest peroxidase activity (33.15; 32.19; 29.69; 25.31 and 23.75 u/ml, respectively). Lower activity of this enzyme (17.19 and 19.41 u/ml) was noted after cultivation of *Dipodascus albidus* and *Sporotrichum pruinosum.*

Lacase activity. The lacase activity of all micromycetes cultivated in n-Czapek medium was low (Fig. 4). Higher lacase activity was exhibited by *Oedocephalum albidum*, *Myrothecium verrucaria* and *Sporotrichum pruinosum* after 4 days of cultivation (0.061, 0.059 and 0.053 ext. coefficient (D), respectively). Lacase activity of all micromycetes studied progressively decreased in the further course of cultivation, and on day 18 of cultivation this enzymatic activity of *Mortierella verticillata*, *Dipodascus armillariae*, *Dipodascus albidus* and other fungi was 0.023, 0.021 and 0.022 D, respectively. Lacase activity slightly increased after 21 days of cultivation again.

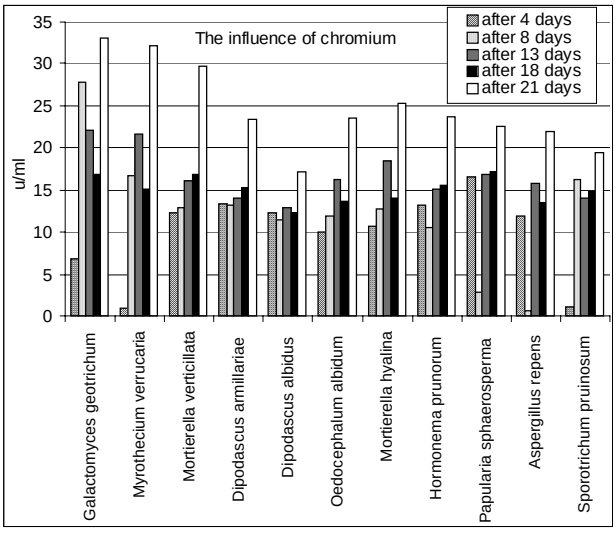

Fig. 3. The influence of chromium on the activity of peroxidases in micromycetes

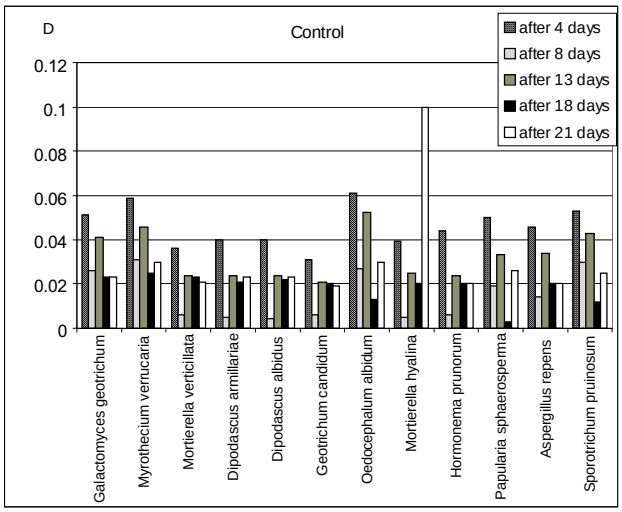

Fig. 4. Lacase activity of micromycetes in n-Czapek medium

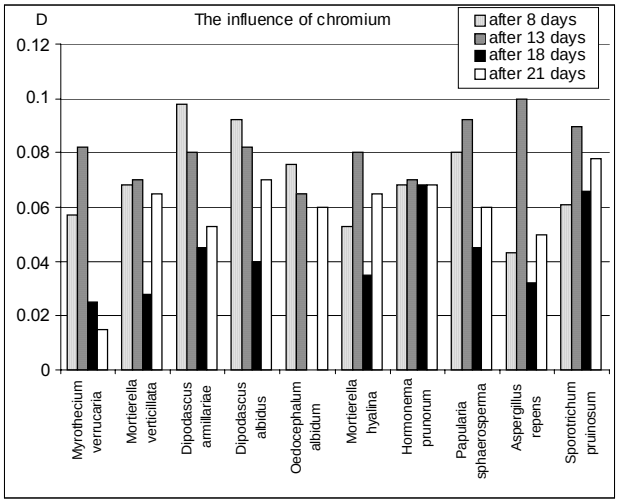

Fig. 5. The influence of chromium on the activity of lacases in micromycetes

The influence of 5 μM $K_2Cr_2O_7$ (Fig. 5) stimulated the lacase activity of all micromycetes studied, except *Oedocephalum albidum* whose enzymatic activity did not manifest at all on day 18 of cultivation; on day 21 its activity was 0.06 D. The influence of Cr^{6+} on the lacase activity of various micromycetes was different. The activity of some micromycetes such as *Dipodascus albidus*, *Dipodascus armillariae*, *Motierella verticillata* (0.098; 0.092; 0.068 D, respectively) was highest after 8 days and of other micromycetes such as *Sporotrichum pruinosum*, *Mortierella hyalina* after 13 days the cultivation (0.09 and 0.08 D, respectively). Lacase activity of all micromycetes studied progressively decreased in the further course of cultivation, remaining higher on the 21st day of *Sporotrichum pruinosum* and *Hormonema prunorum* cultivation (0.078 and 0.068 D, respectively).

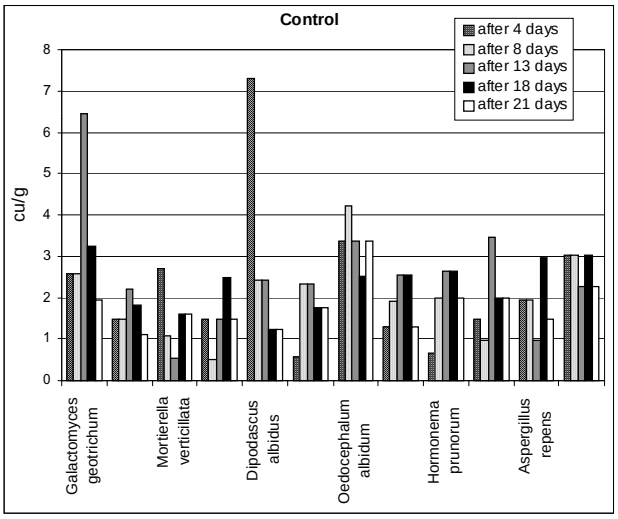

Fig. 6. Tyrosinase activity of micromycetes in n-Czapek medium

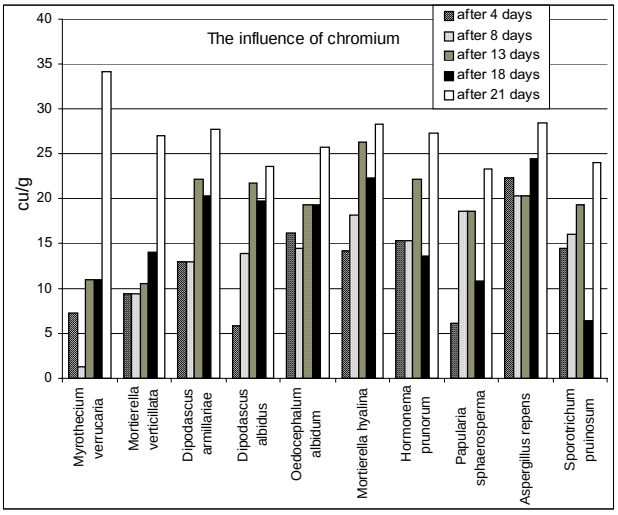

Fig. 7. The influence of chromium on the activity of tyrosinases in micromycetes

Tyrosinase activity. Tyrosinase activity of micromycetes in the n-Czapek medium was different (Fig. 6): *Galactomyces geotrichum* reached its maximum on the 13th day (6.45 conditional unit/g (cu/g)), *Myrothecium verrucaria* (1.83 cu/g) on the 18th day, *Mortierella verticillata* (2.71 cu/g) on the 4th day of cultivation, and so on. The highest tyrosinasic activity was observed in *Galactomyces geotrichum* (6.45), *Oedocephalum albidum* (4.21 on the 8th day of cultivation) and *Sporotrichum pruinosum* (3.02 cu/g) on the 4th, 8th and 18th days of cultivation.

The concentration of 5 μM $K_2Cr_2O_7$ stimulated the tyrosinase activity of almost all micromycetes studied (Fig. 7). The tyrosinasic activity in all micromycetes studied was determined after 21 days of cultivation. The highest tyrosinase activity was shown by *Myrothecium verrucaria* (34.15), *Aspergillus repens* (28.44) and *Mortierella hyalina* (28.31 cu/g) after 21 days of cultivation.

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MIKROMICETŲ – FENOLOKSIDAZIŲ PRODUCENTŲ SAVYBĖS

S a n t r a u k a

Ištirta 12 mikromicetų – fenoloksidazių producentų: *Galactomyces geotrichum, Myrothecium verrucaria, Mortierella verticillata, Dipodascus armillariae, Dipodascus albidus, Geotrichum candidum, Oedocephalum albidum, Mortierella hyalina, Hormonema prunorum, Papularia sphaerosperma, Aspergillus repens, Sporotrichum pruinosum* peroksidazinis, lakazinis ir tirozinazinis aktyvumas, kultivuojant

juos n-Čapeko terpėje ir terpėje, į kurią buvo įnešta 5 mM Cr^{6+} .

Nustatyta, kad dėl visų tirtų mikromicetų Cr^{6+} sumažėjo biomasės kiekis. *Myrothecium verrucaria, Aspergillus repens, Dipodascus armillariae* ir *Papularia sphaerosperma* biomasės sumažėjimas sudarė atitinkamai 3,3; 4,14; 3,7; 3,13 karto, palyginti su kontrole.

Ištirta, kad 5 mM Cr^{6+} koncentracija suaktyvino visų tirtų mikromicetų peroksidazę, ir fermentas aktyvumo maksimumą pasiekė 21-ą kultivavimo parą. Didžiausiu peroksidaziniu aktyvumu pasižymėjo *Galactomyces geotrichum, Myrothecium verrucaria, Mortierella verticillata, Mortierella hyalina,* ir *Hormonema prunorum* (atitinkamai 33,15; 32,19; 29,69; 25,31 ir 23,75 akt. vnt./ml).

Nustatyta, kad mikromicetų lakazinis aktyvumas dėl 5 mM Cr^{6+} buvo permainingas. Mikromicetų *Dipodascus albidus, Dipodascus armillariae* ir *Mortierella verticillata* aktyvumo maksimumas buvo pasiektas po 8 kultivavimo parų (atitinkamai ext. koef. 0,098; 0,092 ir 0,068). Kitų mikromicetų lakazinis aktyvumas buvo didesnis po 13 kultivavimo parų.

Visų tirtų mikromicetų tirozinazinio aktyvumo maksimumas buvo nustatytas 21-ą kultivavimo parą. Didžiausiu fermentiniu aktyvumu pasižymėjo *Myrothecium verrucaria* (34,15), *Aspergillus repens* (28,44) ir *Mortierella hyalina* (28,31 sąl.vnt./g).