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Effect of Ni^{2+} on phenoloxidase activity of micromycetes

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The peroxidase activity of all micromycetes cultivated in the medium containing 0.005 M $NiCl_2$ was low and manifested only in the later period of growth. The highest peroxidase activity was observed in *Aspergillus repens* (1.56 au/ml). In the medium with the concentration of 0.01 M $NiCl_2$ no peroxidase activity was found, except *Mortierella verticillata* in which the maximum (3.97 au/ml) was reached on the 4th day of cultivation.

The concentration of 0.005 M $NiCl_2$ stimulated the tyrosinase activity of all micromycetes studied. The highest tyrosinase activity was observed in *Myrothecium verrucaria*, *Sporotrichum pruinosum*, *Galactomyces geotrichum* and *Oedocephalum albidum* (59.2–121.5 cu/ml) in the whole period of growth. The major concentration (0.01 M) of $NiCl_2$ stimulated the tyrosinase activity in all micromycetes. The influence of 0.01 M of $NiCl_2$ increased the enzymatic activity of all micromycetes 3.74 to 141.8 times in comparison with the control. During the whole period of cultivation, the highest enzymatic activity was observed in *Oedocephalum albidum*, and its maximum was reached (357.28 cu/ml) on the 18th day of cultivation.

The highest laccase activity in the medium containing 0.005 M $NiCl_2$ was observed after 21 days of cultivation (it increased 1.3 to 3.3 times in comparison with the control). The major concentration (0.01 M) of $NiCl_2$ decreased the laccase activity in all micromycetes, except *Galactomyces geotrichum* in which the enzymatic activity on the 18th day of cultivation increased 1.5 times in comparison with the control.

Key words: nickel, micromycetes, peroxidase, laccase, tyrosinase

INTRODUCTION

A great amount of various plant remnants accumulates on the Earth. Cellulose and lignin are the main components of plant remnants. Chemically, they are hard to destruct complex polymeric materials of organic origin, which have accumulated an enormous amount of photosynthetic energy (Kelly, 1992; Blanchette, 2000).

Various enzymes extracted during the functional activity of microorganisms catalyse the processes of plant remnant conversion.

Destruction of plant remnants by microorganisms is a complicated biochemical and physiological process with many enzymatic systems involved. The most difficult problem is to ascertain the degradation of lignin. The main enzymes produced by fungi are phenoloxidases – high redox potential ligninolytic peroxidases (lignin peroxidase, manganese peroxidase) tyrosinase and laccase which participate in lignin degradation (Glenn, Gold, 1985; Bonnarme, Jeffries, 1990; Perez, Jeffries, 1990; Score et al., 1997; Ohkuma et al., 2001).

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The functioning and enzymatic activities of fungi are affected by numerous environmental factors. Fungi of many species are sensitive not only to the quantity and quality of nutrient elements in the surroundings of their development, but also to many other environmental stimuli, e. g., the presence of heavy metals in the zone of their existence (Connolly, Jellison, 1997; Martino et al., 2000; Pečiulytė, 2001).

Among soil organisms, fungi are most resistant to pollution by heavy metals. Heavy metals have a deep negative impact on soil microorganisms. Since metals can be potent growth inhibitors of microorganisms, it is not surprising that the fungal diversity of metal-contaminated sites is reduced. The mechanisms that allow fungi to tolerate toxic metal concentrations include both avoidance through exclusion of metal ions from the cytoplasm and tolerance of high intracellular concentrations (Martino et al., 2000).

Some of heavy metals are required by the fungi for metabolism, whereas others have no known biological role and can be toxic. Toxic levels of metals often occur in wood or soils as a result of industrial pollution. Whereas fungi have metabolic requirements for trace metals, the same metals are often toxic at concentrations only a few times greater than those required. Heavy metals in the soil can also influence

secretion and/or activity of extracellular enzymes. The metals required by fungi include copper, iron, manganese, molybdenum, zinc, and nickel (Jellison et al., 1997).

Nickel is a biologically essential metal for microorganisms, required for the synthesis of certain enzymes, but it becomes toxic above a certain concentration. Nickel as a waste product enters the environment at rather large amounts (Levinskaitė et al., 2000).

The aim of the study was to investigate the influence of Ni^{2+} on the increase of biomass and on the phenoloxidase (peroxidase, laccase and tyrosinase) activity of micromycetes – phenoloxidase producers.

MATERIALS AND METHODS

Micromycetes – producers of phenoloxidases used in the experiments – were isolated from polymeric materials and fields. The following strains of micromycetes were isolated, identified and investigated: *Galactomyces geotrichum* (E. E. Butler., L. J. Petersen) Redhead & Malloch (current name *Geotrichum candidum var. citri-aurantii* (Ferraris) Cif. & F. Cif.), *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar, *Mortierella verticillata* Linnem, *Dipodascus armillariae* W. Gams, *Dipodascus albidus* Lagerh, *Geotrichum candidum* Link, *Oedocephalum albidum* (Preuss) Sacc., *Mortierella hyalina* (Harz) W. Gams, *Hormonema prunorum* (Dennis & Buhagiar) Hermanides-Nijhof, *Papularia sphaerosperma* (Pers.) Höhn (current name *Arthrinium phaeospermum* (Corda) M. B. Ellis), *Aspergillus repens* (Corda) Sacc., *Sporotrichum pruinosum* J. C. Gilman & E. V. Abbott. Identification was performed following Domsch et al. (1980).

To estimate the influence of Ni^{2+} on peroxidase, laccase and tyrosinase activity, fungi were cultivated in a liquid standard Czapek medium (control) and in a Czapek medium with addition of $NiCl_2$ at a concentration of 0.005 and 0.01 M. Each flask was inoculated with two 9 mm discs cut from 7 day cultures of the fungi grown on malt extract agar plates at 28 °C. Micromycetes were cultivated at 28 °C for 21 days on a shaker. The contents were filtered through filter paper, and the filtrate was centrifuged at 5000 g. The supernatant was used for enzymatic activity estimation. The increase of biomass and the peroxidase, laccase and tyrosinase activity were investigated.

Initially, enzymatic activity was evaluated by the method of Lyr (Lyr, 1958).

Activity of ligninolytic enzymes was evaluated according to the oxidation rate of specific substrata. P-phenylenediaminechloride was used as a substratum to estimate the activity of laccase (Ravin, Harward, 1965). The reaction was measured by monitoring the increase in absorbance at 490 nm. The assay method of peroxidase activity is based on the colorimetric evaluation of the oxidation product o-dianisidine in the presence of H_2O_2 (Билай, 1982). Peroxidase activity is expressed as activity units (au) / ml. Tyrosinase activity was measured spectrophotometrically using a method based on the estimation of the optical density of reaction products formed during oxidation of pyrocatechin over a given period (Ермаков и др., 1987). Enzymatic activity is expressed as conditional units (cu) / ml.

The obtained results were computed using Microsoft Excel XP.

RESULTS AND DISCUSSION

The greatest amount of biomass on a standard Czapek medium was defined after *Myrothecium verrucaria*, *Aspergillus repens, Papularia sphaerosperma* and *Dipodascus armillariae* (9.82; 7.33; 7.28 and 7.24 g/l respectively) cultivation (Fig. 1).

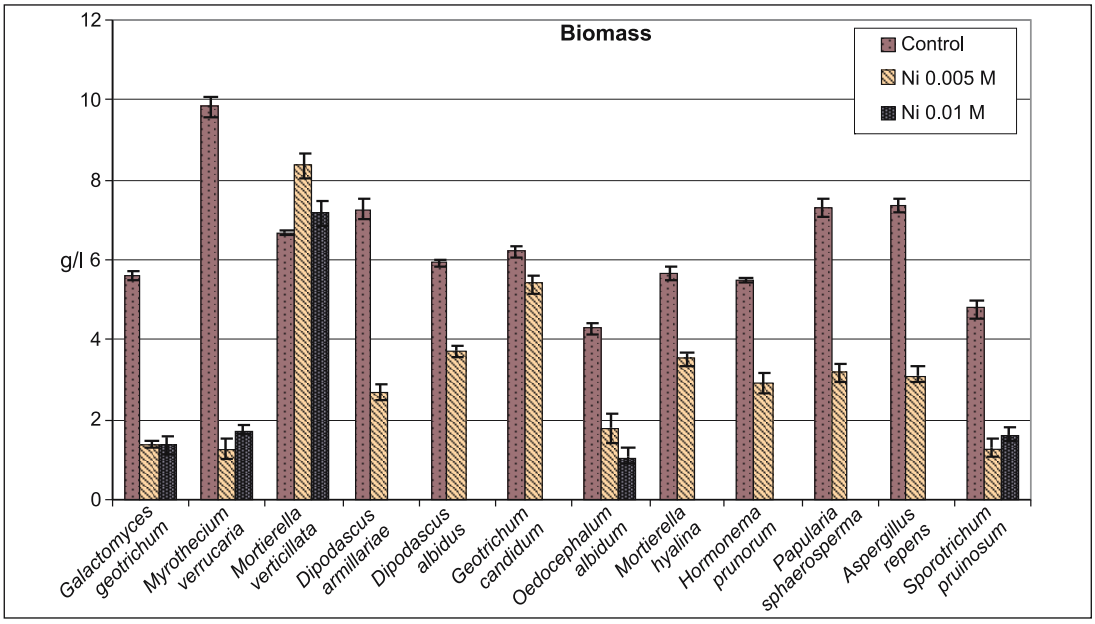

Fig. 1. Effect of nickel on biomass increase in micromycetes

The metal concentration of 0.005 M and 0.01 M $NiCl_2$ reliably reduced the amount of biomass of all micromycetes in comparison with the control, except *Mortierella verticillata* whose amount of biomass slightly increased.

Some works showed that a significant inhibition of C-biomass occurred in soils highly contaminated by heavy metals (Šmejkalova et al., 2003). Microbial biomass is a sensitive parameter and can be used as an indicator of changes in organic matter composition earlier than it could be registered in another way (Brookes, 1995). The inhibition of C-biomass in soils highly contaminated by heavy metals supports the data of Brookes and McGrath (1984) who show only a half content of microbial biomass in soil contaminated by heavy metals compared to uncontaminated soils. Dias et al. (1998) observed an even higher than 80 % inhibition of C-biomass by heavy metals. The synthesis of microbial biomass in soils polluted by heavy metals can be less effective than in non-polluted soils due to the stress caused by heavy metals.

The peroxidase activity of all micromycetes cultivated in the medium containing 0.005 M $NiCl_2$ was low and manifested only in the later period of growth (Fig. 2). However, under the influence of this concentration peroxidase activity was marginally increased (1.1 to 9.7 times in comparison with the control), except *Mortierella verticillata* whose enzymatic activity was 2.1 times lower in comparison with the control on the 21st day of cultivation. The highest peroxidase activity was observed in *Aspergillus repens* (1.56 au/ml).

In the medium with the concentration of 0.01 M, $NiCl_2$ peroxidase activity was not found, except *Mortierella verticillata* in which the maximum was reached (3.97 au/ml) on the 4th day of cultivation. However, this enzymatic activity progressively decreased in the further course of cultivation and on the days 13 and 21 did not manifest at all. The effect of Ni on soil dehydrogenase, protease, endoglucanase, cellobiohydrolase and β-glucosidase activities was analyzed by other researchers (Milošević et al., 2002). The results showed that the enzymatic activities were very sensitively affected by the medium and high Ni rates.

The concentration of 0.005 M $NiCl_2$ stimulated the tyrosinase activity of all micromycetes. The highest tyrosinase activity was observed in *Myrothecium verrucaria*, *Sporotrichum pruinosum*, *Galactomyces geotrichum* and *Oedocephalum albidum* (59.2–121.5 cu/ml) in the whole period of growth. The maximum of this activity *Myrothecium verrucaria* reached on the 4th and *Sporotrichum pruinosum* on the 21st day of cultivation. The tyrosinase activity of other micromycetes was different and increased 1.9 to 75.3 times in comparison with the control (Figs. 3 and 4). The lowest tyrosinase activity was defined in *Mortierella verticillata*, *Dipodascus albidus* and *Geotrichum candidum* during the whole period of cultivation.

The influence of nickel, zinc, iron and other microelements on the level of α-amylase activity was measured to determine the microelements that increased the levels of enzymatic activity. The results showed that in most treatments

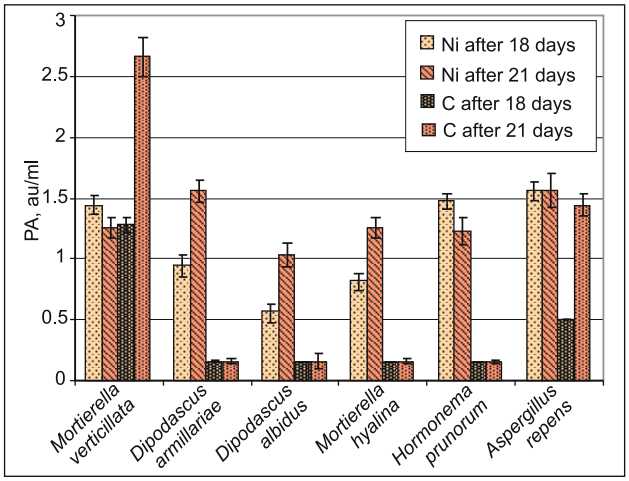

Fig. 2. Effect of nickel (0.005 M) on peroxidase (PA) activity of micromycetes (C – control)

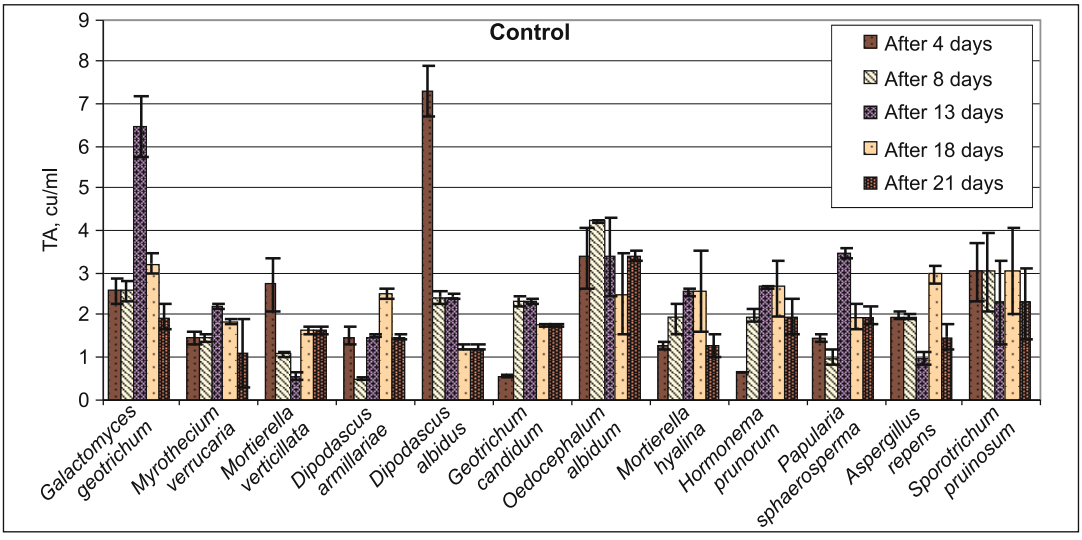

Fig. 3. Tyrosinase activity (TA) of fungi cultivated on standard Chapek medium

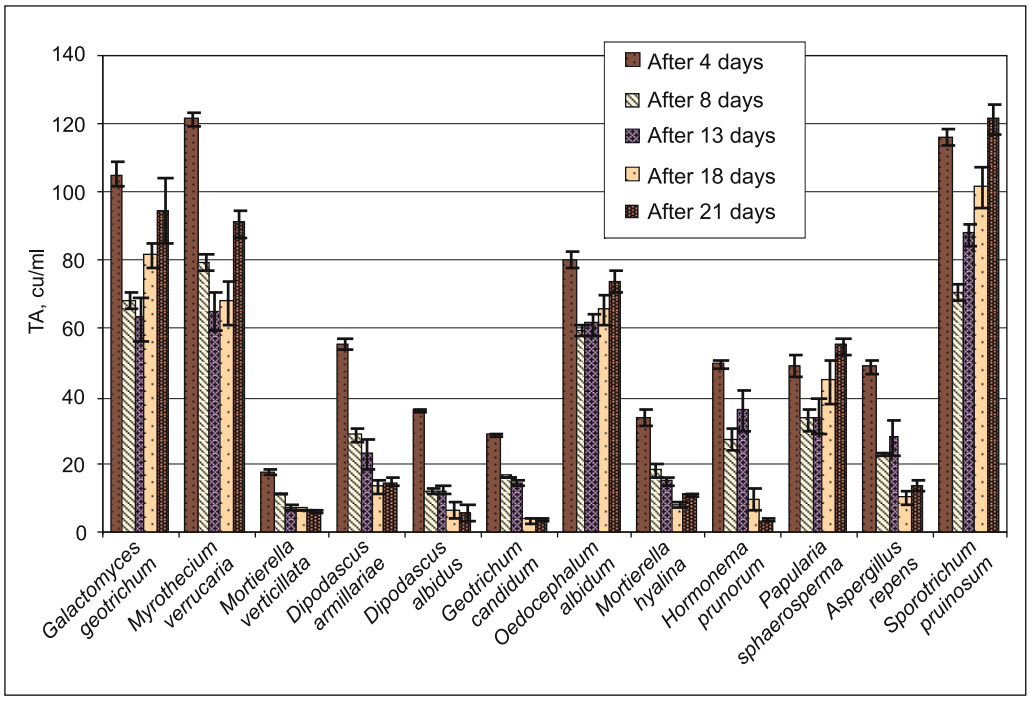

Fig. 4. Effect of nickel (0.005 M) on tyrosinase (TA) activity of micromycetes

the level of enzymatic activities was higher than the control. The decrease of enzymatic activity under the influence of the mentioned microelements was not significantly lower compared with the control (Gorbani et al., 2003).

A higher major concentration (0.01 M) of $NiCl_2 \cdot 6H_2O$ stimulated the tyrosinase activity of all micromycetes (Fig. 5). The influence of 0.01 M of $NiCl_2$ increased the enzymatic activity of all micromycetes 3.74 to 141.8 times in comparison with the control. However, this enzymatic activity was decreased on the 4th day of cultivation by some species of micromycetes 4.2 to 1.4 times in comparison with the concentration of 0.005 M $NiCl_2$.

The tyrosinase activity of almost all micromycetes was highest on the 18th day of cultivation. During the whole period of cultivation the highest enzymatic activity was observed in *Oedocephalum albidum*, and its maximum (357.28 cu/ml) was reached on the 18th day of cultivation. The lowest tyrosinase activity (6.06 to 18.18 cu/ml) was defined in *Mortierella verticillata* in the whole period of cultivation.

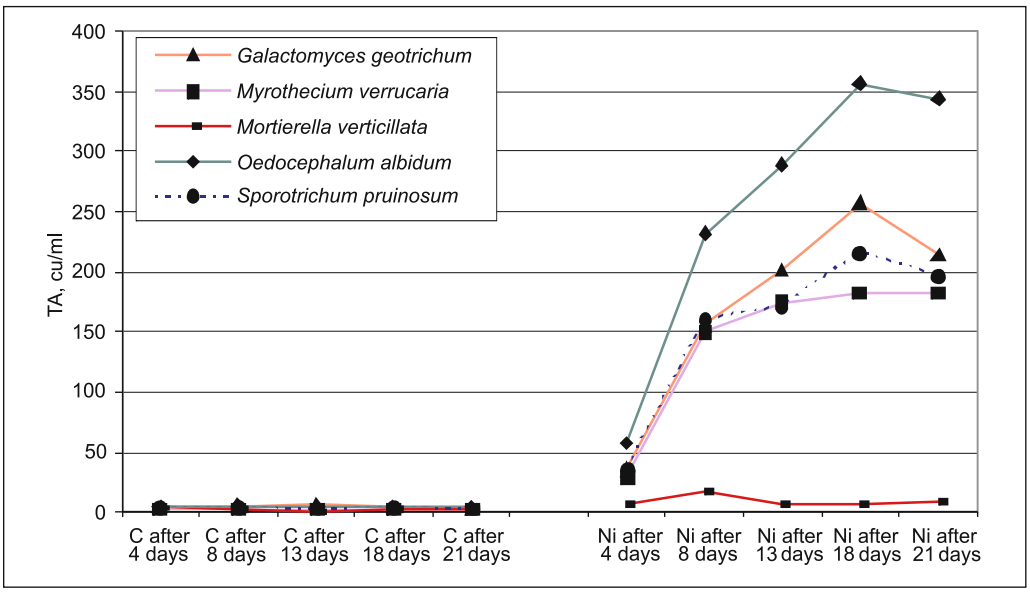

Fig. 5. Effect of nickel (0.01 M) on tyrosinase (TA) activity of micromycetes (C – control)

The influence of 0.005 M $NiCl_2$ on the laccase activity of various micromycetes was different: in some of them it increased and in others decreased (Figs. 6 and 7). The laccase activity was low during the whole period of cultivation of micromycetes and on the 8th day did not manifest at all.

The highest laccase activity in the medium containing 0.005 M $NiCl_2$ was observed after 21 days of cultivation: it increased 1.3 to 3.3 times in comparison with the control.

A higher concentration (0.01 M) of $NiCl_2 \cdot 6H_2O$ decreased the laccase activity of all micromycetes, except *Galactomyces geotrichum* whose enzymatic activity on the 18th day of cultivation increased (1.5 times in comparison with the control) (Fig. 8). This concentration decreased the laccase activity of other micromycetes 1.1 to 99 times in comparison with the concentration of 0.005 M $NiCl_2$.

Our results show that the effect of $NiCl_2$ on phenoloxidase activity was different.

The peroxidase activity of all micromycetes cultivated in the medium containing 0.005 M $NiCl_2$ was low, however, marginally increased 1.1 to 9.7 times in comparison with the

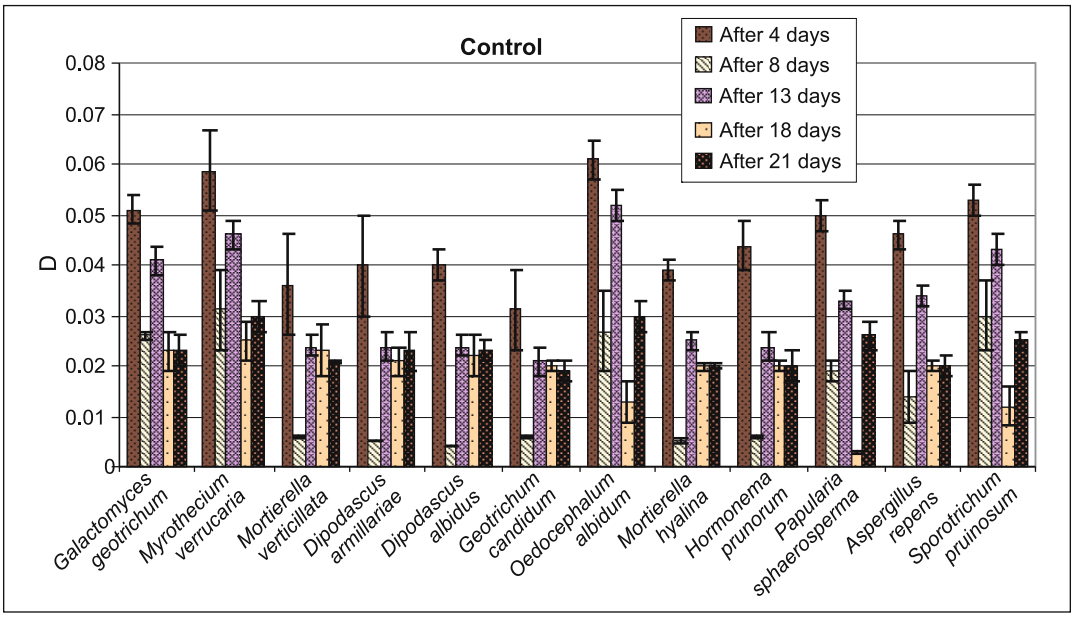

Fig. 6. Laccase activity (LA) of fungi cultivated on standard Chapek medium

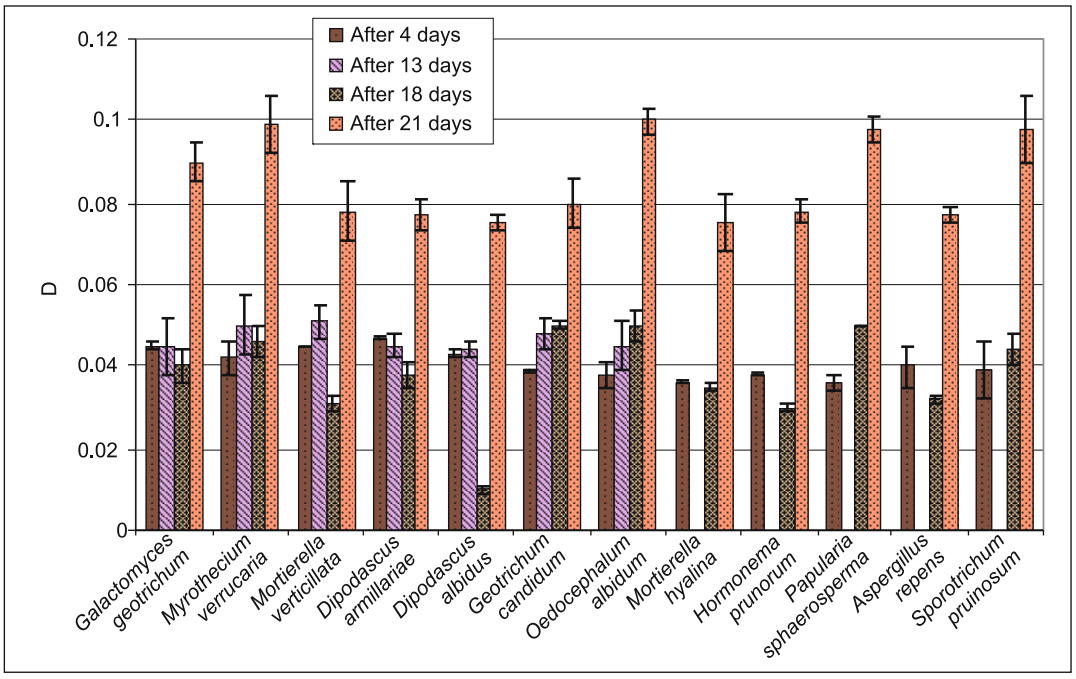

Fig. 7. Effect of nickel (0.005 M) on laccase (LA) activity of micromycetes

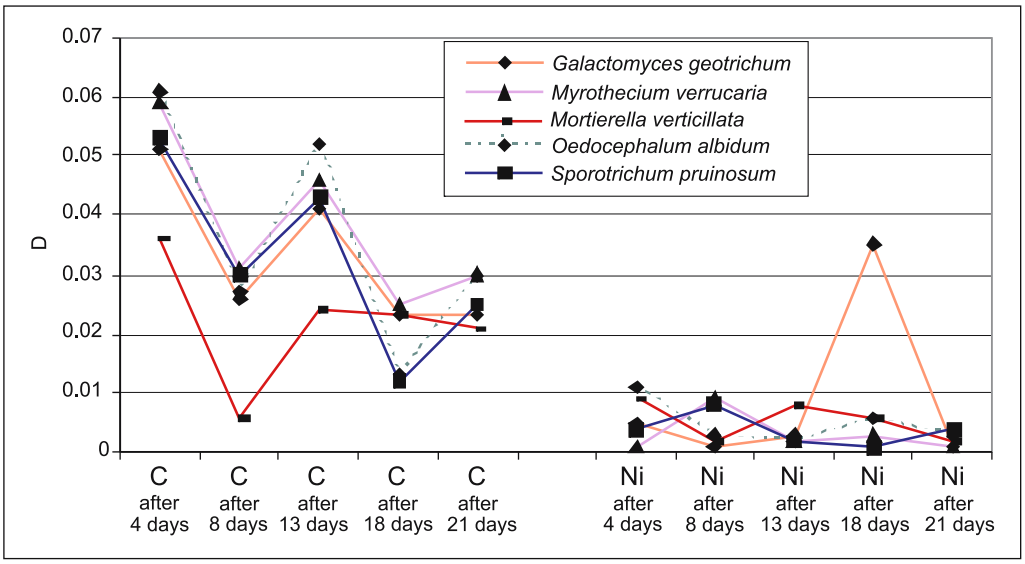

Fig. 8. Effect of nickel (0.01 M) on laccase (LA) activity of micromycetes (C – control)

control, except in *Mortierella verticillata*. In the medium with the concentration of 0.01 M $NiCl_2$ no peroxidase activity was found, except in *Mortierella verticillata*.

Both concentrations (0.005 M and 0.01 M) of $NiCl_2$ stimulated the tyrosinase activity of all micromycetes.

The effect of 0.005 M $NiCl_2$ on the laccase activity of various micromycetes was different: in some of them it increased, whereas in others decreased. Laccase activity was low during the whole cultivation period of micromycetes. A higher concentration (0.01 M) of $NiCl_2$ decreased the laccase activity of all micromycetes, except *Galactomyces geotrichum*.

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Ni^{2+} ĮTAKA MIKROMICETŲ FENOLOKSIDAZINIAM AKTYVUMUI

S a n t r a u k a

Ištirta Ni^{2+} įtaka mikromicetų fenoloksidazių (peroksidazės, lakazės ir tirozinazės) aktyvumui. Nustatyta, kad esant 0,005 M $NiCl_2$ koncentracijai tiriamų mikromicetų peroksidazinis aktyvumas buvo nedidelis ir pasireiškė tik vėlesnėse augimo stadijose. Didesniu peroksidaziniu aktyvumu išsiskyrė *Aspergillus repens* (1,56 a.v./ml). Peroksidazinis aktyvumas terpėje su 0,01 M Ni^{2+} visai nepasireiškė, išskyrus *Mortierella verticillata*, kurio aktyvumas po 4-ių kultivavimo parų buvo didžiausias (3,97 a.v./ml).

0,005 M $NiCl_2$ koncentracija skatino visų tirtų mikromicetų tirozinazinį aktyvumą. Dideliu tirozinaziniu aktyvumu terpėje su nikeliu visą augimo periodą pasižymėjo *Myrothecium verrucaria*, *Sporotrichum pruinosum*, *Galactomyces geotrichum* ir *Oedocephalum albidum* (59,2–121,5 sąl.v./ml). Didesnė (0,01 M) $NiCl_2$ koncentracija skatino visų tirtų mikromicetų tirozinazinį aktyvumą. Dėl jos įtakos tirtų mikromicetų fermentinis aktyvumas padidėjo nuo 3,74 iki 141,8 karto, palyginus su kontrole. Didžiausiu tirozinaziniu aktyvumu visą kultivavimo laiką išsiskyrė *Oedocephalum albidum*, kurio aktyvumo maksimumas 18-ą kultivavimo parą buvo 357,28 sąl.v./ml.

Didžiausias lakazinis aktyvumas terpėje su 0,005 M Ni^{2+} buvo nustatytas po 21-os mikromicetų kultivavimo paros (padidėjo nuo 1,3 iki 3,3 karto, palyginus su kontrole). Didesnė (0,01 M) $NiCl_2$ koncentracija sumažino visų tirtų mikromicetų lakazinį aktyvumą, išskyrus *Galactomyces geotrichum*, dėl kurio įtakos šis aktyvumas 18-ą kultivavimo parą padidėjo iki 1,5 karto, palyginus su kontrole.

Raktažodžiai: Ni^{2+}, mikromicetai, peroksidazė, lakazė, tirozinazė