BIOLOGIJA. 2004. Nr. 2. P. 33–36


# Phenoloxidase activity of fungal complexes under hard-phase conditions

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Peroxidase, laccase and tyrosinase activity and lignin degradation were investigated in rye straw after 20, 30 and 60 days of cultivation of the fungal complexes *Galactomyces geotrichum – Myrothecium verrucaria, Galactomyces geotrichum – Sporotrichum pruinosum, Myrothecium verrucaria – Sporotrichum pruinosum, Dipodascus armillariae – Verticillium fungicola* and *Papularia sphaerosperma – Fusarium redolens.*

Lignin degradation was found to depend on the fungal cultivation time. The fungal complexes *Galactomyces geotrichum – Myrothecium verrucaria* and *Galactomyces geotrichum – Sporotrichum pruinosum* decreased lignin content most significantly (2.4 and 1.9 times, respectively in comparison with the control) in rye straw (after 60 days). Under the influence of other fungal complexes the decrease made up 1.7 to 1.25 times in comparison with the control.

The highest peroxidase activity (247.6 a.u./g) was fixed after eight days of the fungal complex *Galactomyces geotrichum – Myrothecium verrucaria* cultivation. The second maximum (192.9 a.u./g) of this complex activity was observed after 20 days of cultivation. The lignin degradation degree in these periods was 14.28% and 12.18%, respectively.

The tyrosinase activity (4.026 c.u./g) was highest after eight days of cultivation of the fungal complex *Galactomyces geotrichum – Sporotrichum pruinosum.* The second activity maximum (2.61 c.u./g) was reached by this complex after 30 days of cultivation. The degree of lignin degradation in these periods was 14.50% and 10.68%, respectively.

The laccase activity of the fungal complexes was very low. Their extinction coefficient changed from 0.003 to 0.260.

**Key words:** fungal complexes, phenoloxidases: peroxidase, laccase, tyrosinase, lignin

## INTRODUCTION

The importance of biological delignification has been amply demonstrated in various fields of biotechnology. Fungi hold a great potential in the paper industry to delignify wood chips, not only saving chemical investment but also reducing pollution hazards [1, 2]. Ligninolytic strains not only prevent pollution, but have also been used for the bioconversion of industrial effluents into useful energy-yielding chemicals [3, 4]. With the removal of lignin barrier cellulose becomes easily accessible for bioconversion. Delignification of forage crop residues enhances their digestibility and improves their nutritive value [5, 6].

Plant remnant bioconversion by microorganisms is a complex biological and physiological process in which many enzymatic systems take part [7–9]. Biosynthesis of enzymes (phenoloxidases, amylases, cellulases, hemicellulases) under hard-phase conditions attracts the attention of investigators. Hard-phase fermentation is cheaper than liquid-phase. Besides, a better fungal growth is noticed, more products are received when more substrate is present, etc. The shortcomming of this method is insufficient standardization and control of the process; besides, cultural growth thermoregulation is rather difficult.

The goal of the work was to elucidate lignin degradation in rye straw, exposing it to fungal complexes under hard-phase fermentation conditions, and to determine the activity of phenoloxidases (peroxidases, laccases and tyrosinases) of fungi-destructors in connection with lignin degradation. We used mineral additives to improve fungal growth and to hasten lignin degradation.

## MATERIALS AND METHODS

The object of the investigation was five fungal complexes and rye straw. Fungi *Galactomyces geotrichum*

(Butl et Petersen) Ditmar ex Fries, *Myrothecium verrucaria* (Alb. et Sweinitz) Ditmar ex Fries, *Sporotrichum pruinosum* (Gilman et Abbott), *Dipodascus armillariae* W. Gams, *Verticillium fungicola* (Preuss) Hassebr., *Papularia sphaerosperma* (Pers) Höhn, *Fusarium redolens* Wr. isolated from various substrata were used for the detection of enzymatic activity and for the examination of lignin degradation degree.

Five fungal complexes were composed and studied:

1. *Galactomyces geotrichum – Myrothecium verrucaria*
2. *Galactomyces geotrichum – Sporotrichum pruinosum*
3. *Myrothecium verrucaria – Sporotrichum pruinosum*
4. *Dipodascus armillariae – Verticillium fungicola*
5. *Papularia sphaerosperma – Fusarium redolens.*

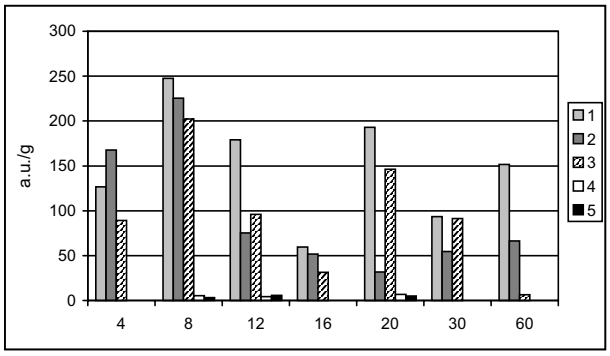


Fig. 1. Peroxidase activity of micromycete complexes during their cultivation on rye straw for 4 to 60 days. Figs. 1–4 – micromycete complexes: *1 – Galactomyces geotrichum – Myrothecium verrucaria*, *2 – Galactomyces geotrichum – Sporotrichum pruinosum*, *3 – Myrothecium verrucaria – Sporotrichum pruinosum*, *4 – Dipodascus armillariae – Verticillium fungicola*, *5 – Papularia sphaerosperma – Fusarium redolens*

Plant remnants were moistened with mineral solution (0.3 g $NH_4NO_3$ and 0.1 g $KH_2PO_4$ was added to 10 g of air-dried material). Fungi were cultivated on plant remnants for 60 days at 28 °C. The activity of phenoloxidases (peroxidases, laccases and tyrosinases) and lignin degradation degree were fixed in biomass after 4, 8, 12, 16, 20 days and in the later fungal complex cultivation stages: after 30 and 60 days.

Peroxidase activity was determined with o-dianisidine reagent [10], laccase – with p-phenylenediamine chloride [11] and tyrosinase – with tyrosine [12]. Lignin amount was estimated in rye straw according to Khudiakova's method [13].

## RESULTS AND DISCUSSION

**Peroxidase activity**. The investigations showed (Fig. 1) the first and highest maximum (247.6 a.u./g) of peroxidase activity of the *Galactomyces geotrichum – Myrothecium verrucaria* complex after eight days of cultivation. The second peroxidase activity maximum (192.9 a.u./g) of this complex was detected after 20 days. The lignin degradation degree during these periods was 14.28% and 12.18%, respectively.

The fungal complex *Galactomyces geotrichum – Sporotrichum pruinosum* reached the first peroxidase activity maximum (225.4 a.u./g) also after eight days of cultivation. During further cultivation the enzymatic activity decreased and reached the second activity maximum (66.51 a.u./g) only after 60 days of cultivation (Fig. 1). In this period the fixed lignin degradation degree was 8.34%.

The fungal complex *Myrothecium verrucaria – Sporotrichum pruinosum,* like the previous two, reached the highest peroxidase activity (202.3 a.u./g) and lignin degradation degree (13.47%) after eight days of cultivation. Afterwards, this activity decreased gradually and its second maximum (91.39 a.u./g) was detected after 30 days of cultivation.

Peroxidase activity of the fungal complexes *Dipodascus armillariae – Verticillium fungicola* and *Papularia sphaerosperma – Fusarium redolens* wasn't high during 20 days and in the later cultivation stages (30 and 60 days) didn't occur at all.

Two peaks of peroxidase activity show that during fungal growth on natural complex polymers uneven increase of micelium and necrosis occur, resulting in uneven enzymatic activity.

**Tyrosinase activity**. The investigations showed that the change regularities of tyrosinase activity of the fungal complexes *Galactomyces geotrichum – Myrotecium verrucaria* were analogous to the change regularities of peroxidase activity during 20 days. The first enzymatic activity maximum (1.03 c.u./g) of this complex was fixed after eight days of cultivation; it decreased to 0.825 c.u./g on day 12th of cultivation and the second activity maximum (1.42 c.u./g) was reached after 20 days (Fig. 2).

The highest tyrosinase activity maximum (4.026 c.u./g) of the fungal complex *Galactomyces geotrichum – Sporotrichum pruinosum* was detected not only after eight days of cultivation but, in contrast to all cultivated fungal **complexes, during 20** days as well. During further cultivation this activity decreased and reached the second activity maximum (2.61 c.u./g) on day 30 of cultivation.

Tyrosinase activity of other three fungal complexes was lower after 20, 30 and 60 days: this activity of the fungal complexes *Dipodascus armillariae – Verticillium*

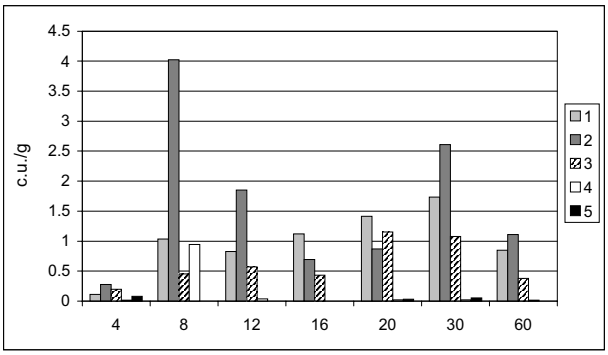


Fig. 2. Tirosinase activity of micromycete complexes during their cultivation on rye straw for 4 to 60 days

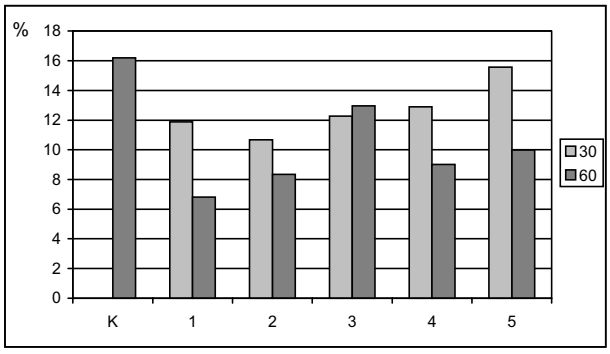


Fig. 4. Change of lignin content in rye straw after cultivation of micromycete complexes for 30 and 60 days

*fungicola* and *Papularia sphaerosperma – Fusarium redolens* didn't show at all on day 16 of cultivation.

**Laccase activity**. The laccase activity of the fungal complexes *Galactomyces geotrichum – Myrothecium verrucaria, Galactomyces geotrichum – Sporotrichum pruinosum* and *Myrothecium verrucaria – Sporotrichum pruinosum* was very low. Their extinction coefficient changed from 0.003 to 0.05 (Fig. 3).

The fungal complex *Dipodascus armillariae – Verticillium fungicola* stood out for a higher laccase activity and reached the maximum activity (extinction coefficient 0.232) on day 12 of cultivation; later it gradually decreased and was 0.11 on day 20. The activity maximum (extinction coefficient 0.260) of the fungal complex *Papularia sphaerosperma – Fusarium redolens* was reached after eight days; afterwards it gradually decreased, slightly increasing (up to the extinction coefficient of 0.120) on day 16 of cultivation. During 30 and 60 cultivation days the laccase activity of these complexes was low.

**Lignin**. A 16.2% lignin content was fixed in the control rye straw. During fungal complex cultivation for 20 days the lignin degradation went on slowly (Fig. 4). In this period the content of lignin was changed more significantly by the fungal complexes *Galactomyces geotrichum – Sporotrichum pruinosum* and *Galactomyces geotrichum – Myrothecium verrucaria*: 1.4 and 1.33 times, respectively, in comparison with control. The complex *Dipodascus armillariae – Verticillium fungicola* changed lignin content least significantly (1.16 times).

The analysis showed that deeper lignin degradation went on in the later stages of fungal cultivation (after 60 days). The fungal complexes *Galactomyces geotrichum – Myrothecium verrucaria* and *Galactomyces geotrichum – Sporotrichum pruinosum* decreased lignin content 2.4 and 1.9 times, respectively in comparison with control. Under the influence of other fungal complexes its content decreased from 1.7 to 1.25 times in comparison with control.

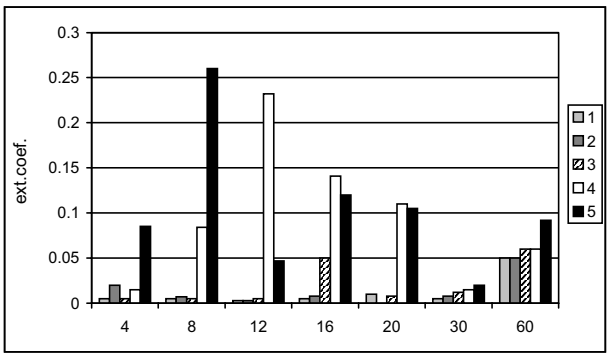


Fig. 3. Laccase activity of micromycete complexes during their cultivation on rye straw for 4 to 60 days

Received 25 February 2003
Accepted 3 May 2004

**References**

1. Eriksson KE , Vallander L. Sven. Papperstiden 1982; 85: 33–8.
2. Kirkpatrick N, Reid ID, Ziomek E, Ho C, Paice MG. Appl Environ Microbiol 1989; 55: 1147–52.
3. Eaton D, Chang HM, Kirk TK. Tappi 1980; 63: 103–9.
4. Hammel KE. Ens Microbial Technol 1989; 11: 776–7.
5. Zadrazil F, Brunert H. Eur J Appl Microbiol Biotechnol 1980; 9: 37–44.
6. Reid ID. Ens. Microbial Technol 1989; 11: 786–803.
7. Galas E, Pyč R, Romanowska I. Acta Biotechnol 1997; 17(4): 339–49.
8. Бабицкая ВГ. Прикладная биохимия и микробиология 1994; 30 (6): 827–35.
9. Varnaitė R. Biologija 2002; 2: 23–5.
10. Билай ВИ. Методы экспериментальной микологии. Киев, 1982.
11. Ravin H, Harward M. The Lancet. 1965; 270 (6920): 726–7.
12. Ермаков АИ, Арасимович ВВ, Ярош НП, Перуанский ЮА и др. Методы биохимического исследования растений. Ленинград, 1987.
13. Худякова ХК. Сельскохозяйственная биология 1984; (8): 120–4.

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**MIKROMICETŲ KOMPLEKSŲ FENOLOKSIDAZINIS AKTYVUMAS TVIRTAFAZĖS FERMENTACIJOS SĄLYGOMIS**

S a n t r a u k a

Ištirta mikromicetų kompleksų *Galactomyces geotrichum–Myrothecium verrucaria; Galactomyces geotrichum–Sporotrichum pruinosum; Myrothecium verrucaria–Sporotrichum pruinosum; Dipodascus armillariae–Verticillium fungicola ir Papularia sphaerosperma–Fusarium redolens* peroksidazės, lakazės ir tirozinazės aktyvumas, taip pat lignino degradacija rugių šiauduose po mikromicetų kultivavimo 20, 30 ir 60 parų.

Nustatyta, kad lignino degradacija priklausė nuo mikromicetų kultivavimo trukmės. Maksimaliai lignino kiekį rugių šiauduose (po 60 parų) sumažino šie mikromicetų kompleksai: *Galactomyces geotrichum–Myrothecium verrucaria, Galactomyces geotrichum–Sporotrichum pruinosum* (atitinkamai 2,4 ir 1,9 karto, palyginus su kontrole). Dėl kitų mikromicetų kompleksų poveikio šis sumažėjimas sudarė nuo 1,7 iki 1,25 karto, palyginus su kontrole.

Didžiausias peroksidazės aktyvumo maksimumas (247,6 a.v./g) buvo nustatytas po mikromicetų komplekso *Galactomyces geotrichum–Myrothecium verrucaria* kultivavimo 8 paras. Antras šio komplekso aktyvumo maksimumas (192,9 a.v./g) buvo po 20 kultivavimo parų. Lignino degradacijos laipsnis šiais periodais atitinkamai sudarė 14,28 ir 12,18%.

Maksimalus tirozinazės aktyvumas (4,026 sąl. v./g) buvo po mikromicetų komplekso *Galactomyces geotrichum–Sporotrichum pruinosum* kultivavimo 8 paras. Antrą aktyvumo maksimumą (2,61 sąl.v./g) šis kompleksas pasiekė po 30 kultivavimo parų. Lignino degradacijos laipsnis šiais periodais atitinkamai siekė 14,50 ir 10,68%.

Mikromicetų kompleksų lakazės aktyvumas buvo nedidelis. Jų ekstinkcijos koeficientas keitėsi nuo 0,003 iki 0,260.