
Enzymatic lignin degradation by micromycetes in plant remnants

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The activity of phenoloxydases (peroxidase, laccase and tyrosinase) of the micromycetes *Galactomyces geotrichum* and *Myrothecium verrucaria*, as well as lignin degradation in rye straw after cultivation of the micromycetes for 20, 30 and 60 days were investigated.

It was established that lignin content in rye straw was more significantly reduced after 30 and 60 days by *Galactomyces geotrichum* (1.88 and 1.92 times, respectively, in comparison with control). The highest peroxidase activity (84.63 u/g) and the highest degradation level of lignin (8.4%) were ascertained after cultivation of *Galactomyces geotrichum* for 60 days and of tyrosinase (0.717 cu/g) after cultivation of this micromycetes for 30 days.

The micromycete *Myrothecium verrucaria* reached the highest peroxidase activity after 30 days of cultivation (84.63 u/g). After this period, lignin degradation level was 9.8% and tyrosinase activity was 0.693 u/g after cultivation for 60 days.

Key words: micromycetes, lignin, phenoloxidas: peroxidase, tyrosinase, laccase

INTRODUCTION

Microorganisms use for their nutrition various substrata, including plant remnants in which the main components are cellulose, hemicellulose and lignin. These polymers comprise 20 to 90% of organic matter in wood, roots and grass stalks. Bioconversion of these raw materials to fuel, fodder, food products and subproducts for chemical and microbiological industry has become of great value as one of the most important trends in biotechnology [1–6].

Bioconversion 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, a component of these substrata, as its enzymatic destruction is not yet clear [7–10].

The aim of the present work was to investigate the degradation of lignin in rye straw by micromycetes under solid-state fermentation conditions, and to elucidate the activities of phenoloxydases (peroxidase, laccase and tyrosinase) produced by fungi-destroyers in relation with lignin degradation. To accelerate the growth of micromycetes and lignin degradation, mineral additives were used.

MATERIALS AND METHODS

The object of the study was rye (*Secale*) straw. Micromycetes – producers of phenoloxydases isolated

from polymeric materials were used in experiments. The experiments were conducted with microorganisms – most successful degraders of the lignin–cellulose complex in plant remnants – *Galactomyces geotrichum* (Butl. et Petersen) Redhead et Malloch and *Myrothecium verrucaria* (Alb. et Schweinitz) Ditmar ex Fries.

The plant remnants were moistened with a mineral medium (0.3 g NH₄NO₃ and 0.1 g KH₂PO₄ were added to 10 g of dry material). Micromycetes on the rye straw were cultivated 20, 30 and 60 days at a temperature of 28 °C. Then changes in lignin content were analysed and the activity of peroxidase, laccase and tyrosinase was determined. Lignin content in rye straw was evaluated following Chudiakova's method [11]. Phenoloxidase activity of the micromycetes was tested during their cultivation under solid-state fermentation conditions.

Peroxidase activity was analysed using o-dianisidine reagent [12], tyrosinase was assessed with pyrocatechin [12] and laccase with p-phenylendiaminohydrochloride [13].

RESULTS AND DISCUSSION

The results showed that lignin made up 16.2% in the control straw. When the micromycetes *Galactomyces geotrichum* were cultivated for 20 days, the content of lignin decreased to 12.35%, and this was

1.3 times lower than in the control case (Fig. 1). A similar, only with slight differences, decrease of lignin content was established after cultivation of the micromycete *Myrothecium verrucaria*.

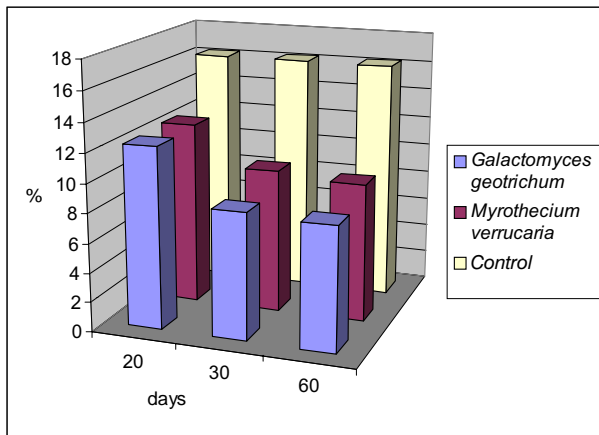


Fig. 1. Changes of lignin content in rye straw after cultivation of micromycetes *Galactomyces geotrichum* and *Myrothecium verrucaria* for 20, 30 and 60 days (% of air-dried matter)

The lignin content was lower 1.29 times in comparison with control.

The phenoloxidase activity of fungal biomass after 20 day cultivation was not high: peroxidase activity of *Galactomyces geotrichum* was 3.93 u/g, and it was slightly higher (5.36 u/g) after *Myrothecium verrucaria* cultivation (Fig. 2). Tyrosinase activity (0.324 cu/g) was also higher after growth of *Myrothecium verrucaria*. The activity of *Galactomyces geotrichum* was 0.249 conditional units (cu/g) (Fig. 3). Laccase activity of the micromycetes was negligible, as the role of this enzyme in the degradation of rye straw lignin is insignificant.

When the micromycetes were cultivated for a longer time (30 days), *Galactomyces geotrichum* diminished the amount of lignin in rye straw 1.88 times and *Myrothecium verrucaria* 1.65 times in comparison with control.

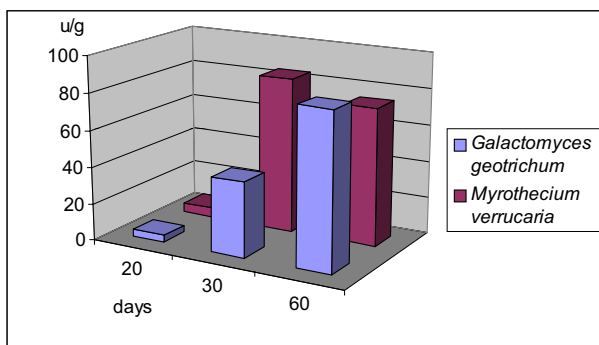


Fig. 2. Changes in peroxidase activity after 20, 30 and 60 days of micromycetes cultivation on rye straw (u/g)

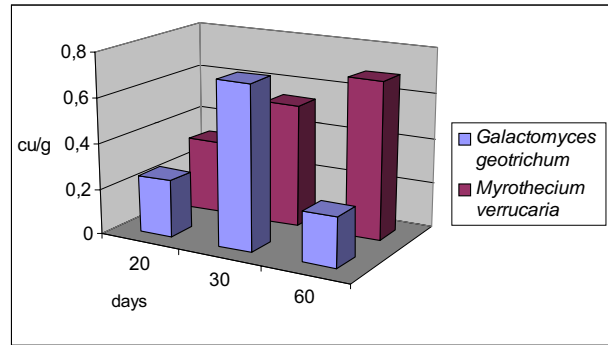


Fig. 3. Changes of tyrosinase activity after cultivation of micromycetes for 20, 30 and 60 days (conditional units/g)

A higher phenoloxidase activity was determined during deeper degradation of lignin.

Peroxidase activity of *Galactomyces geotrichum* after solid-state fermentation for 30 days was found highly increased and reached 40.62 u/g. Peroxidase activity of *Myrothecium verrucaria* was twice higher in comparison with *Galactomyces geotrichum* and reached 84.63 u/g. This shows that when the cultures get older, oxidative processes catalyzed by peroxidase take place.

After 30 days the tyrosinase activity, contrary to the peroxidase activity, was higher after cultivation of *Galactomyces geotrichum* (0.717 cu/g).

The laccase activity of the micromycetes tested was rather low. The extinction coefficient of *Galactomyces geotrichum* was 0.05, and that of *Myrothecium verrucaria* was not detected at all.

When micromycetes were grown for 60 days, deeper degradation of lignin was established. Lignin content after cultivation of *Galactomyces geotrichum* for 60 days decreased 1.92 times and after cultivation of *Myrothecium verrucaria*, on the contrary, increased from 0.54 to 0.69 cu/g.

No laccase activity of the micromycetes after 60 days was observed.

The alteration of the enzymes was probably caused by the unequal growth of mycelium and its dying what manifest in the unequal activity of enzymes

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FERMENTINĖ LIGNINO DEGRADACIJA MIKROMICETAIS AUGALŲ ATLIEKOSE

S a n t r a u k a

Ištirtas mikromicetų *Galactomyces geotrichum* ir *Myrothecium verrucaria* fenoloksidazių – peroksidazės, lakazės ir

tirozinazės – aktyvumas ir lignino degradacija rugių šiauduose po 20, 30 ir 60 parų mikromicetų kultivavimo.

Nustatyta, kad lignino kiekį rugių šiauduose po 30 ir 60 kultivavimo parų labiau sumažino *Galactomyces geotrichum* (atitinkamai 1,88 ir 1,92 karto palyginus su kontrole).

Maksimalus peroksidazinis aktyvumas (84,63 a.v./g) ir lignino degradacijos laipsnis (8,4%) yra nustatytas po mikromiceto *Galactomyces geotrichum* kultivavimo 60 parų, o tirozinazinis (0,717 sąl.v./g) – po šio mikromiceto kultivavimo 30 parų.

Mikromicetas *Myrothecium verrucaria* peroksidazinio aktyvumo maksimumą (84,63 a.v./g) pasiekė po 30 parų kultivavimo; šiuo periodu yra nustatytas lignino degradacijos laipsnis (9,85%); didesnis šio mikromiceto tirozinazinis aktyvumas (0,693 sąl.v./g) pastebėtas po 60 parų kultivavimo.