BIOLOGIJA. 2004. Nr. 3. P. 43–46


# Biodegradation of plant remnants by micromycetes

**R. Varnaitė,**
**V. Raudonienė**

*Institute of Botany,*
*Žaliųjų Ežerų 49,*
*LT-08608 Vilnius, Lithuania*

Degradation of lignin and cellulose in rye straw was investigated when the micromycetes *Galactomyces geotrichum*, *Myrothecium verrucaria* and *Sporotrichum pruinosum* were cultivated.

*Galactomyces geotrichum* was found to be most successful in rye straw bioconversion. This strain after 60 days of cultivation on rye straw diminished the lignin content 1.92 and cellulose 9.6 times in comparison with control.

*Myrothecium verrucaria* was found to be more successful in the degradation of the lignin–cellulose complex in rye straw. After a 60-day cultivation it diminished the lignin content 1.65 times. *Sporotrichum pruinosum* was more efficient in cellulose degradation. It diminished the cellulose content 2.4 times in comparison with control.

Analysis of destruction metabolites showed that anhydride of chemipin acid, veratraldehyde, veratryl alcohol, ketonic compound, palmitic and linoleic acids and their derivatives were the main components after cultivation of the micromycetes *Galactomyces geotrichum* and *Myrothecium verrucaria*. Palmitic, linoleic acids and their derivatives, dioxiglucose prevailed after cultivation of *Sporotrichum pruinosum*. The ester of guaiacolglyceril coniferil was detected among compounds of high molecular mass. We could conclude that the oxidation processes were faster in substrates on which micromycetes *Galactomyces geotrichum* and *Myrothecium verrucaria* had been cultivated.

**Key words**: micromycetes, lignin, cellulose, destruction metabolites

## INTRODUCTION

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 (lignin peroxidase, manganese peroxidase and laccase), which participate in lignin degradation [1–5].

Various compounds such as alcohols, ketonics, organic acids, compounds of phenolic origin, amino and neutral compounds are formed during disintegration of plant remnants. For example, white-rot fungi synthesize aromatic alcohols such as veratrol, anizol and chloranizol or *de novo* they are synthesized from aromatic amino acids or phenolic compounds. All of them (first of all veratrol) are indispensable elements of a lignolytical system [6].

Microbial ligninolytic systems have been used in improving the digestibility and nutritive value of animal feeds [7], degradation of toxic pollutants [8, 9], bioconversion of lignin into useful organic compounds [10], and in increasing the fermentability of lignocellulosic residues [11].

There are a few works dealing with decomposition of lignin-cellulose complex in plant remnants by mycelium fungi [12–16].

Delignification of wheat straw by complexes of micromycetes from various taxonomic groups and the role of phenoloxidases in the degradation process of these substrates were investigated [12–14]. Differences of lignin degradation in rye straw and flax boons with the participation of macro- and micromycetes was ascertained [15]. The demetoxilation and oxidation of lignin component become deeper during cultivation of macromycetes. A considerable difference of the enzyme complex of fungi-destructors was ascertained [16].

The ability of micromycete to utilize lignin–cellulose complex in plant remnants is different. Some micromycetes can carry out deeper hydrolysis of this complex in a short term, meanwhile some of them need much time to induce small changes in the structure of this complex.

The aim of the present work was to investigate the degradation of lignin-cellulose complex in rye (*Secale*) straw by solitary micromycetes and to carry out a qualitative analysis of the decay product of this complex.

## MATERIALS AND METHODS

The object of the study was three strains of micromycetes and rye (*Secale*) straw. Micromycetes isolated from various materials were used in experiments. The following strains were isolated, identified and investigated: 1. *Galactomyces geotrichum* (Butl. et Petersen) Redhead et Malloch – isolated from soil (Botany Gardens of Kaunas); 2. *Myrothecium verrucaria* (Alb. et Schweinitz) Ditmar ex Fr. – isolated from roots of cereal plants (Dotnuva); 3. *Sporotrichum pruinosum* Gilman et Abbott. – isolated from a glued together multi-layer film (sort of 3LARK TU 6-19-051-237-79).

The plant remnants were fine-cut and moistened with a mineral solution (0.3 g $NH_4NO_3$ and 0.1 g $KH_2PO_4$ were added to 10 g of dry material) and sterilized at 121 °C for 30 min. Then the plant material was inoculated with fungi. Micromycetes were cultivated on straw for 30, 60 and 90 days at a temperature of 28 °C. Then the degradation of the lignin–cellulose complex content and the qualitative composition of the decay product were analyzed.

Lignin content was evaluated in rye straw following Khudiakova's method [17].

Cellulose content was evaluated in rye straw following Kürschner's and Hafer's method [18].

Elution of destruction metabolites of rye straw was made with 96° ethyl alcohol. Analysis of destruction metabolites was performed applying gas chromatography (HP-5890) with a mass detector (HP 5971A).

## RESULTS AND DISCUSSION

**Degradation of lignin–cellulose complex of rye straw.** The results showed that lignin made up 16.2% in the control straw (Fig. 1). When the micromycete *Galactomyces geotrichum* was cultivated for 30 days on rye straw, the content of lignin decreased to 8.6%, *i.e.* became 1.3 times lower than in the control. When the micromycete was cultivated for a longer time, the content of lignin changed and after 60 days was 1.92 times lower in comparison with control. The results indicated [19] that degradation of lignin by the micromycete in the initial stage of cultivation (20 days) changed the content of lignin very slightly. It was in line with data of other authors [20] that degradation of this polymer appears in the further course of cultivation of micromycetes.

The cultivation of the micromycete *Myrothecium verrucaria* on rye straw for 30 days diminished the content of lignin 1.55 times in comparison with control and after 60 days reduced it to 9.8%. This was 1.65 times lower than in control.

The minimum decrease of lignin (in comparison with the above-mentioned strains of micromycetes) was observed after a cultivation 30-day of the micromycete *Sporotrichum pruinosum*. Its content decreased only 1.15 times in comparison with control. The same lignin degradation degree as after cultivation of *Myrothecium verrucaria* was observed in the further course of cultivation (60 days), and its content decreased to 9.8%.

Investigations of cellulose degradation in rye straw (Fig. 2) showed that degradation of this polymer, like that of lignin, was most rapid after cultivation of *Galactomyces geotrichum*. After 30 days of cultivation the cellulose content diminished 2.76 and after 60 days even 9.6 times in comparison with control.

Degradation of cellulose was noticed after cultivation of micromycete *Sporotrichum pruinosum*; after 30 days the cellulose content was 45.58% and after 60 days 21.78%. The minimum content was observed after *Myrothecium verrucaria* cultivation: after 30 and 60 days it was respectively 1.07 and 1.29 times lower in comparison with control.

**Destruction metabolites of plant remnants.** Destruction metabolites, which affect enzyme systems

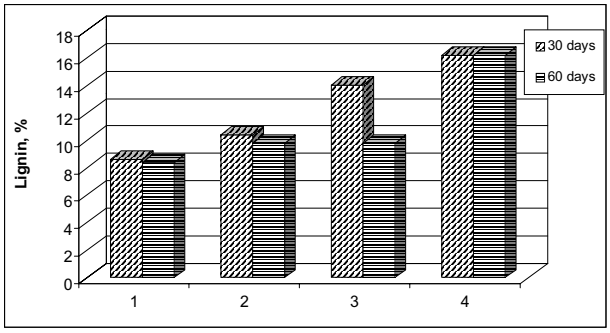


Fig. 1. Changes of lignin content in rye straw after cultivation of micromycetes for 30 and 60 days (% of air-dried matter): *1 – Galactomyces geotrichum*, *2 – Myrothecium verrucaria*, *3 – Sporotrichum pruinosum*, *4* – control

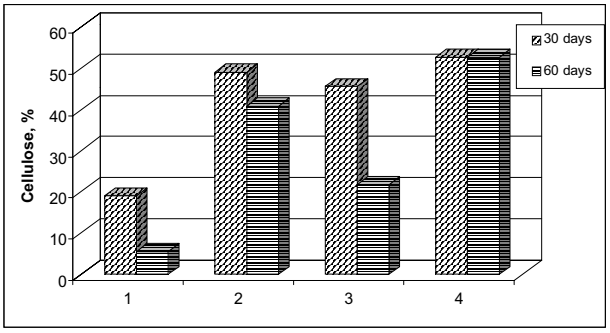


Fig. 2. Changes of cellulose content in rye straw after cultivation of micromycetes for 30 and 60 days (% of air-dried matter): *1 – Galactomyces geotrichum*, *2 – Myrothecium verrucaria 3 – Sporotrichum pruinosum*, *4* – control

involved in the degradation of this complex, are produced during degradation of plant remnants.

Destruction metabolites of the micromycetes that are the most successful degraders of the lignin–cellulose complex in plant remnants were investigated after 90 days of cultivating *Galactomyces geotrichum*, *Myrothecium verrucaria* and *Sporotrichum pruinosum* on plant remnants. Compounds of different nature were detected in the solution of an alcoholic extract.

The research showed (Fig. 3) that after cultivation of *Galactomyces geotrichum* and *Myrothecium verrucaria* the main component was the anhydride of chemipin acid, which is formed from natural compounds during their deep oxidation process (time of escape 13.8 min). Also, the following compounds were detected: veratralaldehyde (time of escape 9.1 min), veratryl alcohol (time of escape 9.9 min), ketonic compound (time of escape 14.07 min), palmitic acid (time of escape 14.8 min), linoleic acid (time of escape 16.6 min) and their derivatives.

The larger amount of palmitic, linoleic acids and their derivatives was ascertained after cultivation of *Sporotrichum pruinosum*. Dioxiglucose was also de-

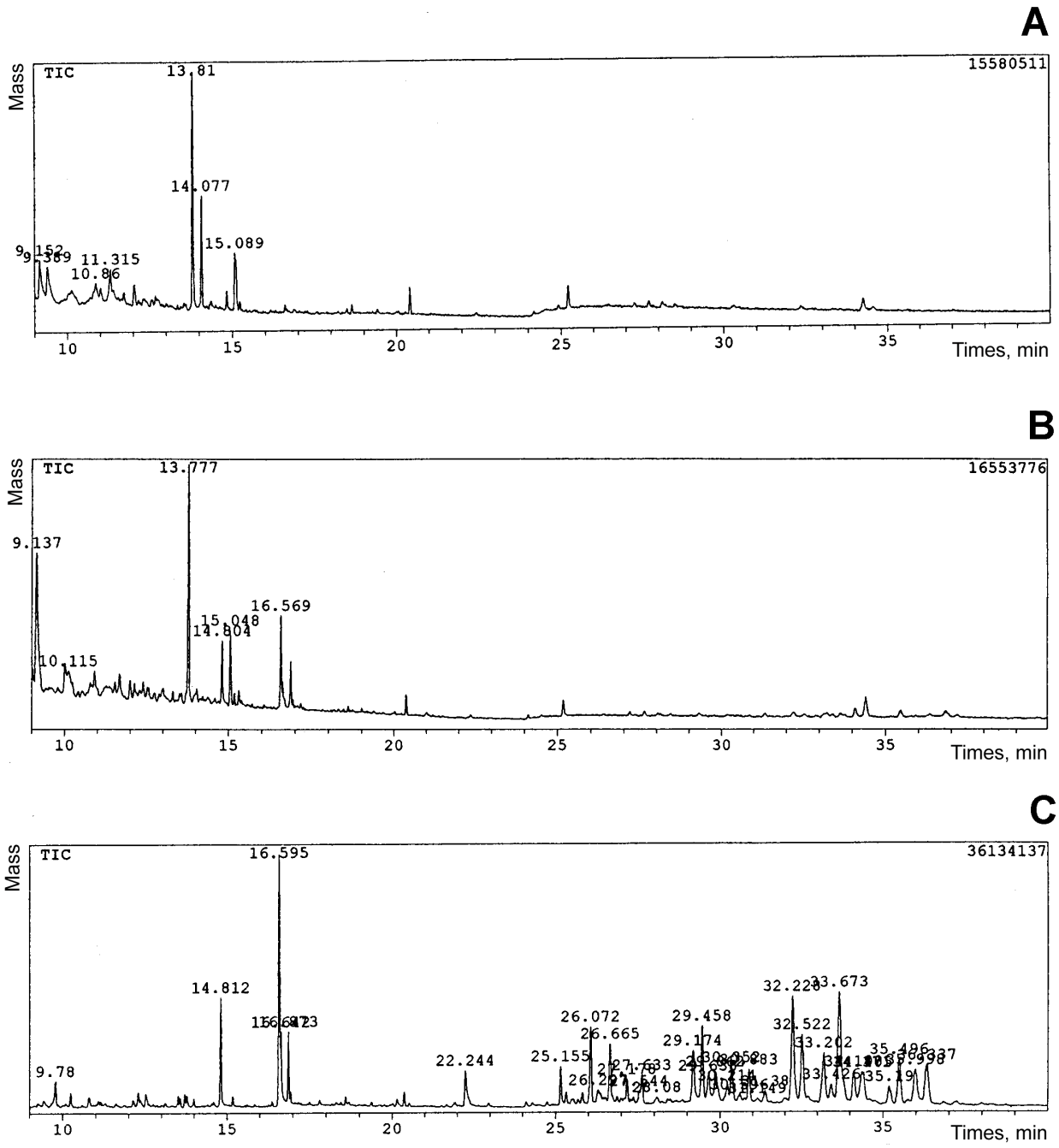


Fig. 3. Chromatographic characteristic of metabolites of plant remnant destruction after cultavation of micromycetes: *A – Galactomyces geotrichum*, *B – Myrothecium verrucaria*, *C – Sporotrichum pruinosum*

tected (time of escape 9.78 min). Most compounds of high molecular mass that have a time of escape over 26 min were not detected, except the ester of guaiacolglyceril coniferil (time of escape 26.07 min).

One can see from the chromatogram that after cultivation of *Galactomyces geotrichum* and *Myrothecium verrucaria* no compounds of high molecular mass were detected in the plant remnant hydrolysis products. Thus, we can conclude that oxidation processes were faster in the substrates on which these micromycetes were cultivated.

Received 29 January 2004
Accepted 7 July 2004

**References**

1. Glenn JK, Morgan MA, Mayfield MB, Kuwahara M, Gold MH. Biochem Biophys Res Comm 1983; 114: 1077–83.
2. Hatakka A, Uusi-Rauva AK. Eur J Appl Microbiol Biotechnol 1983; 17: 235–42.
3. Glenn JK, Gold MJ. Arch Biochem Biophys 1985; 242: 329–41.
4. Bonnarme P, Jeffries TW. Appl Envirl Microbiol 1990; 36: 210–7.
5. Perez J, Jeffries TW. Appl Envir Microbiol 1990; 56: 1806–12.
6. Jong Ed de, Field Jim A, Bont Jan AM de. FEMS Microbiol Rev 1994; 13(2–3): 153–88.
7. Zadrazil F. Eur J Appl Microbiol 1980; 9: 243–8.
8. Field JA, de Jong ED, Costa GF, de Bont JAM. Appl Envir Microbiol 1992; 58: 2219–26.
9. Schutenzendubel A, Majcherczyk A, Johaanes C, Huttermann A. Int Biodeter Biodegr 1999; 43: 93–100.
10. Chen CL, Chang HM, Kirk TK. J Wood Chem Technol 1983; 3: 35–57.
11. Jonsson LJ, Palmquist E, Nilvebrant B, Hagerdal H. Appl Microbiol Biotechnol 1998; 49: 694–7.
12. Daljit S. Arora Biodegradation 1995; 6: 57–60.
13. Daljit S Arora, Mukesh Chander, Paramjit Kaur Gill. Int Biodeter Biodegr 2002; 50: 115–20.
14. Kelley J. Outlook of Agriculture 1992; 21(2): 105–8.
15. Бабицкая ВГ, Щерба ВВ, Осадчая ОВ, Латышева СГ. Химия древесины 1990; 6: 83–8.
16. Бабицкая ВГ. Прикладная биохимия и микробиология 1994; 30(6): 827–35.
17. Худякова ХК. Сельскохозяйственная биология 1984; 8: 120–4.
18. Ермаков АИ, Арасимович ВВ, Ярош НП, Перуанский ЮА и др. Методы биохимического исследования растений. Ленинград, 1987.
19. Varnaitė R, Raudonienė V. Ekologija 2002; 4: 16–20.
20. Irbe I, Andersone I, Andersons B, Chirkova J. Int Biodeter Biodegr 2001; 47: 37–45.

**R. Varnaitė, V. Raudonienė**

**AUGALINIŲ ATLIEKŲ BIODEGRADACIJA NAUDOJANT MIKROMICETUS**

S a n t r a u k a

Ištirta lignino ir celiuliozės degradacija rugių šiauduose kultivuojant ant jų mikromicetus *Galactomyces geotrichum*, *Myrothecium verrucaria* ir *Sporotrichum pruinosum*.

Nustatyta, kad perspektyviausias kamienas rugių šiaudų biokonversijoje yra *Galactomyces geotrichum*. Po 60 kultivavimo parų ant rugių šiaudų šis kamienas lignino kiekį juose sumažino 1,92, o celiuliozės – 9,6 karto, palyginus su kontrole.

Kitas perspektyvesnis mikromicetų kamienas rugių šiaudų degradacijai buvo *Myrothecium verrucaria*, lignino kiekį po 60 kultivavimo parų sumažinęs iki 1,65, o celiuliozės degradacijai – *Sporotrichum pruinosum*, sumažinęs jos kiekį 2,4 karto, palyginus su kontrole.

Metabolitų destrukcijos tyrimas, po mikromicetų *Galactomyces geotrichum* ir *Myrothecium verrucaria* kultivavimo pagrindinis komponentas buvo chemipino rūgšties anhidridas, veratraldehidas, veratrilo alkoholis, ketoninis junginys, palmitino, linolo rūgštys ir jų dariniai. Po *Sporotrichum pruinosum* kultivavimo nustatyti didesni kiekiai linolo ir palmitino rūgščių bei jų darinių, dioksigliukozė. Iš didesnės molekulinės masės junginių išsiskyrė gvajakolglicerilo koniferilo esteris. Taigi oksidaciniai procesai sparčiau vyksta substratuose, ant kurių buvo kultivuojami *Galactomyces geotrichum* ir *Myrothecium verrucaria*.