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Cellulose degradation in rye straw by micromycetes and their complexes

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The ability of micromycetes *Galactomyces geotrichum, Myrothecium verrucaria,Sporotrichum pruinosum, Chaetomium globosum, Mortierella verticillata, Verticillium fungicola, Mortierella humilis* as well as their complexes *Galactomyces geotrichum–Myrothecium verrucaria, Galactomyces geotrichum–Sporotrichum pruinosum, Sporotrichum pruinosum–Myrothecium verrucaria, Chaetomium globosum–Mortierella verticillata* and *Verticillium fungicola–Mortierella humilis* to degrade cellulose in plant remnants during 30 and 60 days of cultivation was investigated.

Cellulose degradation in plant remnants was found to depend on the duration of micromycete growth. The lowest cellulose levels after 60 days were detected after cultivation of *Galactomyces geotrichum* (5.48%) and *Myrothecium verrucaria* (12.34%) (8.12 and 3.6 times in comparison with control).

As regards micromycete complexes, cellulose content was lowered most significantly by *Galactomyces geotrichum–Sporotrichum pruinosum* (9.92%) and *Sporotrichum pruinosum–Myrothecium verrucaria* (10.02%), i. e. 4.5 and 4.4 times versus control.

Key words: micromycetes, cellulose, degradation

INTRODUCTION

Degradation of the natural complex of organic compounds (lignin and cellulose) is an important problem of biology and soil science. Resources of these polymers in nature are rather rich. From the chemical viewpoint, these complex polymers are heavily degrading materials which have accumulated huge resources of photosynthesis energy. About 60% of this energy is attributed to cellulose and hemicellulose. Under natural conditions, these polymers are tightly linked with lignin. The latter compound is very resistant to external factors, thus, energy accumulated in its compounds is hardly available to the biota of the environment. If these links can be weakened, plant remnants would be easier available to other chains of the ecological system, and biological activity would increase (Blanchette, 2000; Hammel, 1989; Score et al., 1997).

During the recent years, production of agricultural products has risen and, along with this, pollution with production waste has also increased. All plant wastes contain large amounts of lignin, cellulose and hemicellulose, however, the amounts of these components and their ratios are different. Waste of plant origin from agricultural activities and other industrial branches is a potential raw material for microbiological conversion. Micromycetes play a great role in the biodegradation of the lignin–cellulose complex. Thus, they can be used in biotechnology.

The main enzymes that take part in the cellulose degradation process are cellulases.

Some features of natural cellulosic materials are known to inhibit their degradation / bioconversion (Solomon et al., 1990, 1999). These are the degree of crystallinity and lignification and the capillary structure of cellulose. The crystallynity and lignification limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan et al., 1987). However, many physical, chemical and microbial pre-treatment methods for enhancing the bioconversion of cellulosic materials have been reported (Ojumu et al., 2003; Kansoh et al., 1999; Kumakura, 1997; Wu, Lee, 1997).

Screening and research of new strains of basidiomycetes able to utilize lignin and cellulose in plant remnants have been carried out (Ахмедова и др., 1994). It has been established that utilization of plant remnants depends on their composition and the cultivation time of fungi.

The aim of the present work was to test the ability of micromycetes participating in the lignin degradation process to degrade cellulose in plant remnants.

MATERIALS AND METHODS

Individually, the following micromycetes were investigated: *Galactomyces geotrichum* (Butler and Petersen) Ditmar ex Fries, *Myrothecium verrucaria* (Alb. and Schwein.) Ditmar ex Fries, *Sporotrichum pruinosum* (Gilman and Abbott), *Chaetomium globosum* Kunze, *Mortierella verticillata* Linnem., *Verticillium fungicola* (Preuss) Hassebr., *Mortierella humilis* Linnem. The following micromycete complexes were used: *Galactomyces geotrichum–Myrothecium verrucaria, Galactomyces geotrichum–Sporotrichum pruinosum, Sporotrichum pruinosum–Myrothecium* *verrucaria*, *Chaetomium globosum–Mortierella verticilla* and *Verticillium fungicola–Mortierella humilis*.

Micromycetes were grown under solid state fermentation conditions on rye straw for 30 and 60 days at a temperature of 28 °C in sterile conditions. To enhance bioconversion of plant remnants, mineral additives were used (to 10 g of air-dried material 0.3 g of NH_4NO_3 and 0.1 g of KH_2PO_4 were added).

Cellulose content in plant remnants was assessed by the modified Kurshner and Hafer method (Ермаков и др., 1987).

The data were computed using the Excel 98 program.

RESULTS AND DISCUSSION

Various micromycete species differently degrade cellulose. It has been found (Kerem et al., 1999) that different micromycete species have specific mechanisms to degrade the lignin–cellulose complex.

The investigation showed (Fig. 1) that after cultivation of individual micromycetes for 30 days on rye straw, a decrease of cellulose content in comparison with control was found in all experiment variants.

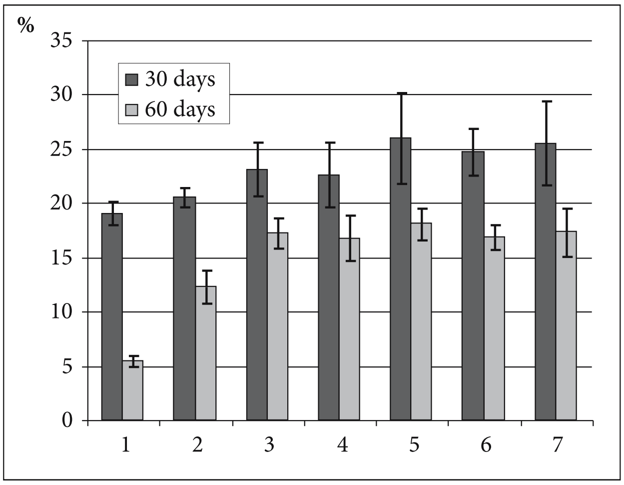

Fig. 1. Changes of cellulose content in rye straw after cultivation of micromycetes for 30 and 60 days. *1 – Galactomyces geotrichum, 2 – Myrothecium verrucaria, 3 – Sporotrichum pruinosum, 4 – Chaetomium globosum, 5 – Mortierella verticillata, 6 – Verticillium fungicola, 7 – Mortierella humilis*

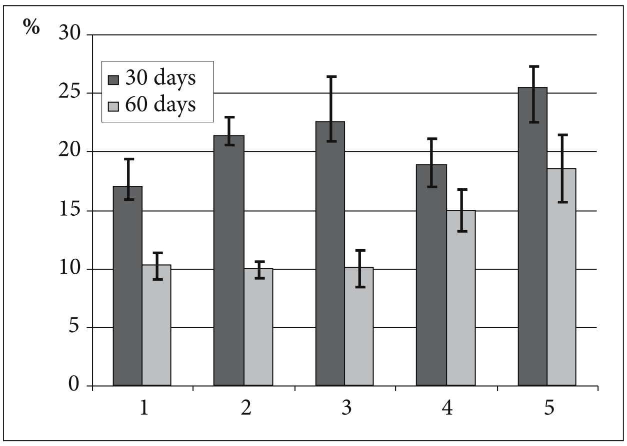

Fig. 2. Changes of cellulose amount in rye straw after cultivation of micromycete complexes for 30 and 60 days. *1 – Galactomyces geotrichum–Myrothecium verrucaria, 2 – Galactomyces geotrichum–Sporotrichum pruinosum, 3 – Sporotrichum pruinosum–Myrothecium verrucaria, 4 – Chaetomium globosum–Mortierella verticillata, 5 – Verticillium fungicola–Mortierella humilis*

In control rye straw, cellulose amounted to 44.52%. Cultivation of *Galactomyces geotrichum* resulted in cellulose decrease to 19.08%. This amount was 2.3 times lower than in control. A significant change of cellulose content was also detected after cultivation of micromycetes *Myrothecium verrucaria* and *Chaetomium globosum*. In 30 days, these micromycetes reduced cellulose content 2.16 and 1.96 times respectively in comparison with control.

Changes in cellulose amount were least when micromycetes *Mortierella verticillata* and *Mortierella humilis* were cultivated (decrease to 26.03 and 25.53%), and cellulose was left 1.7 and 1.74 times less than in control.

While cultivating micromycetes longer (for 60 days), deeper cellulose degradation occurred. Like in the 30-day experiment, *Galactomyces geotrichum* reduced the maximum cellulose amount also after 60 days – to 5.48%. This was 8.12 times less in comparison with control. *Myrothecium verrucaria* reduced cellulose content 3.6 times in this period. Cellulose comprised 12.34% in the obtained biomass. Cellulose was degraded significantly after growth of *Chaetomium globosum* and *Verticillium fungicola*, too (16.8 and 16.85% respectively). As in the 30-day experiment, the least changed cellulose after 60 days was found while cultivating *Mortierella verticillata* and *Mortierella humilis* (cellulase decreased 2.46 and 2.57 times respectively).

The investigation of micromycete complexes showed (Fig. 2) that after the 30-day cultivation cellulose degraded most significantly (to 16.97%) when the *Galactomyces geotrichum–Myrothecium verrucaria* complex was used. Cellulose content in this case was 2.62 times lower than in control. The *Chaetomium globosum–Mortierella verticillata* complex reduced cellulose content in the biomass to 18.81%, i. e. 2.36 times lower than in control.

The lowest cellulose decrease (to 25.42%) was established after cultivation of the *Verticillium fungicola–Mortierella humilis* complex.

During the growth of micromycete complexes for 60 days, cellulose content decreased also in the other range – from 9.92 to 18.56%. Cellulose content changed most significantly after cultivation of *Galactomyces geotrichum–Sporotrichum pruinosum* (4.48 times) and *Sporotrichum pruinosum–Myrothecium verrucaria* (4.44 times) complexes and was least reduced after growing the *Verticillium fungicola–Mortierella humilis* complex (2.39 times).

The micromycete cellulases studied – endoglucanase (Cx) and β-glucosidase – were found to have a different activity depending on the cultivation time and characteristics of micromycetes (Raudonienė, 2003).

Nearly all study micromycetes showed the lowest activity of endoglucanases in the initial period of cultivation, while the activity of β-glucosidase in this period was maximal.

CONCLUSIONS

1. Cellulose degradation depends on micromycete cultivation time. After 60 days of cultivation, micromycete *Galactomyces geotrichum* reduced the content of cellulose in waste 8.12 times in comparison with the control sample.

2. The *Galactomyces geotrichum–Sporotrichum pruinosum* micromycete complex reduced the content of cellulose in waste down to 9.92% and the *Sporotrichum pruinosum–Myrothecium verrucaria* complex to 10.02%. These numbers were respectively 4.5 and 4.4 times lower than in the control sample.

3. For plant remnant biodegradation, it is advisable to use micromycete *Galactomyces geotrichum* and the *Galactomyces geotrichum – Sporotrichum pruinosum* complex.

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CELIULIOZĖS DEGRADACIJA RUGIŲ ŠIAUDUOSE PAVIENIAIS MIKROMICETAIS IR JŲ KOMPLEKSAIS

Santrauka

Ištirta mikromicetų *Galactomyces geotrichum, Myrothecium verrucaria, Sporotrichum pruinosum, Chaetomium globosum, Mortierella verticillata, Verticillium fungicola, Mortierella humilis* ir jų kompleksų *Galactomyces geotrichum–Myrothecium verrucaria, Galactomyces geotrichum–Sporotrichum pruinosum, Sporotrichum pruinosum–Myrothecium verrucaria, Chaetomium globosum–Mortierella verticillata* ir *Verticillium fungicola–Mortierella humilis* gebėjimas ardyti celiuliozę augalų atliekose po jų kultivavimo 30 ir 60 parų.

Nustatyta, kad celiuliozės degradacija augalų atliekose priklausė nuo mikromicetų kultivavimo trukmės. Maksimaliai celiuliozės kiekį po 60 kultivavimo parų sumažino *Galactomyces geotrichum* (iki 5,48%) ir *Myrothecium verrucaria* (iki 12,34%) (atitinkamai 8,12 ir 3,6 karto mažiau, palyginus su kontrole).

Iš mikromicetų kompleksų maksimaliai celiuliozės kiekį pakeitė *Galactomyces geotrichum–Sporotrichum pruinosum* (iki 9,92%) ir *Sporotrichum pruinosum–Myrothecium verrucaria* (iki 10,02%). Tai buvo 4,5 ir 4,4 karto mažiau, palyginus su kontrole.

Raktažodžiai: mikromicetai, celiuliozė, degradacija