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Destruction of hemicellulose in rye straw by micromycetes

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The ability of micromycetes to destruct hemicellulose and xylane – the main hemicellulose polysaccharide, – in herbal waste was investigated. We found that hemicellulose was most destructed after 60 days of micromycete cultivation. Its quantity after *Chaetomium globosum* cultivation was reduced down to 6.35%, *Myrothecium verrucaria* to 7.22%, *Galactomyces geotrichum* to 7.92% and *Mortierella verticillata* to 7.99%. The change of hemicellulose content was least after *Sporotrichum pruinosum* and *Acremonium strictum* cultivation. The numbers of fungi were 2.3 and 2.5 times lower than in the control sample.

The more intensive xylane destruction in liquid fermentation conditions after 15 and 20 days was determined in samples with *Myrothecium verrucaria* (the content of sugars increased up to 37.3 and 75.93 mg%) and *Galactomyces geotrichum* (26.4 and 38.83 mg%).

Key words: micromycetes, hemicellulose, xylan

INTRODUCTION

Herbal waste of agriculture and industry, based on lignin and cellulose, is a potential raw material for microbiological conversion. All the types of herbal waste have a large content of cellulose, hemicellulose and lignin, but the percentage of these components depends on the type of raw material (Detroy, Julian, 1981; Hammel, 1989). Hemicellulose, the second most renewable biomass polymer next to cellulose, represents about 20–35% of the biomass of plant material. It can by converted to a number of value-added fermentation products such as fuel ethanol, xylitol, butanediol, and lactic acid. To this end, the polymer needs to be converted to sugars (Saha, 2002; 2003).

In the meantime, more and more attention is paid to enzymatic hydrolysis of hemicellulose and xylanes because of the progress of biotechnologies. Enzymes that hydrolyze hemicellulose are endo- and exo- types of L-arabinonases, D-galactonases, D-manonases and D-xylanases (Linko, 1982; Fodge et al., 1999). Xylan, the main component of hemicellulose, consists of a b-1-,4-linked D-xylosil residues backbone branched with other pentoses, hexoses and uronic acids. Xylanases and the associated debranching enzymes produced by a variety of microorganisms, including bacteria, yeasts and filamentous fungi, bring about the hydrolysis of hemicelluloses (Gilbert, Hazlewood,1993; Maheshwari et al., 2000).

The number of facts about the utility of xylose degradation in the enzymatic pathway is increasing. Xylose from the enzymatically hydrolysed xylanes present in agricultural, wood conversion and cellulose industry waste, from plant waste confirms the economic profitability of xylite and furfurole which have a wide application in chemical and pharmaceutical industries. It can serve as a carbon source or an indicator for enzyme synthesis (e. g. glucosoisomerase) in microorganisms used in microbiological industries. These enzymes improve the quality of animal and bird crude feed (Abdel-Sater, El-Said, 2001).

Enzymes participating in the degradation of herbal cell wall xylanes are an object of interest of chemists and biochemists investigating the action mechanisms of carbohydrases (Perlin, Reese, 1963), the composition of xylanes and xylanooligosaccharides (Preece, MacDougall, 1958), elucidating the role of endoxylanases (Delker, Richards, 1976) and exoxylanases (Reese et al., 1973) in the hydrolysis of xylanes.

Xylanes get into soil from rotting seeds, herbal waste and play an important role in nature's carbon cycle. Xylose, as a product of xylane degradation by xylanase action, is a source of energy to soil microorganisms from the genera *Fusarium, Penicillium, Trichoderma* and others and can produce hydrolytic enzymes.

The purpose of this work was to investigate the ability of lignin and cellulose degrading micromycetes to destruct hemicellulose and xylanes.

MATERIALS AND METHODS

Rye straw (*Secale*) was an object of this study. Micromycetes – producers of phenoloxydases – were used in this experiment. The following micromycetes – biodestructors of cellulose–lignin complexes in plant waste – were isolated, identified and investigated:

1. *Galactomyces geotrichum* (E. E. Butler et L. J. Petersen) Readhed et Malloch
2. *Myrothecium verrucaria* (Alb. et Schwein.) Ditmar
3. *Sporotrichum pruinosum* J. C. Gilman et E. V. Abbott
4. *Mortierella verticillata* Linnem.
5. *Chaetomium globosum* Kunze

6. *Fusarium redolens* Wollenw.

7. *Acremonium strictum* W. Gams.

Plant waste was moistened with mineral medium (0.3 g of NH_4NO_3, and 0.1 g of KH_2PO_4 was added to 10 g of herbal material) for a better growth of micromycetes. Micromycetes on straw were cultivated for 30 and 60 days at 28 °C in sterile conditions, and then the change of the quantity of hemicellulose was analysed. The method for hemicellulose quantity determination was taken from Yermakov (Ермаков и др., 1987). The quantity of reducing sugars was determined by the Bertran method (Ермаков и др., 1987). For the analysis of xylane destruction, micromycetes were cultivated in liquid fermentation conditions. The Czapek medium in which glucose was replaced with 0.5% of xylane was prepared. Micromycetes were not cultivated in the control sample. In these conditions, micromycetes were cultivated for 5, 10, 15 and 20 days, and then the change of the content of reducing sugars was analysed.

The data were computed using the Excel 98 program.

RESULTS AND DISCUSSION

Analysis showed that there was 23.94% of hemicellulose in the control sample of rye straw. The statistical data showed that all of the micromycetes had reduced reliably the content of hemicellulose in rye straw after 30 days of cultivation (Figs. 1, 2). The biggest decrease of hemicellulose quantity was in the sample with *Fusarium redolens* (down to 9.07%), *Sporotrichum pruinosum* (12.06%) and *Mortierella verticillata* (12.41%). These numbers are 2.63; 1.98; 1.92 times lower respectively to control sample. During the further cultivation (after 60 days), the quantity of hemicellulose in straw was still reliably falling, except *Fusarium redolens* which during cultivation didn't change straw hemicellulose content (reduced unreliably) (Figs. 1, 3). In this period, the biggest decrease (down to 6.35%) was observed in a sample with *Chaetomium globosum, Myrothecium verrucaria* (7.22%), *Galactomyces geotrichum* (7.92%) and *Mortierella verticillata* (7.99%), i. e. respectively 3.77, 3.15, 3.02, and 2.99 times lower than in the control sample.

According to other researchers (Ахмедова и др., 1994), when cultivated on sunflower straw for 30 days, *Panus tigrinus* reduced hemicellulose content from 28.9% (in control sample) to 12.1%, *Pleurotus ostreatus* to 7.3%, *Fomes fomentarius* to 14.7%, *Inonotus hispidus* to 10.6%. The active degradation of the biopolymer was in progress for the first 10 days; later it slackened.

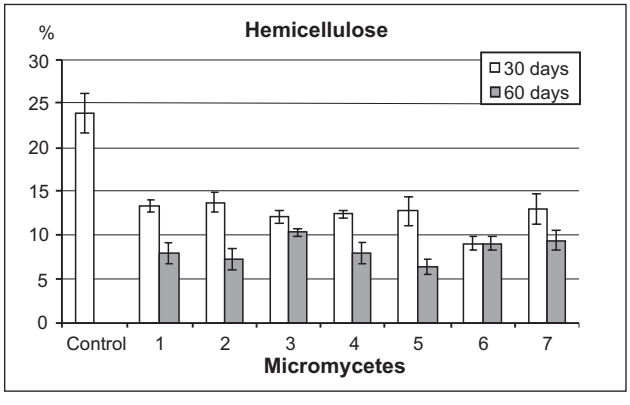

Fig. 1. Destruction of hemicellulosis in rye straw by micromycetes: *1 – Galactomyces geotrichum, 2 – Myrothecium verrucaria, 3 – Sporotrichum pruinosum, 4 – Mortierella verticillata, 5 – Chaetomium globosum, 6 – Fusarium redolens, 7 – Acremonium strictum*

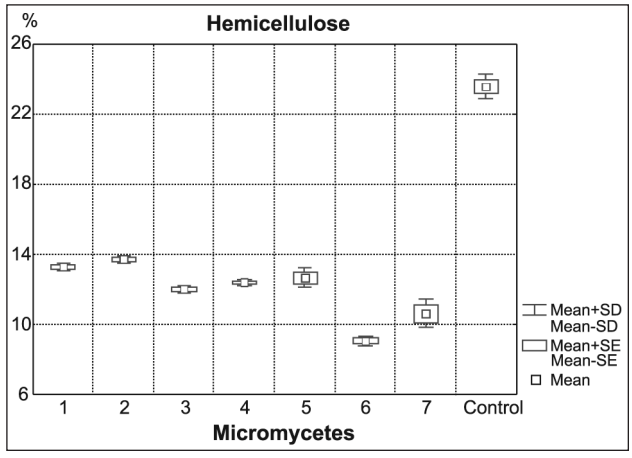

Fig. 2. Distribution of micromycetes in accordance with hemicellulose content in straw after 30 days of cultivation

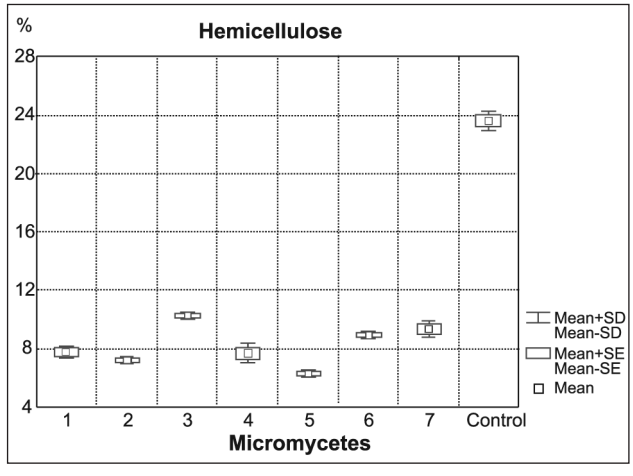

Fig. 3. Distribution of micromycetes in accordance with hemicellulose content in straw after 60 days of cultivation

In our investigation with micromycetes, the maximal reduction of hemicellulose content after 30 days of cultivation (down to 9.07%) was found in the sample with *Fusarium redolens*.

There are attempts to use hemicellulose destruction for practical purposes.

Investigations by other authors (Badal, Saha, 2003) have shown that the conversion of hemicellulose to fuels and chemicals is problematic. Various pretreatment options as well as enzymatic saccharification of lignocellulosic biomass to fermentable sugars have been investigated. There are data on pretreatment and enzymatic saccharification of corn fiber and development of a novel and improved enzymes such as endoxylanase, β-xylosidase and α-L-arabinofuranosidase for hemicellulose bioconversion. The barriers, progress, and prospects of developing an environmentally benign bioprocess for large-scale conversion of hemicellulose to fuel ethanol, xylitol, 2,3-butanediol and other value-added fermentation products have been highlighted.

The main component of hemicellulose in cereals is xylane. In the meantime, more attention is paid to investigation of its structure and characteristics, and the results increase our knowledge of hemicellulose characteristics. Xylose is a product of pentosane hydrolysis. This monosaccharide is the main structural component in the xylane chain.

Abdel-Sater, El-Said (2001) screened xylan-decomposing fungi in two agricultural and one industrial waste. Twenty-six species representing 13 genera were identified from rice straw, wheat straw and sugarcane bagasse on the medium used. The results revealed that 93.3% of the isolates tested could degrade xylan, and the highest activity against xylan was shown by *Aspergillus, Fusarium, Penicillium* and *Trichoderma*.

The statistics shows that after cultivation of all the micromycetes, as a result of xylan destruction, the amount of reduced sugars reliably increased in comparison with the control during the cultivation. In accordance with xylane destruction, micromycete strains can be distributed into three statistically reliable groups (Fig. 5). In our study (Fig. 4), after 5 days the highest content of sugars was found in samples with *Mortierella verticillata* (29.06 mg%) and *Chaetomium globosum* (23.33 mg%), and the lowest quantity was found in a sample with *Acremonium strictum* (1.8 mg%).

In the further cultivation (after 10 days), the maximum content of sugars (28 mg%) was found in a sample with *Myrothecium verrucaria* and *Mortierella verticillata* (19.1 mg%), however, in this period the content of carbohydrates in a sample with *Mortierella verticillata* had fallen 1.52 times in comparison with the level of 5 days cultivation after.

After 15 days, the level of sugars in a sample with *Myrothecium verrucaria* increased to 37.3 mg% and with *Galactomyces geotrichum* to 26.4 mg%; other micromycete strains fell into a group where the content of reduced sugars varied from 3.13 to 12.9 mg% (Figs. 4, 6). In the samples with other micromycetes, the quantity of sugars decreased.

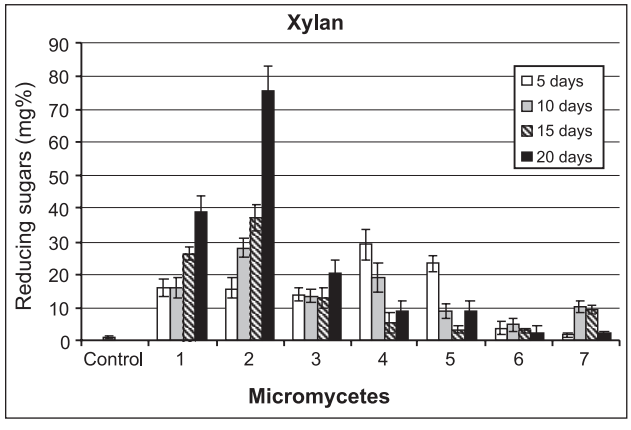

Fig. 4. Content of reducing sugars after xylane destruction by micromycetes: *1 – Galactomyces geotrichum, 2 – Myrothecium verrucaria, 3 – Sporotrichum pruinosum, 4 – Mortierella verticillata, 5 – Chaetomium globosum, 6 – Fusarium redolens, 7 – Acremonium strictum* after 5, 10, 15 and 20 days of cultivation

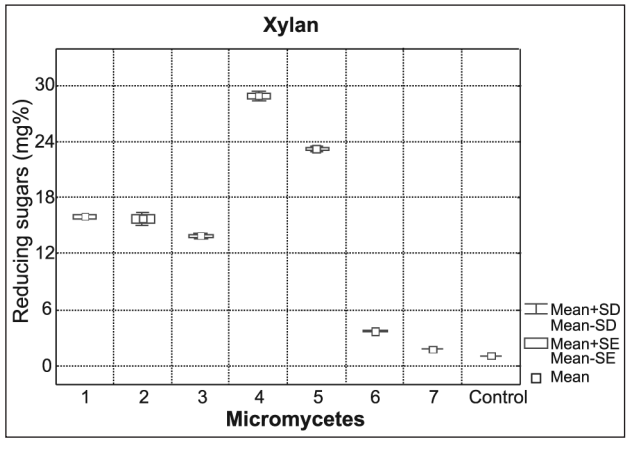

Fig. 5. Distribution of micromycetes in accordance with the content of reduced sugars after xylane destruction after 5 days of cultivation

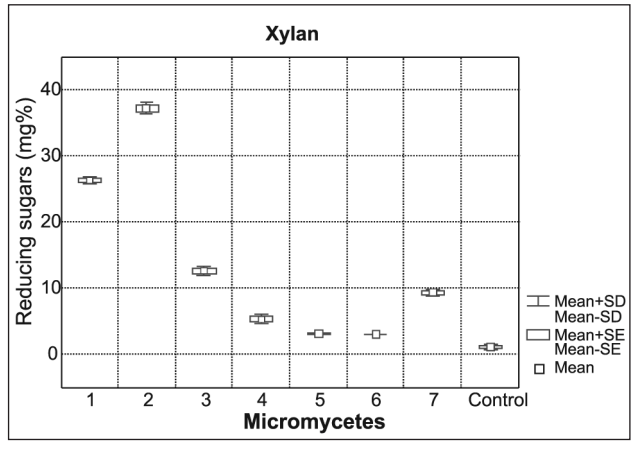

Fig. 6. Distribution of micromycetes in accordance with the content of reduced sugars after xylane destruction after 15 days of cultivation

After 20 days of cultivation of *Myrothecium verrucaria*, the content of sugars increased to 75.93 mg% and of *Galactomyces geotrichum* to 38.83 mg%. In samples with *Sporotrichum pruinosum* and *Mortierella verticillata*, no increase of sugars was noted. *Acremonium strictum* reduced the content of sugars.

Thus, micromycetes *Chaetomium globosum, Myrothecium verrucaria*, *Galactomyces geotrichum*, which deeply degrade lignin and cellulose in plant remnants (Варнайте, Раудонене, 2003; Varnaitė, Raudonienė, 2005), are able to perform a deeper degradation of hemicellulose and xylane in the later stages of their cultivation.

CONCLUSIONS

1. Hemicellulose destruction depends on the duration of micromycete cultivation. After 60 days of cultivation, *Chaetomium globosum* reduced the content of hemicellulose to 6.35%, *Myrothecium verrucaria* to 7.22%, and *Galactomyces geotrichum* down to 7.92%.

2. *Myrothecium verrucaria* and *Galactomyces geotrichum* were the most active micromycetes for xylane destruction after 15 and 20 days.

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References

1. Abdel-Sater M. A., El-Said A. H. M. 2001. Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. *International Biodeterioration and Biodegradation.* Vol. 47. N 3. P. 15–21.
2. Dekker R. F. H., Richards G. N. 1976. Hemicellulases: their occurence, purification, properties and mode of action. *Adv. Carbohyd. Chem. Biochem*. Vol. 32. P. 277–352.
3. Detroy R. W., Julian G. S. 1981. Biomass conversion: Fermentation chemicals and fuels. *Crit. Rev. Microbiol*. Vol. 10. P. 203–228.
4. Fodge Douglas W., Anderson David M., Pettey Thomas M. 1997. *Biotechnol. Adv*. Vol. 15. N 1. P. 96.
5. Gilbert H. J., Hazlewood G. P. 1993. Bacterial cellulases and xylanases. *J. Gen. Microbiol*. N 139. P. 187–194.
6. Hammel K. E. 1989. Organopollutant degradation by ligninо lytic fungi. *Enzyme Microb. Technol*. N 11. P. 776–777.
7. Linko M. 1982. Regulation of enzymatic hydrolysis of polisacharides. In: Krumphanzl V. et al. (eds.). *Overproduction of microbial products*. L.: Academic Press. P. 601–610.
8. Maheshwari R., Bjardwai G., Bhat M. K. 2000. Thermophilic fungi, their physiology and enzymes. *Microbiol. Mol. Biol. Rev*. N 64. P. 461–488.
9. Perlin A. S., Reese E. T. 1963. Dimension of the substrate site involved in the enzymolysis of a polysaccharide. *Can. J. Biochem. Physiol*. Vol. 41. P. 1842–1846.
10. Preece J. A., MacDaugall J. 1958. Enzymatic degradation of cereal hemicelluloses: II. Pattern of pentosan degradation. *J. Inst. Brew*. Vol. 64. P. 489–498.
11. Reese E. T., Maguire A., Parrish F. W. 1973. Production of β-D-xylopyranosides by fungi. *J. Microbiol*. Vol. 19. P. 1065–1074.
12. Saha B. C. 2002. Hemicellulose bioconversion [abstract]. *Society of Industrial Microbiology*. P. 82.
13. Saha B. C. 2003. Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*. Vol. 30. P. 279–291.
14. Varnaitė R., Raudonienė V. 2005. Enzymatic lignin degradation in rye straw by micromycetes. *International Biodeterioration and Biodegradation*. Vol. 56. P. 192–195.
15. Ахмедова З., Белецкая О., Далимова Г., Халикова М., Азимходжаева М., Давранов К., Шарипова А. 1994. Отбор и культивирование целлюлозо- и лигнинразрушающих грибов. *Микробиология*. Т. 63. Вып. 6. С. 829–935.
16. Варнайте Р., Раудонене В. 2003. Биодеградация растительных отходов микромицетами. *Микология и фитопатология.* Т. 37. Вып. 2. С. 49–52.
17. Ермаков А., Арасимович Н., Ярош Ю., Перуанский Г., Луковникова М., Иконникова М. 1987. *Методы биохимического исследования растений.* Агропромиздат. 430 с.

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HEMICELIULIOZĖS DESTRUKCIJA RUGIŲ ŠIAUDUOSE MIKROMICETAIS

S a n t r a u k a

Ištirtas mikromicetų – fenoloksidazių producentų gebėjimas ardyti hemiceliuliozę augalų atliekose ir ksilaną – pagrindinį hemiceliuliozės polisacharidą. Nustatyta, kad gilesnė hemiceliuliozės destrukcija vyko po 60 mikromicetų kultivavimo parų. Jos kiekis šiame periode labiausiai sumažėjo po *Chaetomium globosum* (iki 6,35%), *Myrothecium verrucaria* (iki 7,99%), *Galactomyces geotrichum* (iki 7,92%) ir *Mortierella verticillata* (iki 7,99%) kultivavimo. Mažiausiai pakitęs hemiceliuliozės kiekis buvo po *Sporotrichum pruinosum* ir *Acremonium strictum* kultivavimo. Tai buvo 2,3 ir 2,5 karto mažiau, palyginus su kontrole.

Gilesnė ksilano destrukcija skystafazės fermentacijos sąlygomis po 15 ir 20 parų buvo nustatyta kultivuojant *Myrothecium verrucaria* (cukrų kiekis padidėjo atitinkamai iki 37,3 ir 75,93 mg%) ir *Galactomyces geotrichum* (atitinkamai 26,4 ir 38,83 mg%).

Raktažodžiai: mikromicetai, hemiceliuliozė, ksilanas