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SEARCHING MICROORGANISMS FOR BIODEGRADATION CELLULOSE-CONTAINING RAW MATERIALS FROM RECYCLED RESOURCES AND AGRICULTURAL WASTE

From the mixed forest floor, 35-55 years old, 213 isolates of fungi were secured. They are decomposers of cellulose belonging to 7 genera – Fusarium, Aspergillus, Trichoderma, Penicillium, Cladosporium, Verticillium and Aureobasidium.

Among them some fungi prevailed over others notably fungi of genus Fusarium – 4 species, fungi of genus Aspergillus- 3 species and one species of Trichoderma, Penicillium, Cladosporium, Verticillium and Aureobasidium.

Fungi Aspergillus niger, Aspergillus versicolor and Aureobasidium pululans showed the greatest activity in the degradation of cellulose and hemicellulose.

Keywords: *microorganisms, fungi, cellulose, degradation, biodegradation, enzymes*

Introduction. It is known that cellulose is one of the most widespread plant polymers in nature. Annual increase of plant organic compounds (plant biomass) in process of biosynthesis is about 2 trillions tons and cellulose accounts to one third of the weight [5]. However, natural cellulose has crystalline structure and its biodegradation is complicated. In addition, lignin belongs to lignocellulose sophisticates its accessibility for enzyme molecules. Therefore for increasing of efficiency of biodegradation cellulose-containing raw materials (CCRM) it is

necessary previously treatment of it by cellulitic and lignolytic enzymes [1, 4]. process of lignocellulose pretreatment by biological agents is the most promising because energy expenditure by using physical, mechanical and chemical methods of previously treatment of CCRM is more than 20% [2, 3].

Object. Screening of species of fungi- decomposers secured from natural cenoses for detecting species promising for previously treatment and further degradation of straw.

Methods. Securing of fungi-decomposers from natural cenoses was carried out while 2009-2011 years. Isolation of pure cultures of fungi selected from forrest floor carried out on Van-Iterson and Czapek mediums. For removal of extraneous microorganisms pattern was sterilized by burning of spirit lamp fire and located on nutrient medium. Duration of incubation was 30-60 days at +25°C. Microorganisms oxidizing cellulose were revealed by Omelyansky method. For detemining of cellulitic activity researched strain were cultivated during 30 days on liquid nutrient medium of such composition (in grams on 100 ml distilled water: KNO_3 -1,25; K_2HPO_4 - 0,5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0,25; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ - 0,005; NaCl - 0,25; $\text{Mn}_2(\text{SO}_4)_3$ - 0,005; KMI (carbomethylcellulose) – 5,0.

For studing morphological, cultural and physiological-biochemical properties of cultures we have used standard microbiological media and methods.

We have carried out quantitative determination of glucose in the hydrolysis after growth of fungi by *Hagedorn-Jensen* method. The study was conducted in the laboratory of of the Department of Technology of storage and processing of agricultural products, Zhytomyr National Agroecological University.

Results of research. For securing of fungi-decomposers in Zhytomyr region and neighboring areas during June-August we have sampled tests out from forrest floor, 35-55 years old, where stable biocenoses are formed. From grass ecosystems tests were sampled also in May-August, when the greatest activity of fungi is observed. Over the years of research we have sampled 210 testes and have secured to pure culture 213 isolates of cellulose-decomposers fungi (table 1).

**Genera of fungi secured from forrest floor to Van-Iterson culture
(by growth on filter paper), 2009-2011 years**

Genus	Quantity of isolates, pc.	Genus	Quantity of isolates, pc.
Fusarium	131	Aspergillus	47
Penicillium	9	Aureobasidium	2
Trichoderma	17	Cladosporium	4
Verticillium	3		

For identification of fungi-decomposers their fundamental biological and morphological features were investigated. In particular, basic systematic characteristics, by which species of fungi were determined, are morphologic peculiarities of macroconidia (formation, sizes and form, quantity of membranes, form and size of top cell, existence of stem, concavity) and of microconidia and chlamydospores.

Most of isolates belong to genus *Fusarium* – 61,5%, to genus *Aspergillus* – 22,1%, to genus *Trichoderma* – 8,0%, fungi of generi *Verticillium* and *Cladosporium* were within 1,4–1,9%. Least of isolates - 0,9% - belong to genus *Aureobasidium*.

Determination of fungi species secured fungi-decomposers cultures showed that among them some fungi predominates over others notably fungi of genus *Fusarium* – 4 species, fungi of genus *Aspergillus*- 3 species and one species of *Verticillium*, *Trichoderma*, *Cladosporium*, *Penicillium*, *Aureobasidium* (fig.1)

Practical interest are fungi of the genus *Trichoderma*, including strain *T. hamatum* ZH- 6, which showed high antagonistic activity relative to agents of fusarirose rot (*Fusarium oxysporum*) and brown rot (*Rhizoctonia solani*) of sugar beet roots. The strain of *T. hamatum* ZH- 6 were characterised by colourless smooth branched hyphae, whitish-green conidium-bearing area. Conidium-bearers forms small pustules in the lower part. Conidia are paly green cylindrical, elongated and narrowed near the top. On the standard Czapek medium colonies of strain *T. hamatum* ZH- 6 at temperature 22 ° C, reach brim of Petri dish for 4-6 days.

Mycelium is colourless, cobweb. Sporification appears at 5-12 day of growth, placing is placing uniform or concentric zones, often only on the edge of colony. Coloring is gray-green, blue-green, sometimes dirty-green or dark green. The reverse side of colony is colourless. Pigment is not allocated into the medium. The smell of the isolate resembles mushroom. Hyphae are colourless, smooth. Conidium-bearers are uncolored, smooth, straight, branched at intervals of equal length, branches are short and thick, placed two-four sometimes one, diverged at right angles to conidium-bearer. Conidia are green-gray, elliptical, smooth.

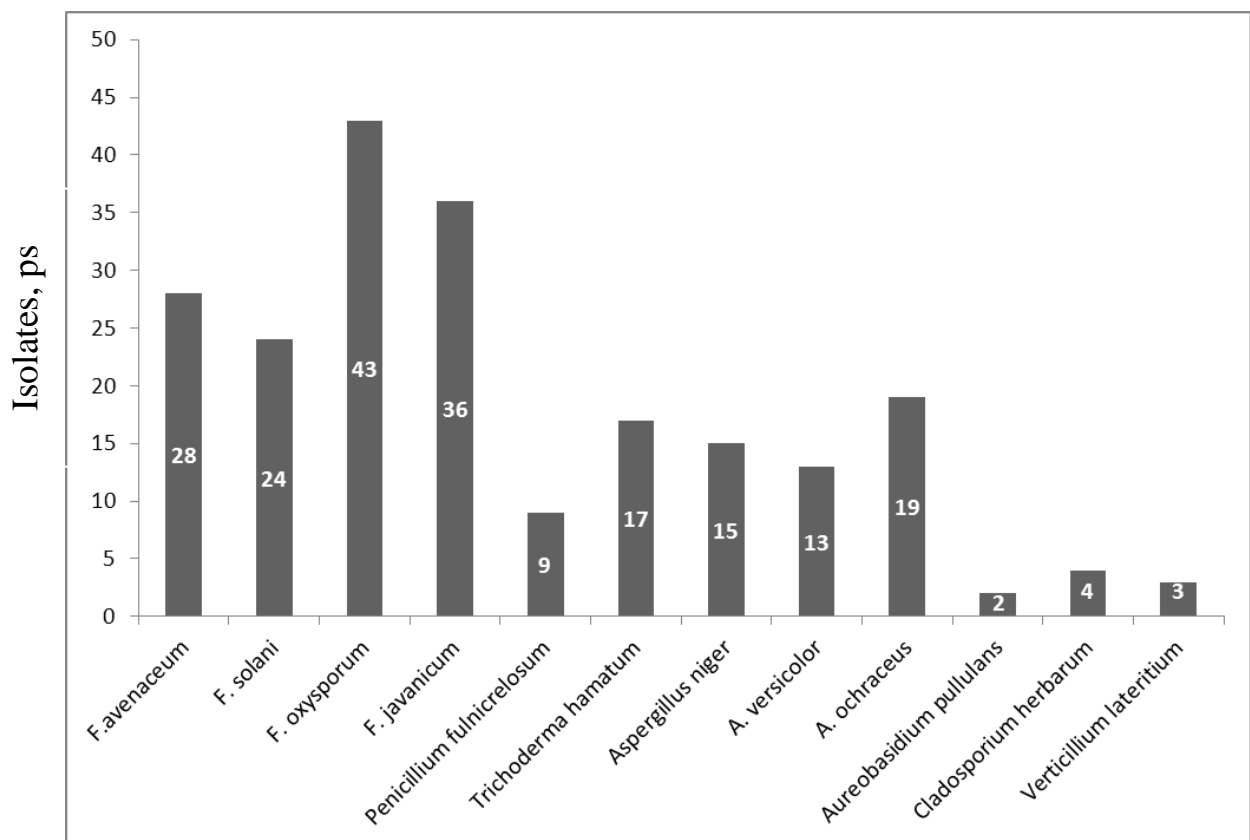


Fig.1. Primary cultures of fungi-decomposers secured from forrest floor, 2009 - 2011 years.

It was found that the largest amount of glucose formed in fungi *Aspergillus niger*, *Aspergillus versicolor* and *Aureobasidium pululans*, and accounted for 0,82, 1,43 and 0,68 mg/ml, accordingly. The strain *Trichoderma hamatum* ZH-6 formed relatively low amount of glucose in the medium - 0.28 mg/ml (tabl. 3).

Cellulitic fungi activity by Hagedorn-Jensen method

Strain	Quantity of glucose, mg/ml
<i>Aspergillus niger</i>	0,82
<i>Aspergillus versicolor</i>	1,43
<i>Aureobasidium pululans</i>	0,68
<i>Penicillium fulnicrelosum</i>	0,48
<i>Aspergillus ochraceus</i>	0,32
<i>Trichoderma hamatum</i> ZH-6	0,28

Research on in-depth study of activity of cellulitic and lignolitic microorganisms enzymes, optimization of their cultivation conditions and search ways to increase efficiency of biodegradation of straw is continued now.

Conclusions.

1. From the mixed forest floor, 35-55 years old, in Zhytomyr region and neighboring areas, where stable microbial communities are formed, 213 isolates of fungi-decomposers were secured. They belong to 7 genera – *Fusarium*, *Aspergillus*, *Trichoderma*, *Penicillium*, *Cladosporium*, *Verticillium* and *Aureobasidium*. Among secured fungi cultures some fungi prevailed over others notably fungi of genus *Fusarium* – 4 species, fungi of genus *Aspergillus* - 3 species and one species of *Trichoderma*, *Penicillium*, *Cladosporium*, *Verticillium* and *Aureobasidium*.

2. Evaluation of cellulose and hemicellulose biodegradation activity by the number of glucose formation show that the most active fungi are *Aspergillus niger*, *Aspergillus versicolor* and *Aureobasidium pululans*. The strain *Trichoderma hamatum* ZH-6 (number in National collection of microorganism strains– IMB F – 100060) is of practical interest too because it show antagonistic properties against fusariose and brown rots of sugar beet roots.

Bibliography

1. Богдановская Ж.Н. Микробиологическая трансформация целлюлозо-содержащих материалов Изд-во «Наука и техника», Минск, 1986, 55 с.
2. Калунянц К.А., Голгер Л.И. Микробные ферментные препараты, М. 1979, 118 с.
3. Михайлова Р.В. Мацерирующие ферменты мицелиальных грибов в биотехнологии, РУП «Издательский дом «Белорусская наука», 2007. - 407 с.
4. Роговин З.А. Химия целлюлозы. - М., 1972, - 519 с.
5. Сеницын А.П., Гусаков А.В., Черноглазов В.М. Биоконверсия лигноцеллюлозных материалов: Учеб.пособие.- М: Изд-во МГУ, 1995, - 224 с.

Анотація

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Пошук мікроорганізмів для біодеградації целюлозовмісної сировини з вторинних ресурсів і відходів сільського господарства

*З лісової підстилки мішаних лісів віком 35–55 років виділено 213 ізолятів грибів-деструктантів, які належали до 7 родів - *Fusarium*, *Aspergillus*, *Trichoderma*, *Penicillium*, *Cladosporium*, *Verticillium* і *Aureobasidium**Penicillium*. Серед них переважали гриби роду *Fusarium* - 4 види, 3 види роду *Aspergillus* і по-одному виду - *Verticillium*, *Trichoderma*, *Cladosporium*, *Penicillium*, *Aureobasidium*. Найбільшу активність в деструкції целюлози і геміцелюлози виявили гриби *Aspergillus niger*, *Aspergillus versicolor* і *Aureobasidium pululans*.*

Ключові слова: мікроорганізми, гриби, целюлоза, деструкція, біодеградація, ферменти

Аннотация

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Поиск микроорганизмов для биодеградации целлюлозосодержащего сырья из вторичных ресурсов и отходов сельского хозяйства

Из лесной подстилки смешанных лесов возраста 35-55 лет выделено 213 изолятов грибов – деструктантов целлюлозы, принадлежащих к 7 родам - Fusarium, Aspergillus, Trichoderma, Penicillium, Cladosporium, Verticillium и Aureobasidium. Среди них преобладали грибы рода Fusarium - 4 вида, 3 вида рода Aspergillus и по одному виду Trichoderma, Penicillium, Cladosporium, Verticillium и Aureobasidium.

Наибольшую активность в деструкции целлюлозы проявили грибы Aspergillus niger, Aspergillus versicolor и Aureobasidium pululans.

Ключевые слова: *микроорганизмы, грибы, целлюлоза, деструкция, биодegradация, ферменты*