THE ANALYSIS OF CELLULASES AND LIGNIN PEROXIDASE ACTIVITY IN *PHANEROCHAETE CHRYSOSPORIUM* GROWN ON MEDIA WITH FIR AND BEECH SAWDUST UNDER THE INFLUENCE OF SOME TRACE ELEMENTS

ANALIZA ACTIVITĂŢII CELULAZELOR ŞI A LIGNIN PEROXIDAZEI LA SPECIA *PHANEROCHAETE CHRYSOSPORIUM*, CULTIVATĂ PE MEDII CU RUMEGUŞ DE BRAD ŞI FAG SUB INFLUENŢA UNOR OLIGOELEMENTE

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Abstract. *The purpose of this paper is the study on the influence of trace elements on cellulase and lignin peroxidase activity in Phanerochaete chrysosporium grown on media with fir and beech sawdust. In order to achieve research, carbon source was replaced from Sabouraud medium with fir and beech sawdust which were added separately and some trace elements such as: boron, molybdenum, manganese, lead, copper, zinc, iron, and a mixed solution with trace elements and control with no trace elements, resulting in final nine final working variants. Measurements were made at 7 days and 14 days after sowing. After analyzing the data obtained showed that the activity of cellulase and lignin peroxidase was stimulated in the second period in the presence of trace elements to both kinds of sawust media. Boron was the most nutrient stimulus, while zinc and trace elements solution had an inhibitory effect.*

Key words: *Phanerochaete chrysosporium*, cellulase, lignin peroxidase, trace elements.

Rezumat. *Scopul acestei lucrari este studiul influenței unor oligoelemente asupra activității celulazelor și a lignin peroxidazei la specia lignocelulozolitică Phanerochaete chrysosporium, cultivată pe medii cu rumeguș de brad și fag. În vederea realizării cercetărilor s-a înlocuit sursa de carbon din mediu Sabouraud cu rumegușuri de brad și fag la care s-au adăugat urmatoarele oligoelemente: bor, molibden, mangan, plumb, cupru, zinc, fier (introduse separat), precum și o soluție amestec de oligoelemente, rezultând în final 9 variante de lucru. Varianta martor nu prezintă oligoelemente. Determinările au fost realizate la 7 zile și respectiv 14 zile de la însămânțare. În urma analizei datelor obținute s-a evidențiat faptul că în prezența oligoelementelor a fost stimulată activitatea celulazelor și a lignin peroxidazei în a doua perioadă de timp la ambele tipuri de mediu de cultur.. Borul a avut un efect stimulator, în timp ce zincul și soluția de oligoelemente au avut un efect inhibitor.*

Cuvinte cheie: *Phanerochaete chrysosporium*, celulaze, lignin peroxidaza, oligoelemente

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INTRODUCTION

Cellulolytic microorganisms such as fungi and bacteria that inhabit diverse habitats play a vital role in the recycling of carbon in nature. In order to produce the white wood rot, fungus *Phanerochaete crysosporium* induces degradation of lignin and cellulose by producing enzymes such as cellulase and ligninase. Cellulose degradation process is problematic due to the semi-crystallin character of cellulose, thus the decomposition process is never an isolated event. The combined action of extracellular enzymes with complementary specificity is essential (Beguin și Aubert, 1994).

Simionescu et al. (1993) have highlighted the important role of trace elements in the processes of celullase biosynthesis and the effect of their transfer between the medium, the solid substrate and mycelium.

Previous studies on cellulolytic activity of fungi grown on waste from the forestry industry have been conducted in the Department of Microbiology, Institute of Biological Science of which include: the evolution of the cellulase complex in the fungus *Alternaria alternata*, cultivated on medium containing wastes from the forestry industry (coniferous and deciduous sawdust) (Manoliu Al.et al., 2005), the influence of electromagnetic field (EMF) on the celullase activity in cellulolytic fungi *Trichoderma viridae* and *Chaetomium globosum* grown on medium containing hardwood sawdust (Manoliu & al., 2007).

The lignolitic system of *Phanerochaete chrysosporium* is not induced by lignin but appears constitutively as culture enters the secondary metabolism, when primary growth ceases because of depletion of nutrients (Schmidt, 2006).

The purpose of this study was to investigate the influence of trace elements on lignin peroxidase and celullase activity in the fungus *Phanerochaete chrysosporium* grown on medium containing pine and fir sawdust.

MATERIAL AND METHOD

The study was conducted on the fungus *Phanerochaete chrysosporium* (HEM no. 5772) acquired by the Institute of Biological Science from the Institute Scientifique Santé Publique, Belgium. Carbon source was replaced in Sabouraud medium with sawdust from beech and pine tree to determine the influence of some trace elements on celullase and lignin peroxidase activity. Trace elements were then added to the medium as follows: B-10 mg, Cu-100 mg, Mn-20 mg, Mo-20 mg, Fe-20 mg, Zn-200 mg, Pb-20 mg. These amounts were calculated by nutrient equivalent of the following compounds: 0,0571 H_3BO_3 g/l, 0.3928 $CuSO_4 \cdot 5H_2O$ g/l, 0.07204 $MnCl_24 \cdot H_2O$, 0.05043 $NaMoO_4 \cdot 2H_2O$ g/l, 0.18 $FeCl_3 \cdot 6H_2O$ 50% ml/l, $CH_3COO_2Pb \cdot 3H_2O$ 0.0366 g/l. The control showed no source of trace elements. Measurement of enzymatic parameters was made at 7 days and 14 days after seeding in both fungus mycelium and culture fluid. Endoglucanase activity was determined on the basis of Peitersen method, the celobio-hidrolase activity was determined using Petterson and Porath's method, celobiose activity was determined using colorimetric method with dinitrosalycilic reagent and lignin peroxidase activity was determined using Tien and Kirk's method (1996) of veratryl alcohol. The enzymatic activity was reported to the amount of total soluble protein determined by Bradford method.

RESULTS AND DISCUSSIONS

Results of our research on the endoglucanase activity activity in the fungus *Phanerochaete chrysosporium* grown on media with pine and beech sawdust and under the influence of trace elements is showed in figure 1. Seven days after seeding enzymatic activity registered values lower than control in media variants with lead, zinc and trace elements solution, and the highest value was registered in the medium variant with boron. After 14 days all media variants showed values higher than control medium and the highest value was recorded in the media containing boron.

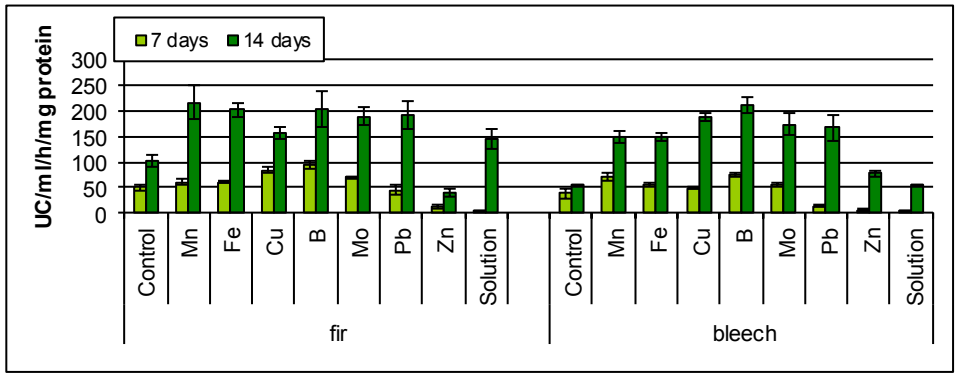

Fig. 1 - Endoglucanase activity in fungus *Phanerochaete chrysosporium* grown on media with pine and beech sawdust under the influence of trace elements

Celobiohidrolase activity in the fungus *Phanerochaete chrysosporium* grown on media with pine and beech sawdust under the influence of trace elements is presented in figure 2.

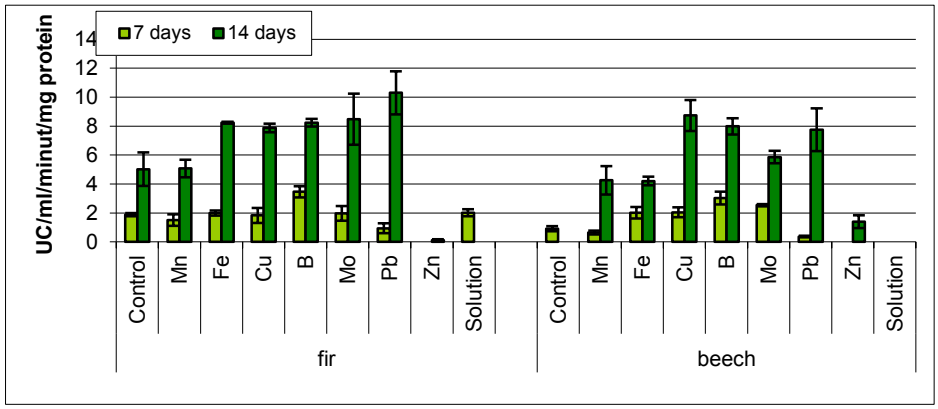

Fig 2 - Celobiohidrolase activity in fungus *Phanerochaete chrysosporium* grown on media with pine and beech sawdust under the influence of trace elements

Seven days after seeding the enzymatic activity was stimulated by the presence of boron in both media, while zinc and trace elments solution had an inhibitory action. At 14 days from seeding celobiohidrolase activity was stimulated in all the media containing trace elemens compared to control media, except for samples with zinc and trace elements that had an inhibitory influence.

Seven days after seeding, β-glucosidase activity (figure 3) was stimulated only in variants of pine sawdust medium under the influence of zinc compared to control medium and in medium containing solution of trace elements and copper the enzymatic activity was completely inhibited. At 14 days after seeding, enzymatic activity was stimulated in media with manganese, iron and trace solution, where they recorded the highest values compared with control variant and in the medium with zinc and copper the enzymatic activity was completely inhibited. On medium with beech sawdust under the influence of trace elements, in which variants with iron and zinc show no enzymatic activity, the highest value was registered in the medium variant with boron at 7 days and in the following period of determination.

We can notice a synergy between endoglucanase and celobiohidrolase activity, and just as stated by Simionescu et al. 1993, *Phanerochaete chrysosporium* is able to synthesise more endoglucanase and celobiohidrolase than celobiose.

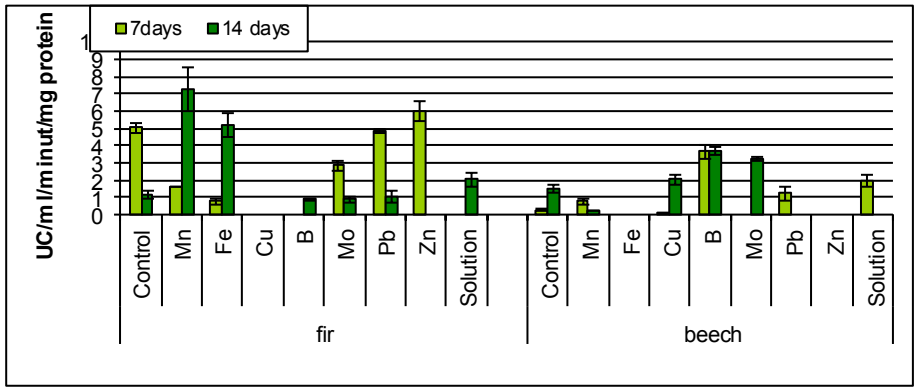

Fig 3 - Celobiose activity in fungus *Phanerochaete chrysosporium* grown on media with pine sawdust under the influence of trace elements

Lignin peroxidase of *Phanerochaete chrysosporium* is an enzyme that acts during secondary metabolism when food resources are depleted and when the culture liquid is influenced by stress factors.

In the medium with pine sawdust under the influence of trace elements (figure 4), lignin peroxidase activity is recorded only in variants with zinc, iron, lead, molybdenum and manganese, the rest of the variants show no enzymatic activity. Lignin peroxidase activity is recorded only in some of the media variants suggesting the presence of toxic factors in the case of variants with manganese,

zinc, lead, molybdenum, and in the variant with iron activity can be stimulated by the fact that iron enters the structure of the enzyme's active site.

Enzymatic activity was stimulated in all variants 14 days after seeding except variant medium with zinc, where the activity is low but in all variants under the influence of trace elements enzymatic activity increased compared to control variant.

On media with beech sawdust under the influence of trace elements seven days after seeding, enzymatic activity was present only in versions of medium containing iron, boron and molybdenum and was weak compared to the control. At 14 days after seeding enzymatic activity increased in all media variants with trace elements except zinc medium, trace elements solution and control version. Lignin peroxidase activity recorded the highest value in the media variant with boron 14 days after seeding.

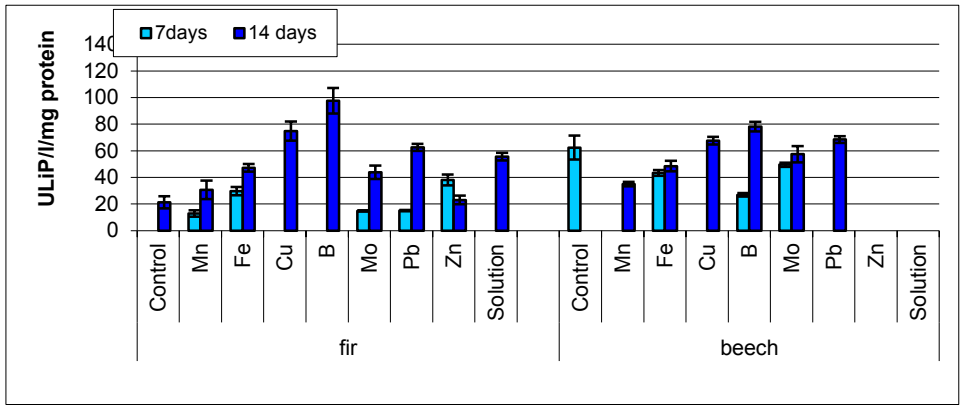

Fig. 4 - Lignin peroxidase activity in fungus *Phanerochaete chrysosporium* grown in media with pine sawdust under the influence of trace elements

Based on our research we can assume that the extracellular enzyme profile may vary depending on the state of degradation of the substrate and the various expressions of enzymes with different specificities may be the most effective way for an organism to biodegrade the substrate in order to obtain optimal nutrients necessary for growth and development.

CONCLUSIONS

1. In the presence of trace elements cellulase activity was stimulated after 14 days from seeding on both media types. Boron was the most stimulating nutrient, while zinc and trace elements solution had an inhibitory effect.

2. Celobiose activity was completely inhibited by the presence of copper in both time intervals on medium with pine sawdust and by the presence of iron and zinc on medium with beech sawdust.

3. In the presence of trace elements, lignin peroxidase activity was stimulated 14 days after seeding in both types of medium. Boron was the most stimulating nutrient.

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