STUDIES ON CATALASE AND PEROXIDASE ACTIVITY IN *PHANEROCHAETE CHRYSOSPORIUM* BURDS. CULTIVATED ON SPRUCE SAWDUST MEDIA

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Key words: ***Phanerochaete chrysosporium,*** catalase, peroxidase, spruce sawdust

Abstract: The aim of this study is to present the results regarding the influence of different spruce sawdust concentrations on catalase and peroxidase activity in ***Phanerochaete chrysosporium***. Determinations were made using mycelium and liquid culture after 7 days and 14 days from seeding. This study showed that the enzymes activity was influenced by spruce sawdust concentration introduced in medium culture and also it was influenced by the fungus age.

INTRODUCTION

Cellulose is the most abundant organic material in nature, always renewing by photosynthesis process and because of its crystalline structure is very resistant to chemical and biological degradation. Also, there are more than 200 species of microorganisms which biodegrades cellulose wastes. The Microbiology laboratory from Biological Research Institute Iasi carried out the research regarding the biology of the cellulolytic fungi for more than 15 years ago. The most important studies were those regarding Krebs cycle's dehydrogenases activity in the cellulolytic species ***Alternaria alternaria*** grown on media containing the deciduous and coniferous sawdust (Manoliu & al., 2002), the analysis of the proteins synthetised by cellulolytic fungi ***Chaetomium globosum*** and ***Alternaria alternaria*** grown on media containing beech and pine sawdust (Oprică & al., 2004), the evolution of cellulase complex in ***Alternaria alternaria*** grown on media containing forestry industry wastes - leafy and coniferous sawdust (Manoliu & al., 2005), the influence of electromagnetic field (EMF) on cellulase activity in cellulolytic fungi ***Trichoderma viridae*** and ***Chaetomium globosum*** grown on media containing leafy sawdust (Manoliu & al., 2007), the influence of magnetic and electromagnetic field on peroxidase activity in ***Chaetomium globosum*** and ***Trichoderma viridae*** grown on media containing leafy and coniferous sawdust (Manoliu & al., 2008).

Phanerochaete chrysosporium Burds. is capable to degrade the lignocellulose, lignin, cellulose and hemicellulose components and that is the reason why he is one of the most intense studied cellulolytic microorganisms (Broda & al., 2006).

In this study we present the influence of different concentration of spruce sawdust on catalase and peroxidase activity in ***Phanerochaete chrysosporium,*** in mycelium and culture liquid.

MATERIAL AND METHODS

The study was preformed using ***Phanerochaete chrysosporium*** (BCCM/IHEM, Culture Collection N^0 5772) from the collection of the Biological Research Institute of Iaşi. In order to investigate the influence of different concentrations of spruce sawdust on catalase and peroxidase activity, the fungus was grown on *Sabouraud* medium with the following composition: peptone 10 g / l, glucose 35g / l, distilled water 1000 ml (Constantinescu, 1974); the carbon source (glucose) was replaced by 3 different concentrations of spruce sawdust resulting 4 work variants: V1 - 20 g / l, V2 - 30 g / l and V3 - 40 g / l, V4 - control (the carbon source from the sample was not replaced). The incubation was made at 28^{0}C and the activity of catalase and peroxidase was determined at 7 and, respectively, 14 days from inoculation, using mycelium and liquid culture.

In order to determine the catalase activity it was used the spectrophotometric method (Artenie & al., 2008); for the peroxidase activity o-dianisidine method (Cojocaru, 2009); the enzymic activity was reported to soluble protein amount which was determined using Bradford method (Artenie & al. 2008).

RESULTS AND DISCUSSIONS

The results regarding the influence of different concentration of spruce sawdust on catalase and peroxidase activity are presented in figures 1-4. Figure 1 represents the catalase activity in fungus mycelium showing that after 7 days from inoculation only on 2 variants there were obtained values bigger than the control sample: V1 – 765,562 UC/mg/min. and V_3 -708,672 UC/mg/min., compared with V_4 – control – 680,054 UC/mg/min.; the smallest value for the catalase activity was obtained at V_2 - 663,450 UC/mg/min.

At 14 days after inoculation, the biggest value for the activity of catalase was determined at V4 - 921.269 UC / mg / min., followed in descending order by V2 - 885.83 UC / mg / min., V1 - 845.19 UC / mg / min., V3 - 807.153 UC / mg / min. Following the dynamics activity of this enzyme, it was noticed that at 14 days after inoculation the enzyme activity increased compared to the values recorded at 7 days after inoculation: at V1 - from 765.562 UC / mg / min. to 845.190 UC / mg / min., V2 - from 663.450 UC / mg / min. to 885.83 UC / mg / min., V3 - from 708.672 UC / mg / min. to 807.153 UC / mg / min., V4 - from 680.054 UC / mg / min. to 921.269 UC / mg / min.
The intensification of catalase activity of the fungus mycelium at 14 days from inoculation may suggest that there is a defense reaction of it against the spruce sawdust.

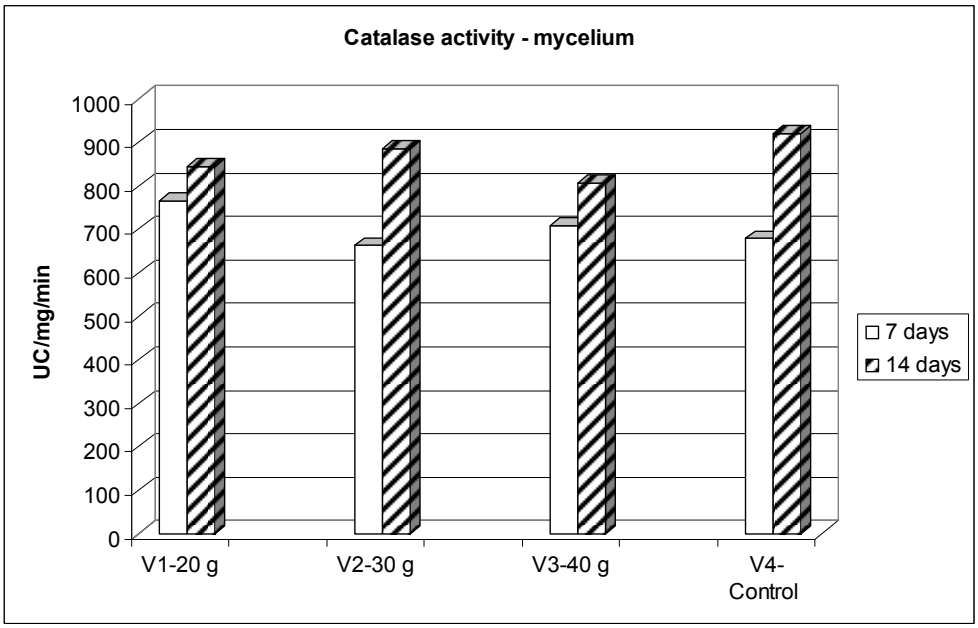

Fig. 1. The influence of different concentration of spruce sawdust on the activity of catalase in *Phanerochaete chrysosporium* - mycelium

The results concerning the influence of different concentrations of spruce sawdust on activity of catalase in liquid culture are presented in figure 2; it can be noticed that at 7 days after inoculation the highest activity of this enzyme was found in V1 - UC 109.79 / ml / min., followed by V3 - 87.89 UC / ml / min., V2 - 45.84 UC / ml / min; in the control (V4) the catalase activity was zero.

At 14 days after inoculation the catalase activity increased significantly but it maintained the same value difference between the variants; the highest activity of this enzyme was noticed at V1 - 516.366 UC / ml / min., followed by V3 - 352.950 UC / ml / min., V2 - 313.950 UC / ml / min., V4 - 0.

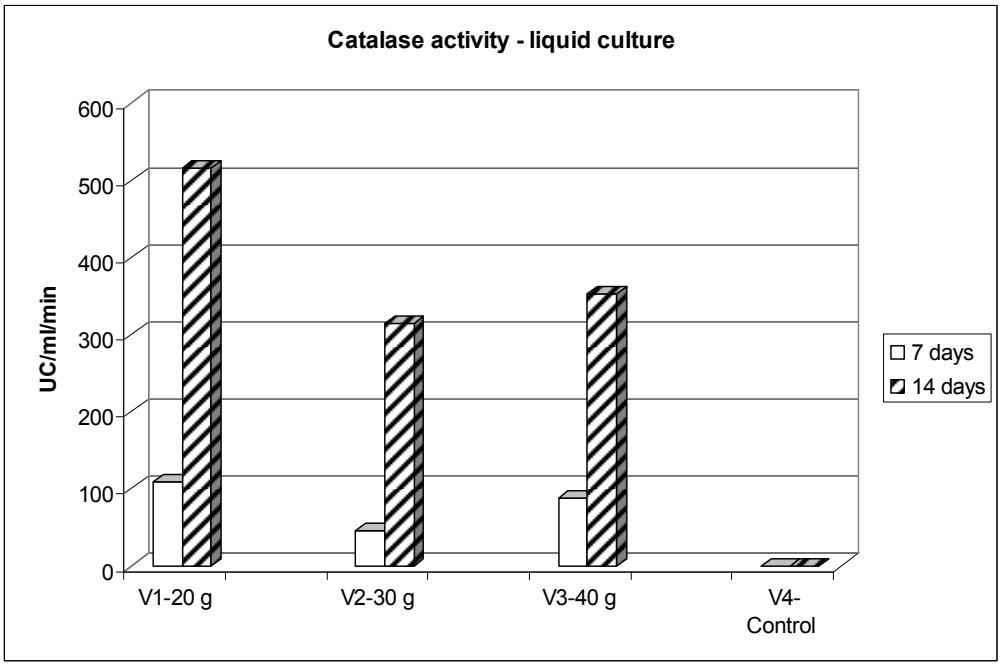

Fig. 2. The influence of different concentration of spruce sawdust on the activity of catalase in *Phanerochaete chrysosporium* – liquid culture

The figure 3 presents the results regarding the influence of different concentrations of spruce sawdust on the peroxidase activity in mycelium, from which results that at 7 days after inoculation the lowest activity of this enzyme was at V2 - 9.5734 x 10^{-3} UP/ mg / min., followed in ascending order by: V1 - 17.64 x 10^{-3} UP / mg / min., V3 - 22.15 x 10^{-3} UP / mg / min., V4 - 31.538 x 10^{-3} UP / mg / min.

At 14 days after inoculation the peroxidase activity in mycelium had the following values: V4 - 26.49 x 10^{-3} UP / mg / min., V3 - 4.0 x 10^{-3} UP / mg / min., V1-3, 36 x 10^{-3} UP / mg / min. and V2 - 0. Following the dynamics of the activity of the catalase it was noticed a decrease of the activity of the peroxidase at 14 days after inoculation, compared with the values obtained at 7 days after inoculation: V1 - from 17.64 x 10^{-3} UP / mg / min. to 36 x 10^{-3} UP / mg / min., V2 – from 9.5734 x 10^{-3} UP / mg / min. to 0, V3 - 22.15 x 10^{-3} UP / mg / min to 4, 0 x 10^{-3} UP / mg / min, V4 – from 31.538 x 10^{-3} UP / mg / min. to 26.49 x 10^{-3} UP / mg / min.

Knowing that 14 days after inoculation there was a progressive increase of catalase activity and a decrease in peroxidase activity, we can concluded that at this time the amount of hydrogen peroxide may be higher and it should be removed by catalase; it is known the fact that in living organisms peroxidase operates on a small quantitie of hydrogen peroxide and catalase removes the excess of peroxide.

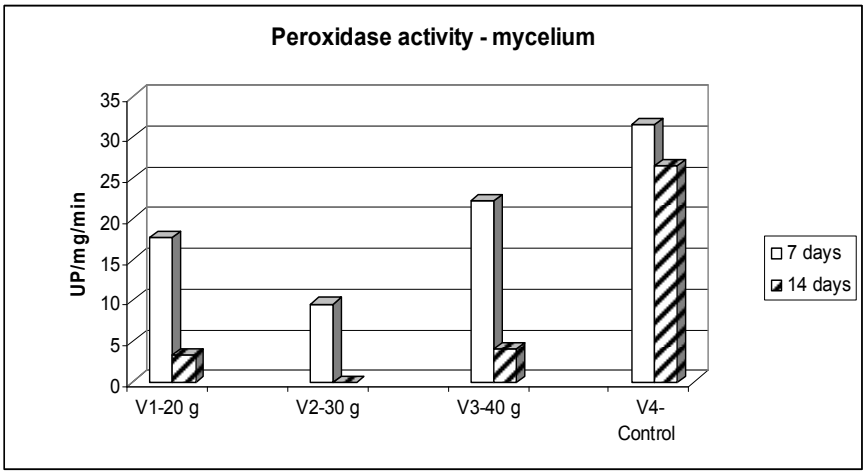

Fig. 3. The influence of different concentration of spruce sawdust on the activity of peroxidase in *Phanerochaete chrysosporium* – mycelium

The data about the influence of spruce sawdust on peroxidase activity in liquid culture are presented in figure 4; we noticed that at 7 days after inoculation, the activity of this enzyme had the following values: V2 - 0.382 UP / ml / min., V1 - 0.322 UP / ml / min., V3 - 0.067 UP / ml / min., V4 - 0.

At 14 days after inoculation the value of peroxidase activity was zero in all variants which contained spruce sawdust in different concentrations; in V4 the enzyme activity was 0.0274 UP / ml / min.

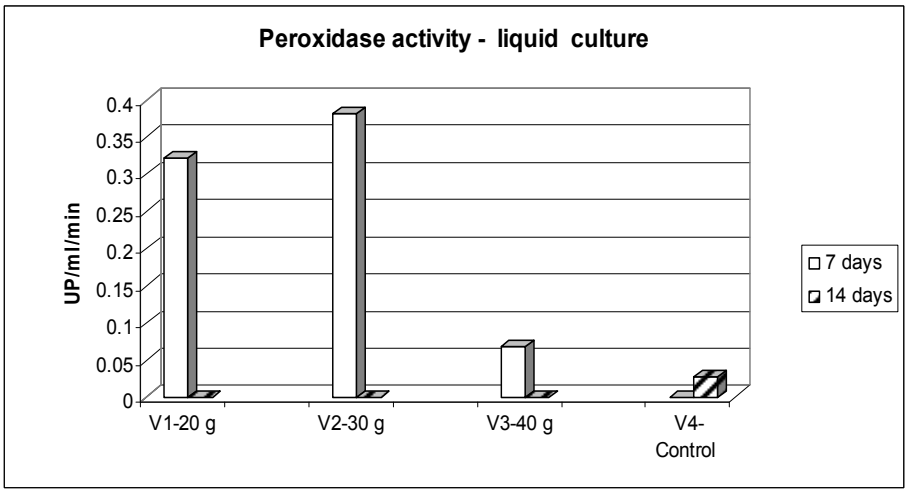

Fig. 4. The influence of different concentration of spruce sawdust on the activity of peroxidase in *Phanerochaete chrysosporium* – liquid culture

CONCLUSIONS

The activity of the catalase in mycelium of ***Phanerochaete chrysosporium*** was stimulated 7 days after inoculation at the variants containing 20 g of spruce sawdust/1.000 ml and

40 g of spruce sawdust /1.000 ml; after 14 days from inoculation the activity of this enzyme was not influenced by any different concentrations of spruce sawdust. The catalase activity in liquid culture was stimulated in all the variants (containing different concentrations of spruce sawdust) both at 7 and 14 days after inoculation.

The activity of the peroxidase in mycelium of ***Phanerochaete chrysosporium*** was inhibited by the presence in the culture medium of various concentrations of spruce sawdust in both intervals of time taken into study; in liquid culture it was stimulated in all variants in different concentrations of sawdust spruce but only after 7 days from inoculation.

REFERENCES

Artenie V., Ungureanu E., Negura Anca Mihaela, 2008. Metode de investigare a metabolismului glucidic si lipidic. Editura PIM, Iaşi, p. 182.

Broda, P., P. R. J. Birch, P. R. Brooks, and P. F. G. Sims, 1996. Lignocellulose degradation by *Phanerochaete chrysosporium*: gene families and gene expression for a complex process. Molecular. Microbiology. 19 : 923 - 932.

Burdshall H H ,1985. A contribution to the taxonomy of the genus Phanerochaete. Mycologia Memoir 10: 61 - 63.

Cojocaru, D. C., 2009. Enzimologie practica. Editura Tehnopress, Iaşi, p. 458.

Constantinescu, O., 1974. Metode şi tehnici în micologie, Editura Ceres, Bucureşti, p. 150.

Manoliu Al., Oprică Lăcrămioara, Olteanu Zenovia, 2002. *Studiul activităţii dehidrogenazelor ciclului Krebs la specia celulozolitică Alternaria alternata în condiţiile creşterii pe medii cu rumeguşuri de foioase şi conifere*, Lucrări Ştiinţifice, Univ. Agronomică şi Mededicină Veterinară “Ion Ionescu de la Brad”, Seria Horticultură, Iaşi, 1 : 687 - 692.

Manoliu Al., Creţu V., Olteanu Zenovia, Oprică Lăcrămioara, Ungureanu E., 2005. *Evoluţia complexului celulazic la specia Alternaria alternată cultivată pe medii conţinând deşeuri din industria forestieră (rumeguşuri de conifere şi foioase)*, Lucrările celui de-al X-lea Simpozion de Microbiologie şi Biotehnologie, Iaşi 15 – 16. X. 2004 – Proceedings of the X – th Symposion of the Microbiology and Biotechnology, Ed. Corson Iaşi, p. 403 – 406.

Manoliu Al., Olteanu Zenovia, Oprică Lăcrămioara, Moisă Mihaela Lăcrămioara, 2007. *The influence of the electomagnetic field (EMF) on cellulases activity in the cellulolytic fungi Trichoderma viride and Chaetomium globosum cultivated on media with deciduous sawdust* – European Society for new Methods in Agricultural Research (ESNA), Proceedings of the XXXVI Annual Meeting, Iaşi, Romania, 10 – 14. 09. 2006, p. 967 – 974.

Manoliu Al., Oprică Lăcrămioara, Olteanu Zenovia, 2008. *Influenţa câmpului magnetic şi electromagnetic asupra activităţii peroxidazei la speciile celulozolitice Chaetomium globosum şi Trichoderma viride cultivate pe medii conţinând rumeguşuri de foioase şi conifere, “Lignicolous fungi: diversity in forest ecosystem and horti-viticultural agroecosystems. Prevention and limitation of wood rots caused by lignicolous fungi”*, Sănătatea Plantelor, 117 : 58 – 63.

Oprică Lăcrămioara, Manoliu Al., Humă Anca, Ungureanu Eugen, 2004. *Analyse des protéines solubles synthetisent par des champignons cellulolytiques Chaetomium globosum at Alternaria alternata cultivént dans les mileux avec des sciures de hêtre et de pin* - Analele Ştiinţifice ale Univ. “Al. I. Cuza” Iaşi, Genetică şi Biologie moleculară, V : 16 – 20.

Sun-Il Kwon, Anne J. Anderson, 2001. Catalase activities of Phanerochaete chrysosporium are not coordinately produced with ligninolytic metabolism: catalase from a white-rot fungus. Current Microbiology 42: 8-11aboratory Press, p. 95 – 115.

Volc J., Kubatova E., Daniel G., Prikrylova V., 1996. Only C-2 specific glucose oxidase activity is expressed in ligninolytic cultures of the white rot fungus ***Phanerochaete chrysosporium***. Archives of Microbiology 165 : 421 – 424.

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