COMPARATIVE STUDIES ON THE ACTIVITY OF CATALASE IN WHITE ROT FUNGUS *PHANEROCHAETE CHRYSOSPORIUM* GROWN ON MEDIA CONTAINING CONIFEROUS AND DECIDUOUS SAWDUST

STUDII COMPARATIVE ASUPRA ACTIVITĂŢII CATALAZEI LA CIUPERCA LIGNOCELULOZOLITICĂ *PHANEROCHAETE CHRYSOSPORIUM* CULTIVATĂ PE MEDII CONŢINÂND RUMEGUŞURI DE CONIFERE ŞI FOIOASE

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Abstract *In the present study we presented the comparative studies in catalase activity in the white rot fungus Phanerochaete chrysosporium grown on media containing various amounts of sawdust: spruce, beech, fir and poplar. In order to conduct these studies was replaced glucose from Sabouraud medium with different amounts of sawdust of 4 species of trees resulting in the final 3 work variants for each species: V1-20 g /l, V2-30 g /l; V3-40 g /l. Catalase activity was determined at 7 and 14 days from inoculation using fungus mycelium and culture fluid. Analyzing the data obtained revealed that this enzyme activity was influenced by type of sawdust, amount of sawdust and the fungus age.*

Key words: *Phanerochaete chrysosporium*, catalase, sawdust, coniferous, decidous

Rezumat *În lucrarea de faţă se prezintă studiul comparativ asupra activităţii catalazei la ciuperca Phanerochaete chrysosporium cultivată pe medii conţinând cantităţi variate de rumeguş de molid, fag, brad şi plop. În vederea efectuării acestor studii s-a înlocuit glucoza din mediul Sabouraud cu diferite cantităţi de rumeguş de la 4 specii de arbori (molid, fag, brad şi plop) rezultând în final 3 variante de lucru pentru fiecare specie: V1-20 g rumeguş /l; V2 -30 g rumeguş /l; V3-40 g rumeguş /l. Activitatea catalazei s-a determinat la 7 şi 14 zile de la însămânţare din miceliul ciupercii şi din lichidul de cultură. Din analiza datelor obţinute s-a evidenţiat faptul că activitatea acestei enzime a fost influenţată de tipul de rumeguş, cantitatea de rumeguş şi de vârsta ciupercii.*

Cuvinte cheie: *Phanerochaete chrysosporium*, catalază, rumeguş, conifere, foioase.

INTRODUCTION

Phanerochaete chrysosporium is a fungus that degrades woody cell wall components including lignin and uses hydrogen peroxide as a substrate in its ligninolytic phase (Sun-Il Kwon and Anne J. Anderson, 2001).

In the Microbiology Laboratory from Biological Research Institute Iaşi the biology of cellulolytic fungi represented the center of research for more than 15 years. The most important studies were those regarding Krebs cycle's dehydrogenases activity in cellulolytic species *Alternaria alternata* grown on media containing deciduous and coniferous sawdust (Manoliu et al., 2002), the evolution of cellulase complex in *Alternaria alternata* grown on media containing forestry industry wastes - deciduous and coniferous sawdust (Manoliu et al., 2005), the influence of magnetic and electromagnetic field on peroxidase activity in *Chaetomium globosum* and *Trichoderma viride* grown on media containing deciduous and coniferous sawdust (Manoliu et al., 2008), the influence of different amount of spruce sawdust on catalase and peroxidase activity in species *Phanerochaete chrysosporium* (Manoliu et al., 2009).

Catalase (H_2O_2: H_2O_2 – oxidoreductase, EC 1.11.1.6) is an antioxidant enzyme, grown in aerobic organisms. Along with peroxidase and glutathione peroxidase, catalase is involved in detoxification of hydrogen peroxide, a reactive oxygen species (ROS), produced by fungi during metabolic activity. Fungi increase their production due to stress factors as lack of food, light, mechanical degradation, and interaction with other organisms. Setting levels of reactive oxygen species is very important during development fungus (Gessler et al., 2007).

MATERIAL AND METHOD

The study was performing on lignolytic fungus *Phanerochaete chrysosporium* (HEM no. 5772) acquared by the Biological Research Institute Iasi from the Institute of Santé Publique Scientifique, Belgium. In order to determine catalase activity, the fungus was grown on Sabouraud medium with following composition: peptone – 10 g, glucose – 35 g, distillated water - 1000 ml (Constantinescu, 1974), in which the carbon source (glucose) was replaced with different amounts of sawdust from 4 trees species and the final result 3 variants for each species: V1-20 g sawdust /l; V2-30 g sawdust /l; V3-40 g sawdust /l. Incubation took place at 28^{o}C and determination of catalase activity was realised at 7 days and 14 days after sowing in fungus mycelium and culture liquid by spectrophotometric method Sinha. Enzyme activity was reported to the amount of soluble protein determinated by Bradford method (Artenie et al., 2008).

RESULTS AND DISCUSSIONS

The results regarding the influence of various concentrations of coniferous and deciduous sawdust on catalase activity are presented in figures 1-6. Thus, figure 1 present the comparative results of catalase activity in mycelium of *Phanerochaete chrysosporium* in variant V1 containing 20g sawdust/l, from which observed that at 7 days after sowing, the increased activity was recorded on medium containing spruce sawdust (139.287 ± 4.872 UC/g/mg protein), and the lowest on medium containing beech sawdust (47.498 ± 6.724 UC/g/mg protein). At 14 days after sowing the highest activity was detected in the variant with the fir sawdust (243.584± 32.506 UC/g/mg protein), while in the variant with the beech sawdust was present the lowest value (128.547 ± 4.408 UC/g/mg protein).

Following the dynamics activity of catalase, we established that enzyme actvity increased to 14 days after sowing compared with values recorded in 7 days from sowing in all four species of trees, the most significant differences between the two periods of growth occured in the variants with fir and beech sawdust.

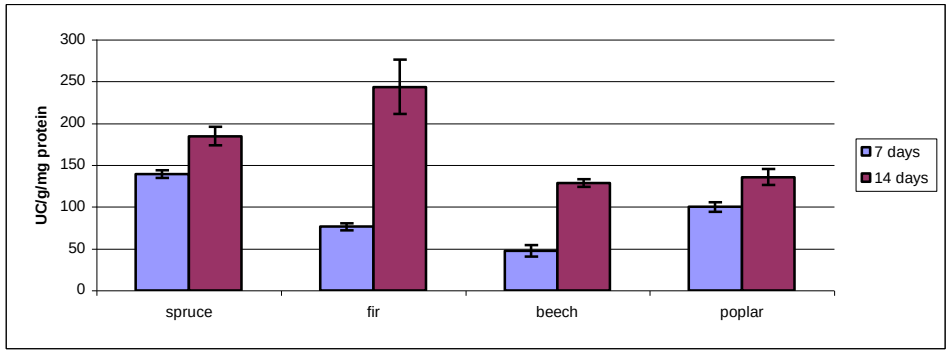

Fig. 1. Catalase activity in *Phanerochaete chrysosporium* - V1-20 g sawdust/l (mycelium)

The data regarding catalase activity in fungus mycelium at variant V2 with 30 g sawdust/l are shown in figure 2, in which it appears that most of enzyme activity at 7 days after sowing was determined on medium containing spruce sawdust (145.034 ± 12.099 UC/g/mg protein) and the lowest activity was recorded in the variant with the beech sawdust (42.936 ± 2.151 UC/g/mg protein). At 14 days after sowing, catalase activity was stimulated in the variant containing fir sawdust (192.796 ± 13.535 UC/g/mg protein), while in the variant with the beech sawdust was highlighted lowest enzyme activity (114.632 ± 15.643 UC/g/mg protein).

Comparing the dynamics of catalase activity in connection with the type of sawdust used as substrate and fungus age, it is noted that there was an intensification of this enzyme in all four trees species in second period of growth especially at medium containing fir and beech.

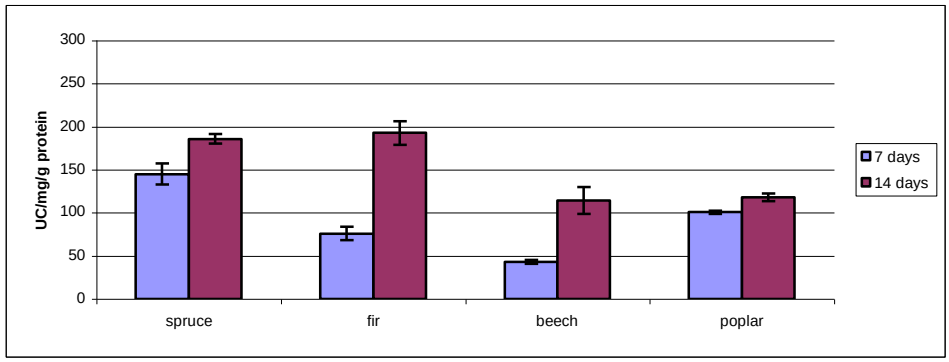

Fig. 2. Catalase activity in *Phanerochaete chrysosporium* – V2-30 g sawdust/l (mycelium)

Maximum values of catalase activity in the fungus mycelium at variant V3 with 30 g sawdust/l (figure 3) were recorded on culture medium containing spruce sawdust at 7 and 14 days after sowing (156.184 ± 12.435 UC/g/mg protein respectively 176.570 ± 8.048 UC/g/mg protein). The lowest values in those two periods of growth were observed in variant with beech sawdust (46.24085 ± 0.900371 UC/g/mg respectively 97.57118 ± 7.026108 UC/g/mg protein).

The dynamics of catalase activity in connection with the culture age showed that at 14 days after sowing compared with results obtained at 7 days increased the activity of this enzyme in all four sawdust types used as substrate.

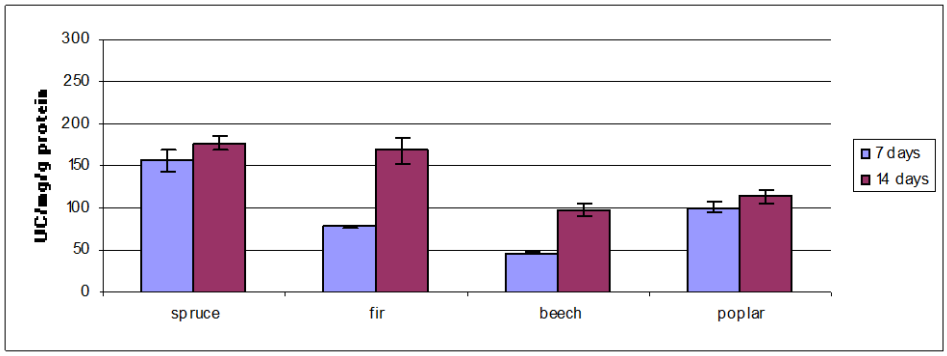

Fig. 3. Catalase activity in *Phanerochaete chrysosporium* – V3-40 g sawdust/l (mycelium)

The catalase activity in liquid culture at the variant V1 is presented in figure 4, wich shows that at 7 days after sowing, the increased value was determined on medium containing poplar sawdust (69.807 ± 5.533 UC/ml/mg protein) and the lowest value occured in variant with beech sawdust (20.650 ± 3.205 UC/ml/mg protein). At 14 days after sowing the maximum activity was detected in the variant with spruce sawdust (113.067 ± 17.702 UC/ml/mg protein), also, and the minimum value is in the variant with poplar sawdust (44.097 ± 14.768 UC/ml/mg protein). At this time it might show that there has been a stimulating catalase activity only at three types of sawdust (spruce, fir, beech), while the poplar has been decreased in activity of this enzyme.

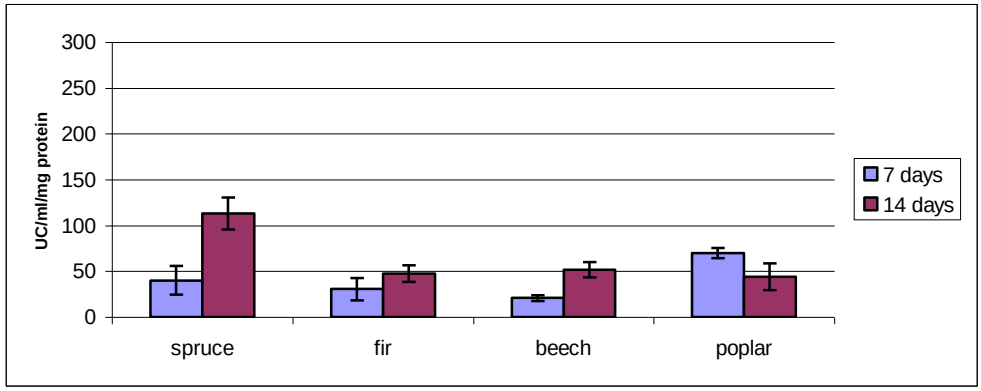

Fig. 4 Catalase activity in *Phanerochaete chrysosporium* –- V1-20 g sawdust/l (liquid culture)

In the variant V2 with 3 g sawdust/l (figure 5), the catalase activity was higher in the poplar sawdust (97.036 ± 30.421 UC/ml/mg protein) and the lowest activity was observed in the spruce sawdust (14.934 ± 1.549 UC/ml/mg protein) at 7 days after sowing. In the next period (14 days after sowing), the highest value was recorded in the variant with fir sawdust (99.590± 5.315 UC/ml/mg protein) and the lowest in the variant with poplar sawdust (61.857 ± 4.404 UC/ml/mg protein).

Folowing the dynamics of this enzyme activity depending on culture age can show that at 14 days after sowing was registred an increased activity in the variants with spruce, fir and beech sawdust, while the variant with poplar sawdust was observed weaking in.

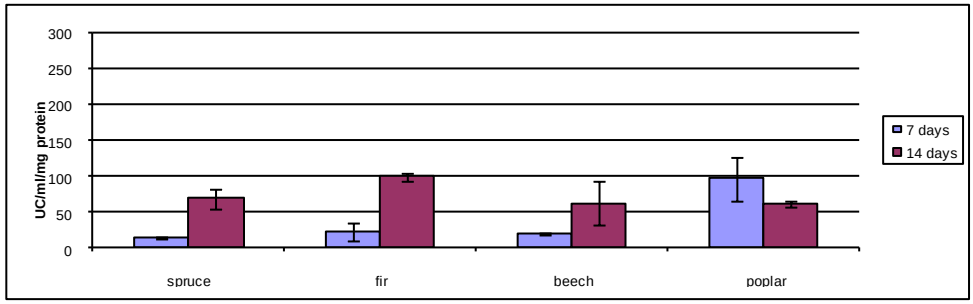

Fig. 5. Catalase activity in *Phanerochaete chrysosporium* – V2-30 g sawdust/l (liquid culture)

The results of catalase activity in liquid culture medium in variant V3 (40 g sawdust/l) are shown in figure 6, from which established that at 7 days from sowing, the increased enzyme activity was recorded in the variant with poplar sawdust (103.020 ± 8.241 UC/ml/mg protein) and the lowest in the variant with beech sawdust (17.891 ± 3.688 UC/ml/mg protein). At 14 days the most intense activity was observed in the variant with beech sawdust (105.056 ± 15.137 UC/ml/mg protein) and the lowest activity in the variant with pine sawdust (38.707 ± 12.835 UC/ml/mg protein).

The dinamics of the catalase activity in culture liquid at 14 days after sowing compared with age of 7 days , shows an increase in enzyme activity only in media containing spruce and beech sawdust, comparatively with the variants with pine and poplar sawdust which decreased.

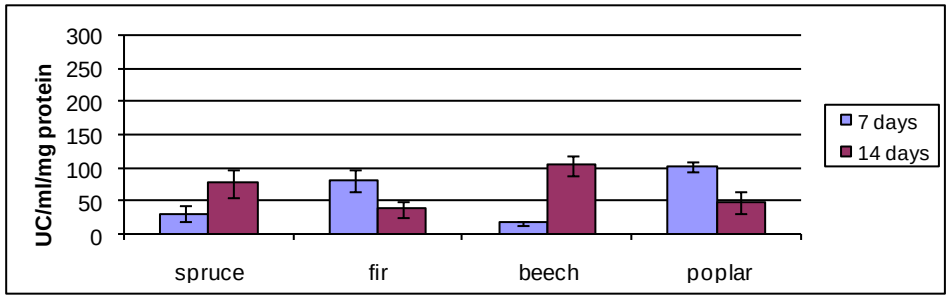

Fig. 6. Catalase activity in *Phanerochaete chrysosporium* V3 - 40 g sawdust/l (liquid culture)

CONCLUSIONS

1. In the fungus mycelium, the catalase activity, in all variants has been stimulated to 7 days from sowing in the samples with spruce and poplar sawdust compared with those containing fir and beech sawdust. At 14 days after sowing the activity of catalase was stimulated at all sawdust types compared with values determined at 7 days, the most significant values was recorded in the variants with coniferous sawdust.

2. In the liquid culture, at 7 days after sowing, the catalase activity was stimulated in all variants which contained poplar sawdust and at 14 days after sowing the catalase activity was stimulated in the variant V1 containing spruce sawdust , variant V2 containing fir sawdust and the variant V3 with the beech sawdust.

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