THE EVOLUTION OF CATALASE AND PEROXIDASE ACTIVITY IN *PHANEROCHAETE CHRYSOSPORIUM* GROWN ON MEDIA CONTAINING BEECH AND FIR SAWDUST AND UNDER THE INFLUENCE OF SOME AMINO ACIDS

ALEXANDRU MANOLIU[*1], MIHAELA BĂLAN[2], LĂCRĂMIOARA OPRICĂ[2], PETRONELA GRADINARIU[1]

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Abstract. The purpose of this paper is to highlight the influence of amino acids (histidine, glutamic acid, serine, valine, methionine, asparagine, α-alanine) on catalase and peroxidase activity in *Phanerochaete chrysosporium* grown on media containing fir and beech sawdust. In view of this research, Sabouraud medium carbon source was replaced by pine and beech sawdust 4 g per 100 ml medium and nitrogen source in the corresponding amino acid with different amounts of 100 mg N / L, resulting in the final eight work variants for each type of sawdust namely V1- valine, V2- histidine, V3- asparagine, V4- methionine, V5-glutamic acid, V6-α-alanine, V7- serine and V8 control who did not introduce any source nitrogen. Determination of catalase activity was done by spectrophotometric Sinha method and the peroxidase with ortho-dianisidine method at 11 days and 18 days after seeding, in fungus mycelium and culture liquid. Following the study found that the activity of these two enzymes was influenced by the type of amino acid, the nature of sawdust and fungus age.

INTRODUCTION

Phanerochaete chrysosporium is a fungus that degrades woody cell wall components including lignin (Gold & Alic, 1993). Lignin depolymerization is in part achieved by a free-radical mechanism based upon radicals generated by extra cellular peroxidase complexes in the presence of external hydrogen peroxide (H2O2) produced by the fungus (Kwon & Anderson, 2001).

The H_2O_2 used as substrate by these peroxidases is produced by several extracellular enzymes (Volc et al., 1996). Hydrogen peroxide could permeate the fungal membrane, and in association with redox-active metal ions damage cellular structures, including DNA (Gold & Alic, 1993). Catalase (EC 1.11.1.6 hydrogen peroxide oxidoreductase) may help protect this fungus against H_2O_2. Often, external sources of H_2O_2 increase catalase activities in microbial organisms (Loewen, 1992).

Green and Gould (1983) working with intact mycelium of *Phanerochaete chrysosporium,* found five to ten times more catalase activity when mycelium was grown on LN than high-nitrogen (HN) medium.

Dynamic activity of catalase and peroxidase in mycelium and liquid culture of cellulolytic fungi was studied in Romania under the influence of magnetic field (Manoliu et al. 2005a), liquid ferric (Manoliu et al, 2005b), bakery waste industry (Manoliu et al., 2006) and on culture media containing spruce sawdust (Manoliu et al. 2009).

Belinky et al., (2003) sustained that the fungus *Phanerochaete chrysosporium* cells produce a high concentration of reactive oxygen species when stimulated by pure oxygen, which appears to be involved in modulating the expression of lignin peroxidase. Fungal resistance to high concentrations of peroxide has been explained by Dosoretz et. al. (1990) and is due to higher activity of catalase and glutathione peroxidase.

In the present paper in presented the evolution of catalase and peroxidase activity in *Phanerochaete chrysosporium* grown on media containing fir and beech sawdust under the influence of some amino acids.

MATERIAL AND METHODS

Organism: Fungus *Phanerochaete chrysosporium* was acquired from the Institute Sciéntifique de Santé Publique, Belgium (HEM no.5772) by Biological Science Research Institute.

Culture conditions: Fungus *Phanerochaete chrysosporium* was grown on Sabouraud medium in which carbon source was replaced by fir and beech sawdust - 4 per 100 ml of medium and nitrogen source (peptone) was replaced with amino acids (glutamic acid, valine, serine, histidine, methionine, α-alanine, asparagine) equivalent to 100 mg of nitrogen per liter for each amino acid, ultimately resulting in eight versions of each and every type of sawdust: V1- valine, V2- histidine, V3- asparagine, V4- methionine, V5- glutamic acid, V6 - α-alanine, V7 – serine V8 – control which not present any nitrogen source. Enzyme determinations were carried out for 11 days and 18 days after seeding in fungus mycelium and culture fluid.

Determination of enzyme activity: Catalase activity was determinate by spectrophotometric Sinha method, and for peroxidase activity was used ortho-dianisidine method (Cojocaru, 2009).

Reporting enzyme activity was the amount of soluble protein determined by Bradford method (Artenie., et al. 2008).

RESULTS AND DISCUSSIONS

The results of catalase activity from the species *Phanerochaete chrysosporium* grown on media containing various amino acids as nitrogen source, and fir and beech sawdust as carbon source, were presented in Figures 1 and 2. In the fungus mycelium, 11 days after sowing, catalase activity was much higher in the medium variant with amino acid compared with control variant at both alternative media with fir sawdust and those with beech sawdust (Figure 1) but different in each type of amino acid, the lowest revealing at the α-alanine variant (167 136 ± 18 239 UC / g / mg protein) grown on medium containing fir sawdust and valine variant on media with beech sawdust (211.500 ± 1.908 UC / g / mg protein).

At 18 days from seeding, in media containing fir sawdust, catalase activity was increased only by variants containing serine (412.227 ± 14.3303 UC / g / mg protein) and histidine (371.483 ± 9.301 UC / g / mg protein) compared with control (312.716 ± 18.819 UC / g / mg protein), while in the medium variant with beech sawdust, enzymatic activity was highly stimulated in all work variants, the highest value was observed at histidine variant (753.225 ± 4.669 UC / g / mg protein) followed by methionine variant (697.715 ± 9.852 UC / g / mg protein), asparagine (635.090 ± 7.694 UC / g / mg protein) and control (626.746 ± 9.236 UC / g / mg protein).

The amplification of catalase activity on the second time in media with beech sawdust compared to those with fir sawdust may suggest an increase of hydrogen peroxide in fungus mycelium, as a defense response to oxidative stress.

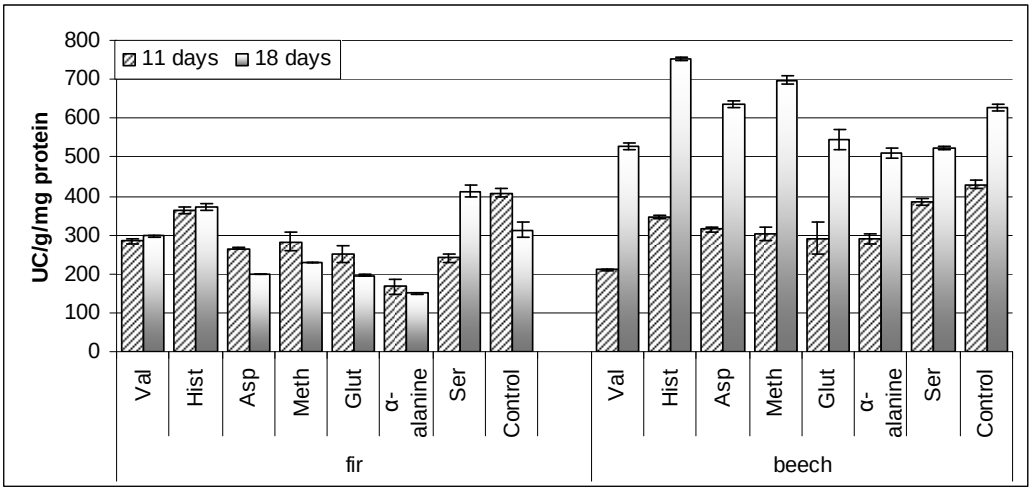

Fig. 1 Catalase activity in fungus *Phanerochaete chrysosporium* cultivated on media containing fir sawdust under the influence of some amino acids - mycelium

In culture liquid, the variants with fir sawdust (Figure 2), catalase activity, at 11 days from seeding, was much increased compared to control (16.074 ± 6.878 UC / ml / mg protein) to variante containing: glutamic acid (37.700 ± 9.846 UC / ml / mg protein), histidine (44.443 ± 10.95 UC / ml / mg protein), asparagine (37 404 ± 12 762 UC / ml / mg protein), α-alanine (40.759 ± 6.309 UC/ml/mg protein) și serin (33.974 ± 3.933 UC/ml/mg protein). The only variants that showed lower values compared with control are: methionine (1.300 ± 0.216 UC / ml / mg protein) and valine (9.863 ± 1.193 UC / ml / mg protein).

Catalase activity, in media containing beech sawdust, 11 days after sowing, was stimulated by the medium variant with glutamic acid (128.693 ± 4.737 UC / ml / mg protein), followed in decreasing order by valine variant (65,599 ± 3762 UC / ml / mg protein), serine (64.816 ± 2.214 UC / ml / mg protein), asparagine (59.749 ± 5.312 UC / ml / mg protein) metionine (50.453 ± 1.387 UC/ml/mg protein) and α-alanine (47.061 ± 1.630 UC/ml/mg protein). Catalase activity was completely inhibited at the variant with histidine and control variant.

At 18 days after seeding, in liquid culture containing fir sawdust, catalase activity decreased at almost all work variants compared to first time, except variant with methionine, valine and control. Instead, in the media containing beech sawdust, the enzymatic activity decreased in most of all variants, compared with first period, while in variant containing α-alanine and control variant was completely inhibited. Only variant containing serine presented an increased activity of catalase compared with 11 days from seeding (92.067 ± 4.605 UC / ml / mg protein).

From data on catalase activity in fungus *Phanerochaete chrysosporium* grown on media containing fir and beech sawdust and under the influence of amino acids, in both the fungus mycelium and culture liquid, can be observed that enzymatic activity had different values, with an increase activity in medium containing beech sawdust compared with medium containing fir sawdust, which may suggest that the media containing beech sawdust in the presence of amino acid biosynthesis can increase hydrogen peroxide and thus fungus response to oxidative stress.

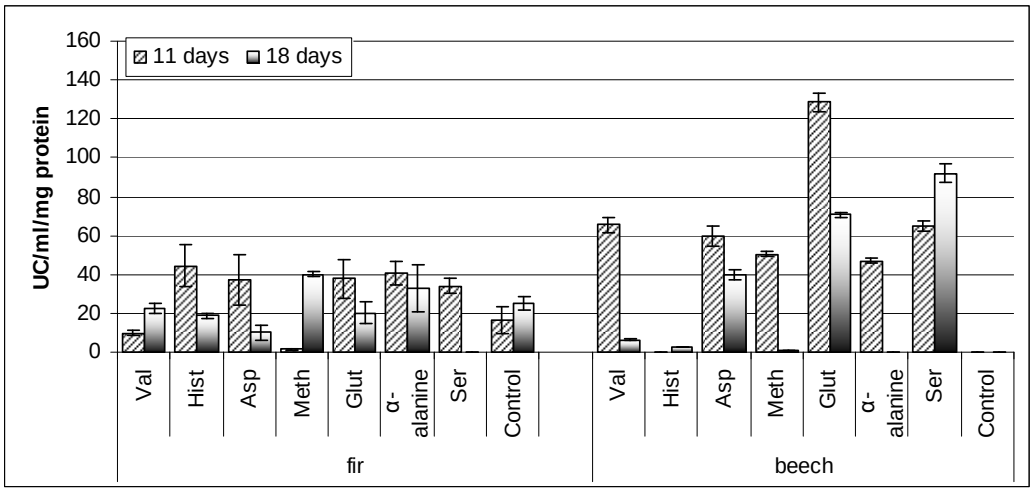

Fig. 2 Catalase activity in fungus *Phanerochaete chrysosporium* cultivated on media containing fir and beech sawdust under the influence of some amino acids – culture liquid

Peroxidase activity in *Phanerochaete chrysosporium* fungus mycelium grown on media containing fir sawdust, (Figure 3), presented variations both in the type of amino acid used as nitrogen source and culture age. Thus, 11 days after sowing, all variants of medium containing sources of nitrogen, showed a much lower activity of enzyme activity compared to the values recorded at control (UP 0.00648 ± 0.0024 g / mg protein), the lowest value observed in the variants containing α-alanine (0.0008 ± 0.000009 UP / g / mg protein) and the histidine to the peroxidase activity was 0 so the first and at the second period of development.

The fungus mycelium *Phanerocahete chrysosporium* grown on media containing beech sawdust, presented a very low peroxidase activity at 11 days from sowing, being present only in the medium variant with asparagine (0.0036 ± 0.0012 UP / g / mg protein) and valine (UP 0.0003 ± 0.00137 g / mg protein), other work variants have been completely inhibited.

At 18 days after sowing, in the media containing fir sawdust, the enzymatic activity is increasing compared with first period at variant with asparagine (UP 0.0065 ± 0.001 g / mg protein), methionine (0.0044 ± 0.0006 UP / g / mg protein), α-alanine (0.0038 ± 0.0009 UP / g / mg protein) and serine (0.00768 ± 0.00096 UP / g / mg protein). On the other variants showed enzymatic activity value of 0.

On medium containing beech sawdust, enzymatic activity is enhanced in control variant (0.096 ± 0.009 UP / g / mg protein), followed by asparagine (0.0178 ± 0.0005 UP / g / mg protein), valine (0.0175 ± 0.00130 UP / g / mg protein), glutamic acid (0.0132 ± 0.0039 UP / g / mg protein), the other variants there was a lack of activity of this enzyme.

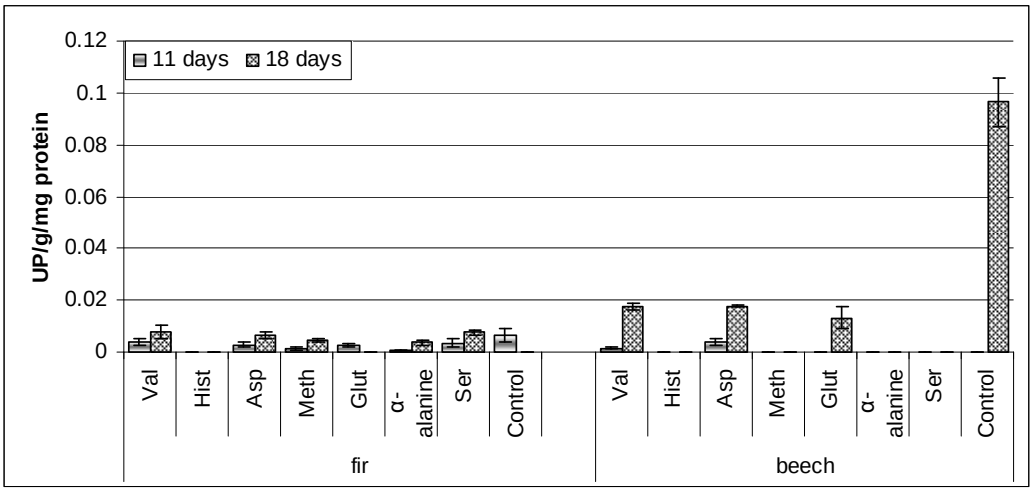

Fig. 3 Peroxidase activity in fungus *Phanerochaete chrysosporium* cultivated on media containing fir and beech sawdust under the influence of some amino acids - mycelium

The data concerning the peroxidase activity, in liquid culture in ligninocellulytic fungus *Phanerochaete chrysosporium*, on medium containing fir sawdust (Figure 4), showed an high values at control variant (0.0061 ± 0.0052 UP / ml / mg protein) followed in decreasing order by variants containing glutamic acid (0.0079 ± 0.001043 UP / ml / mg protein), histidine (0.0087 ±

0.0023 UP / ml / mg protein) and methionine (0.0072 ± 0.0003 UP / ml / mg protein), the other variants were showed lower values and at the valine variant the enzymatic activity was completely inhibited.

At 18 days after seeding peroxidase activity has increased over the first period on all environmental variations, the highest value is observed in α-alanine (0.0206 ± 0.0032 UP / ml / mg protein), followed by histidine (0.0195 ± 0.0043 UP / ml / mg protein) and control (0.015 ± 0.0031 UP / ml / mg protein).

In liquid culture medium containing sawdust beech, the enzymatic activity was very weak at 11 days from seeding to control variant (0.0302 ± 0.0068 UP / ml / mg protein), metionine (0.0098 ± 0.0049 UP / ml / mg protein), asparagine (0.00056 ± 0.0001 UP / ml / mg protein) and serine (0.00050 ± 0.000101 UP / ml / mg protein). In the second period, peroxidase activity was completely inhibited at all work variants taken in study.

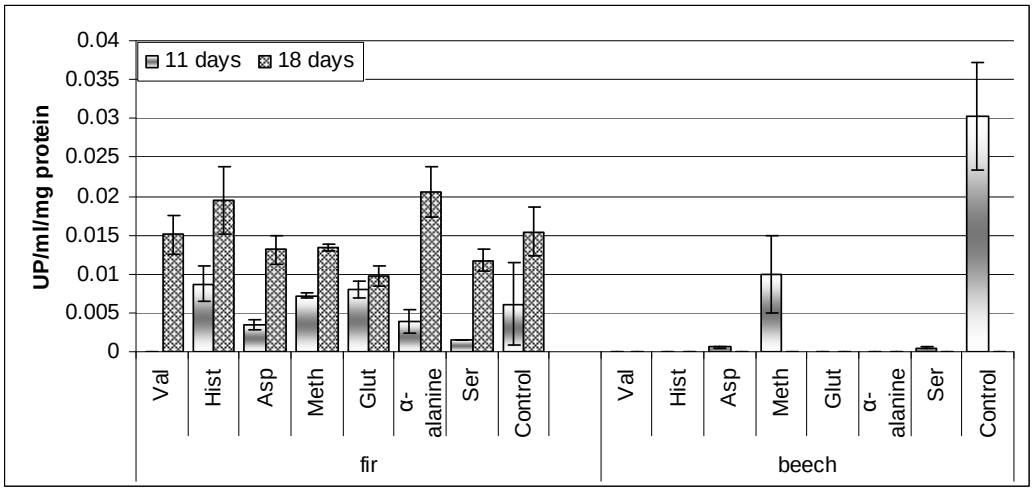

Fig. 4 Peroxidase activity in fungus *Phanerochaete chrysosporium* cultivated on media containing fir and beech sawdust under the influence of some amino acids – culture liquid

CONCLUSIONS

Catalase activity, in the fungus mycelium, 11 days after sowing, was lower at variants containing amino acids compared with control variant in both types of sawdust. At 18 days after seeding, the enzimatic activity was enhanced by the presence of histidine and valine variants with fir sawdust, while at variants of beech sawdust, was stimulated in presence of histidine, methionine and asparagine.

In the liquid culture, at 11 days from seeding, the activity of catalase was increased by variants containing fir sawdust under the influence of histidine, gluamic acid, asparagine, serine and α-alanine, but in media containing beech sawdust, the emzymatic activity was inhibited at the variant with histidine and control. At 18 days after sowing, catalase activity was stimulated only by methionine and α-alanine in culture media containing fir sawdust, and the culture media with beech sawdust most intense activity took place under the influence of serine.

Peroxidase activity in the fungus mycelium, the first time, was inhibited by histidine variants of medium containing fir sawdust and stimulated by asparagine and valine in the medium variant beech sawdust. At 18 days after seeding, peroxidase activity was stimulated in variants with valine, serine, asparagine, methionine and α-alanine on media containing fir sawdust, while the in work variants with beech sawdust, the enzymatic activity was present only at variants containing valine, asparagine, glutamic acid and control.

At 11 days from sowing, in culture liquid, peroxidase activity was stimulated by media containing fir sawdust in the presence of histidine, glutamic acid and methionine, and at 18 days after seeding by α-alanine and histidine. On media containing beech sawdust, peroxidase was present only in the first period to variants with asparagine, methionine, serine and control, while in the second period enzymatic activity was completely inhibited.

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[1] Biological Research Institute, Iaşi.

[2] "Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology.

* alexandru.manoliu@uaic.ro