COMPARED ANALYSIS OF CATALASE AND PEROXIDASE ACTIVITY IN CELLULOLYTIC FUNGUS *TRICHODERMA REESEI* GROWN ON MEDIUM WITH DIFFERENT CONCENTRATIONS OF GRINDED WHEAT AND BARLEY STRAWS

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Keywords: *Trichoderma reesei*, catalase, peroxidase, barley straws, wheat straws

Abstract: The purpose of this study was to assess the evolution of catalase and peroxidase activity in *Trichoderma reesei* grown on medium containing grinded wheat and barley straws. Carbon source of cultivation medium - glucose was replaced by various concentrations of grinded wheat and barley straws, finally resulting three experimental variants as follows: V1 = 20 g/l, V2 = 30 g/l, V3 = 40 g/l. În addition to these variants a control sample was added in which composition remainded unchanged. The catalase activity was determined by spectrophotometric Sinha method (Artenie et al., 2008) while peroxidase activity was assesed using the o-dianisidine method (Cojocaru, 2009). Enzymatic determinations were carried out at 7 and 14 days from inoculation, in both fungus mycelium and culture liquid. The enzymatic assay showed significant differences between determinations intervals and work variants. Enzyme activity is influenced by the age of fungus and by the different nature of the substrate used.

INTRODUCTION

Trichoderma reesei is a mesophilic soft-rot ascomycetous fungus producing high levels of cellulases and hemicellulases, commercially used to modify and hydrolyze plant cell walls polysaccharides (Levasseur, 2010). It is an ubiquitos soil dweller, able to transform a wide variety of organic materials of both natural and xenobiotic origin.

All aerobic organisms use molecular oxygen (O_2) for respiration and energy supply. At the same time they have to face the toxic side effects of O_2, the production of reactive oxygen species (ROS), such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-).

Hydrogen peroxide is a by-product of all living organisms which rely on respiration for energy production. The main site of H_2O_2 production is the mitochondrion (Turrens, 2003). Hydrogen peroxide has a cytotoxic effect on the cell due to its ability to damage macromolecules, including lipids, DNA, and proteins (Jamieson, 1998).

Compared with other reactive oxygen species (ROS), H_2O_2 is less toxic, but is able to diffuse into different compartments from its original production sites before reaching its target (Branco, 2004). Detoxification of H_2O_2 is a fundamental aspect of the cellular antioxidant response in which catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) play a major role. These enzymes, commonly designated as hydroperoxidases are involved in the metabolism of hydrogen peroxide (Levy et al., 1991).

The present study followes the line of previous researches on catalase and peroxidase activity of cellulosolytic fungi under the influence of magnetic field (Manoliu et al., 2005a), liquid ferric (Manoliu et al., 2005b), bakery waste industry (Manoliu et al., 2006).

Wheat and barley straws are important agriculture byproducts. These residues represent an abundant, inexpensive and readily available source of renewable lignocellulosic biomass used for the production of alternative fuels. The filamentous fungus *Trichoderma reesei* is used in enzyme pretreatment processes of the lignocellulosic biomass (Rosgaard et al., 2007).

Chemical composition of wheat and barley straws was assessed on previous studies (Antogiovanni, Sargentini, 1991; Graham, Aman, 1984). Wheat straws consist of 35-45% cellulose, 20-30% hemicellulose and 8-15% lignin (Saha, Cota, 2006); barley straws are made of 33% cellulose, 28, 1 % hemicellulose and 14.9 % lignin (Graham, Aman, 1984).

Extracellular hydrogen peroxide has been involved in the degradation of the crystalline cellulose component of plant cell walls (Veness, Evans, 1989).

To determine how the fungus protects itself against detrimental effects of reactive oxygen species, catalase and peroxidase activity was examined on culture medium containing grinded wheat and barley straws.

MATERIAL AND METHODS

Strain and cultivation: Trichoderma reesei was acquired from the Institute Sciétific de Santé Publique, Belgium by Biological Science Research Institute, Iaşi. The fungus was cultivated on potato dextrose agar plates (PDA)

for 7 days at 28°C. For enzymatic assay we used Sabouround liquid medium with the following composition: peptone-10g, glucose-40g and distilled water-1000 ml (Constantinescu, 1974) in which we replaced the carbon source-glucose with different concentration of grinded wheat and barley straws, ultimately resulting four variants for each type of straw: V1-20 g/l, V2-30 g/l , V3-40 g/l and V4 in which the carbon source was not replaced. Wheat and barley straws were collected from a field near Iaşi, in Miroslava. They were kept in polyethylene bags away from humidity. Prior to addition to culture medium, wheat and barley straws were grinded in an electric grinder.

Enzyme assay. Peroxidase and catalase activity was assessed at 7 and 14 days after fungal inoculation in both mycelium and culture liquid. Peroxidase activity was assessed on the basis of ortho-dianisidine method (Cojocaru, 2009), while catalase activity was determined by spectrophotometric Sinha method (Artenie et al. 2008).

RESULTS AND DISCUSSION

The results of catalase activity in the fungus *Trichoderma reesei* grown on medium containing grinded wheat straws and barley straws are depicted in figure 1 and 2. In the fungus mycelium, at 7 days from inoculation the catalase activity was higher in variants containing various concentrations of grinded wheat straws and barley straws compared to the control sample with glucose as a solely carbon source. No increase in catalase activity correlated to grinded straw concentration was recorded. For example, V1 containing grinded barley straws recorded catalase activity of 2461 UC/g/min compared to 2057 UC/g/min in V2.

At 14 days from inoculation all variants showed a slightly decrease in catalase activity. The media variants with grinded wheat and barley straws recorded higher values compared to the control sample.

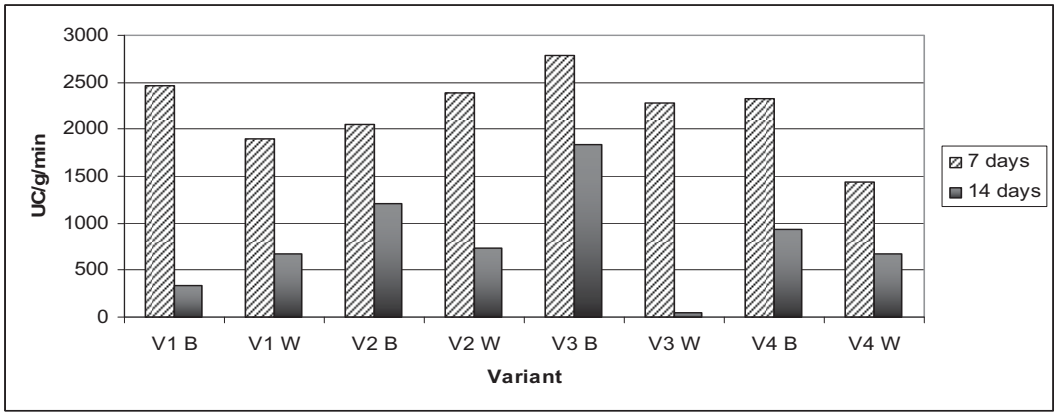

Fig.1 Catalase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws-mycelium (B-barley, W-wheat)

Variant 3 medium containing grinded barley straws recorded the lowest value of 49, 14 UC/g/min. In variants containing grinded wheat straw the catalase activity increased simultaneous with carbon source concentration, but was lower than the activity recorded at seven days.

In culture liquid, at 7 days from inoculation, all variants containing grinded barley straws as carbon source recorded lower values of enzyme activity compared to control variant. These results are similar to medium variants with grinded wheat straws, where control sample recorded the highest value of 488 UC/ml/min. In variants with grinded barley straws the activity was constants when correlated with straw concentration. In contrast, in media variants with grinded wheat straws recorded fluctuating values with a value of 192,14 UC/ml/min in V2 variant and a value of 14,92 UC/ml/min in V3.

The catalase activity in culture liquid at 14 days from inoculation was stimulated in medium containing grinded barley straws compared to control sample. Catalase activity also increased compared to values recorded previously at 7 days. In contrast, the catalase activity in control sample decreased, reaching a value of 68, 69 UC/g/min. In variants with grinded wheat straws enzymatic activity incresed compared to datas recorded at 7 days, but decreased in control variant (259 UC/g/min). Grinded wheat concentration did not influenced catalase activity.

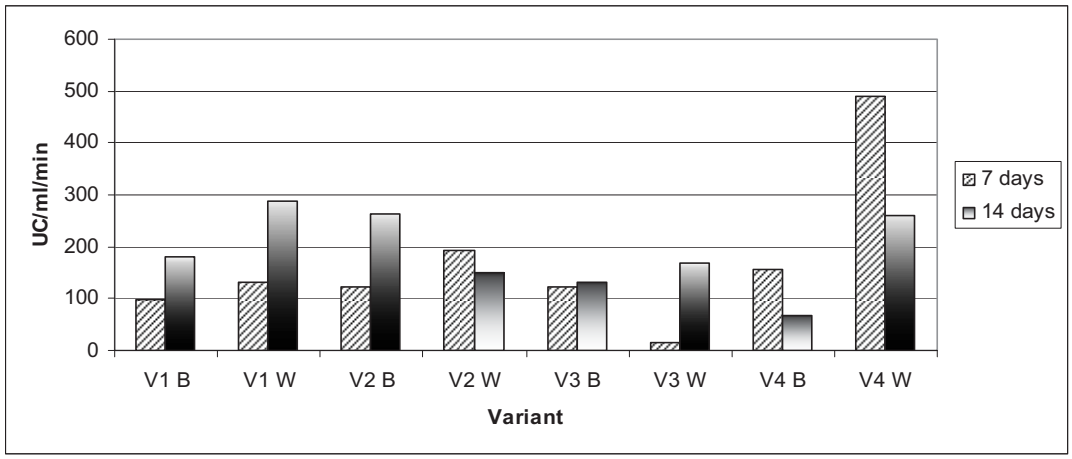

Fig. 2 Catalase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – culture liquid (B-barley, W-wheat)

When comparing datas recorded in both mycelium and liquid culture we conclude that catalase activity increased in mycelium at 7 days from inoculation, and decreased in the second interval. In contrast in liquid culture enzymatic activity decreased at 7 days and increased at 14 days. Even if the overall enzyme assay analysis show a trend in enzymatic activity, the liquid culture recorded low catalase activity when compared to mycelium of *Trichoderma reesei* in both determination intervals.

The peroxidase activity in *Trichoderma reesei* was assessed in both mycelium and liquid culture at 7 and 14 days from inoculation of medium containing grinded barley and wheat straws (Figure 3 and 4).

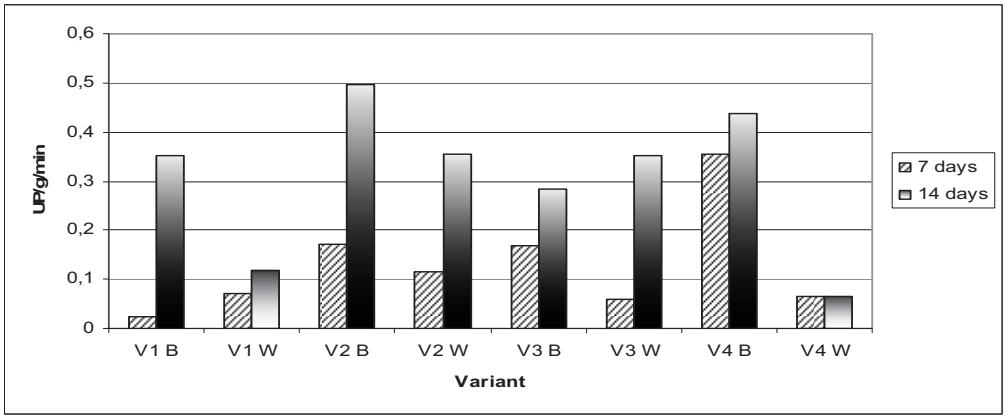

Fig.3. Peroxidase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – mycelium (B-barley, W-wheat)

In mycelium, peroxidase activity in variants with grinded barley straws was lower than in control sample. In contrast, in variants with grinded wheat straws enzymatic activity recorded a lower value in control sample (0, 06 UP/g/min). Peroxidase activity was influenced by carbon source concentration in variants with grinded barley straws. In constrast, media containing various concentration of grinded wheat straws did not effect enzymatic activity.

At 14 days from inoculation enzymatic activity increased in both variants with grinded wheat and barley straws. In variants with grinded wheat straws the peroxidase activity was higher than control variant compared to 7 days enzyme assessment. In medium with grinded barley straws peroxidase activity fluctuated compared to control, with V3 (0, 059 UP/g/min) being higher than control variant (0, 0656 UP/g/min).

Different carbon source concentration added to Sabouround medium did not correlate with enzymatic activity, various values being recorded in both media containing wheat and barley straws. The control sample showed similar peroxidase activities patterns at 7 and 14 days from inoculation.

The peroxidase activity was assessed in liquid culture at 7 days from inoculation and the activity was overall. The values recorded in medium containing different concentration of wheat and barley grinded straws varied, in some cases being higher than control variant (V1 in barley is 0, 1234 UP/ml/min compared to V4 0, 07822 UP/ml/min).

At 14 days from inoculation, the peroxidase activity increased compared to the previous recorded activity at 7 days, reaching its highest in V3 medium with grinded barley straws (0, 12968 UP/ml/min). *Trichoderma reesei* grown on medium with grinded barley straws had an enzymatic activity higher than control, whereas variants with grinded wheat straws recorded just one value above control (V3- 0, 175 UP/ml/min).

Overall peroxidase activity was lower in liquid culture than in mycelium, but in both cases it increased at 14 days from inoculation.

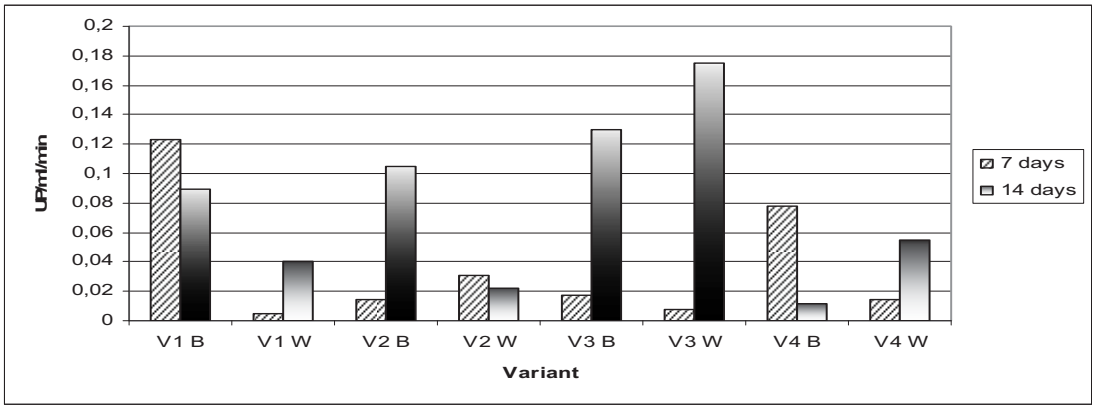

Fig.4. Peroxidase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – culture liquid (B-barley, W-wheat)

CONCLUSIONS

Assessment of catalase activity in the mycelium of *Trichoderma reesei* grown on media with barley and wheat straws showed a slightly increase in enzymatic activity at 7 days from inoculation, and a decrease in activity in the second interval. In contrast, data collected from the liquid culture indicated a decrease in enzymatic activity at 7 days and an increase at 14 days.

The increase in catalase activity from mycelium recorded at 7 days was not correlated with substrate concentration, though at 14 days we recorded an increase in enzymatic activity simultaneously with the carbon source concentration. Enzymatic activity in culture liquid at 7 days from inoculation was lower in all variants compared to control. The catalase activity was stimulated in liquid culture at 14 days in experimental variants with grinded barley straws and in those with grinded wheat straws.

The peroxidase activity recorded from the fungus mycelium at 7 days was lower in variants with grinded barley straws than control. Peroxidase activity was influenced by carbon source concentration in variants with grinded barley straws. At 14 days from inoculation enzymatic activity increased in both variants with grinded wheat and barley straws.

Peroxidase activity recorded in the liquid culture at 7 days was overall low and the data recorded in medium containing different concentration of wheat and barley grinded straws varied. At 14 days from inoculation, the peroxidase activity increased compared to the enzymatic activity recorded at 7 days.

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