THE ANALYSIS OF CATALASE AND PEROOXIDASE ACTIVITY IN SAPROPHYTIC FUNGUS *RHIZOPUS NIGRICANS* GROWN ON MEDIUM WHITH DIFERENT CONCENTRATION OF GRINDED CORN CARYOPSIS

TAMARA BARBĂNEAGRĂ[1*], ALEXANDRU MANOLIU[2], MIHAELA CRISTICA[1], ELENA CIORNEA[1], ELENA TUTU[1]

Keywords: *Rhizopus nigricans*, catalase, peroxidase, corn caryopsis

Abstract. The purpose of this study was to assay catalase and peroxidase activity in the saprophytic fungus *Rhizopus nigricans*, grown on mediums containing grinded corn caryopsis, which, in our experiments have replaced carbon source – sucrose in composition of liquid culture medium Czapeck Dox, resulting in the final three experimental variants: V1 = 20 g/l, V2 = 30 g/l, V3 = 40 g/l, while the control variant composition remained unchanged. Measurements were made at three time intervals: 5 days and 10 days and 15 days after inoculation, using fungus mycelium and culture liquid. Determination of catalase activity was performed using Sinha method (Artenie Vl., *et al.,* 2008), and determination of peroxidase was carried out on the basis of ortho-dianisidine method (Cojocaru D.C., 2009). The results showed significant deferens in dynamic of enzyme activity depending on the concentration of carbon source introduced into the medium and age of the fungus.

INTRODUCTION

The filamentous fungus *Rhizopus nigricans* is an obligate aerobe that is frequently found in decayin of organic matter rich in complex carbohydrates. This organism has de ability to thrive in such environments because of its simple growth requirements and capacity to produce numerous hydrolytic enzymes (Skory, C.D., *et al*, 2009).

Fungal cells must deal with a wide variety of potentially toxic environmental challenges during the course of their proliferation (Moye-Rowley, S. W., 2003).

The use of oxygen as the respiratory substrate is frequently reported to lead to the development of oxidative stress, mainly due to oxygen-derived free radicals, which are collectively termed as reactive oxygen species (ROS) (Qiang, Li *et al*, 2009).

The involvement of oxygen in metabolic processes in fungi is coupled to its activation and formation of number of highly reactive compounds such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical OH^- (Sigler, K., *et al*., 1999).

The development of fungi proceeds in immediate contact with the environment. Therefore, they are constantly subjected to physical and chemical stress factors. All aerobic organisms generate reactive oxygen species, especially through aerobic respiration. Reactive oxygen species (ROS) are formed by fungi in the course of metabolic activity. ROS production increases in fungi due to various stress agents such as starvation, light, mechanical damage, and interactions with some other living organisms. Regulation of ROS level appears to be very important during development of the fungal organism (Gessler N.N. *et al*., 2007).

High reactivity of ROS is responsible for oxidation of proteins, lipids, and acids. Consequently, systems defending against ROS by repair or resynthesis of damaged molecules are present in the cell. Nevertheless, impairment of intracellular redox status, as a result of an increase in generation of oxygen radicals exceeding the cellular capacity to neutralize them, can generate the oxidative stress. Intracellular ROS increase is accompanied by the cessation of growth, and it provokes morphological changes leading to cell adaptation to changes in life conditions as well as the decrease in intracellular oxidants (Belozerskaya T.A.. *et al*, 2006).

Characteristic of the oxidative stress in fungi are a massive protein oxidation with their subsequent degradation, glycosylation, and carbonylation; (Kritskii, M.S., 1982; Hansberg, W., *et al*., 1990; Aguirre J, *et al*. 2005; Gessler, N.N., *et al*., 2006). The metabolic rearrangement leads to the cessation of growth and the synthesis of secondary metabolites in fungal cells, many of which are antioxidants (Bai, Z., *et al*., 2003; Sokolovskii V.Yu. and Belozerskaya T. A., 2000; Yoshida Y. and Hasunuma K., 2004).

Hydrogen peroxyde is the most stable of the oxygene reactive species (ROS) and is a strong nucleofilic oxidant. Hydrogen peroxide is degraded by catalase and peroxidase, enzymes that act synergistically to protect cells.

Fungi are reported to be high producers of catalases (Klotz, M.G. *et al*, 1997; Eremin, A.N., *et al*, 2000; Kurakov, A.V., *et al*, 2001), and different types of catalases and catalase genes have been isolated (Isobe, K., *et al*, 2005).

Also, a large number of peroxidases have been identified in fungal species and are being characterized at the molecular level (Conesa, A., *et al*, 2002).

There are essential nutrients, that participate together with enzymes in antioxidant processes, delaying or totally inhibiting oxidation of the substrate and acting at different levels of oxidative sequence (Halliwell B. and Gutteridge J.M.C., 2007, Sarikurkcu C. et al., 2010). Possessing mechanisms to adapt to oxidative stress (Tanaka C., Izumitsu K., 2010), represented by an endogenous antioxidant system, fungi are able to release exoenzime in the extracellular space to minimize the negative impact of reactive oxygen species.

The objective of this paper, following the line of other research (Manoliu Al. e*t al*., 2005, 2006, 2010) regarding the influence of environmental factors on enzyme activity, aimed at analyzing the activity of these biochemical parameters of oxidative stress in the *Rhizopus nigricans* species grown in laboratory conditions on medium containing different concentrations of grinded corn caryopsis, corn grain representing one of most extensive cultivated cereal species, due to its high nutritional value, being used in human food, animal feed and raw materials for various industries.

MATERIALS AND METHODS

The study was conducted on the species *Rhizopus nigricans*. The fungus has been isolated from germinated wheat cariopses, which were taken from the storage place of the Enterprise of Cereal Products from Chişinău, Republic of Moldova.

Pure culture was obtained after several cycles of growth on PDA solid medium. Identification of *Rhizopus nigricans* species was based on morphological characteristics of the mycelium from culture plates and by making microscopic preparations.

To determine the activity of both enzymes was used Czapek Dox liquid medium with the following composition: sucrose 30 g, NaNO3 2 g, K2HPO4 1 g, KCl 0.5 g, MgSO4. 7H2O 0.5 g, FeSO4. 7H2O 0.01 g, distilled water 1000 ml (Constantinescu O., 1974). The culture medium composition was modified by replacing the carbon source - sucrose, with different amounts of grinded corn caryopsis, resulting in the final three experimental variants: V1 = 20 g/l, V2 = 30 g/l, V3 = 40 g/l, plus a control version, in which composition of medium remained unchanged. Medium was distributed in Erlenmeyer flasks in quantities of 100 ml. In each flask was placed a disk of 8 mm in diameter from 5 days old culture of *Rhizopus nigricans*. The flasks were incubated in the thermostat, set at 28 ˚ C. Enzyme determinations were performed at three time intervals from inoculation of the fungus: at 5, at 10 and 15 days, using fungus mycelium and culture liquid.

Determination of catalase activity was performed using Sinha method (Artenie Vl., *et al*., 2008), and determination of peroxidase was carried out on the basis of ortho-dianisidine method (Cojocaru D.C., 2009).

RESULTS AND DISCUSSIONS

Results on the influence of different concentrations of grinded corn caryopsis, which were introduced into the culture medium, on catalase and peroxidase activity in mycelium and culture liquid of the saprophytic fungus *Rhizopus nigricans*, are shown graphically in figures 1-4.

The dynamics of peroxidase activity in the mycelium (figure 1), recorded, in the first period after inoculation, higher values for all variants compared to the control variant (UP 0.0945 / g / min), enzymatic activity increased in relation to the concentration of grinded corn caryopsis. The maximum value was reached in variant V3 (0.3466 UP / g / min), followed in descending order by V2 version (0.3079 UP / g / min) and variant V1 with a value very close to that of the control value – 0.0984 UP / g / min.

In the second study period, preroxidase activity recorded an increase, the values were much higher than those recorded in the first period. Experimental variants showed higher values compared to the control variant (0.0978 UP / g / min), the maximum enzyme activity occurring in the version with 30 g/l grinded corn caryopsis (1.0689 UP / g / min), followed by a close value in variant V1 with 20 g/l of grinded caryopsis (1.0269 UP / g / min) and the minimum value was found in variant V3 - UP 0.3906 / g / min.

In the third period from insemination, with the ageing of the fungus, there is a decrease of this enzyme activity in all experimental variants (0.2172 UP / g / min - for version V1, 0.6485 UP / g / min – for V2 version and 0.6397 UP / g / min for the variant V3), probably due to

consumption of nutrients from the medium. In all variants were recorded values higher than the value recorded in control variant - 0.0678 UP / g / min.

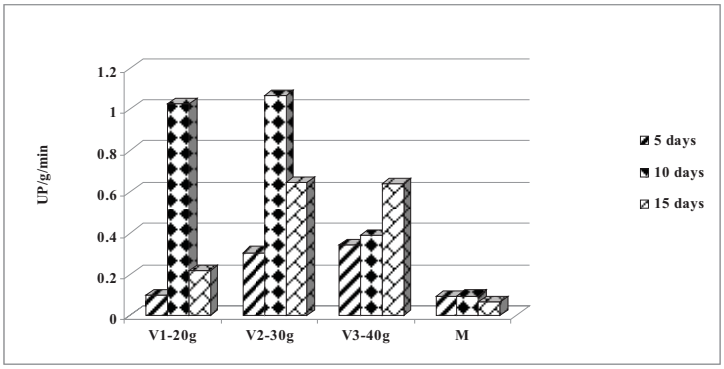

Figure 1. Peroxidase activity in mycelium of *Rhizopus nigricans* species grown on medium with grinded corn caryopsis

Further was determined peroxidase activity in culture liquid at 5, 10 and 15 days, as it is known, fungi are able to synthesize and discharge a series of compounds into the environment, including enzymes.

As shown in figure 2, peroxidase activity recorded at 5 days after insemination of fungus, quite low values for all variants with grinded corn caryopsis, the values are comparable to that of the control version (0.0176 UP / ml / min), ranging between 0.0231 UP / ml / min and 0.0775 UP / ml / min.

At 10 days after inoculation an increase in enzyme activity takes place in all variants, except the control version in which value remained almost unchanged (0.01718 UP / ml / min). The maximum value of peroxidase activity was recorded in V2 variant (0.3109 UP / ml / min) followed in descending order by the variant V3 (0.0718 UP / ml / min) and variant V1 (0.0515 UP / ml / min).

The ageing of fungus entailed a decrease in enzymatic activity at 15 days after inoculation, and in the control variant it was almost completely inhibited (0.0014 UP / ml / min). Maximum value occurred in variant treated with 30 g/l grinded corn caryopsis (0.2478 UP / ml / min), followed decreasingly by the version in which the medium contains 40 g/l corn caryopsis (0.0391 UP / ml / min) and variant with 20 g/l grinded caryopsis (0.0304 UP / ml / min).

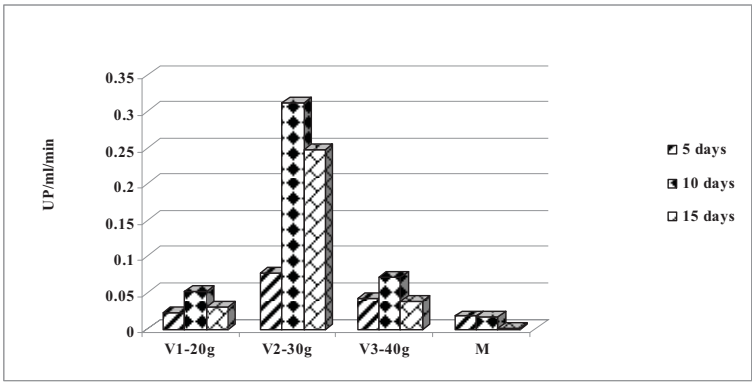

Figure 2. Peroxidase activity in culture liquid of *Rhizopus nigricans* species grown on medium with grinded corn caryopsis

Data on the dynamics of catalase in the mycelium of fungus *Rhizopus nigricans,* grown on medium with different concentrations of grinded corn caryopsis are reproduced graphically in figure 3.

In the first study period, catalase activity in the mycelium showed higher values in all experimental variants compared with control (209.234 cu / g / min). In V1 version was found maximum value (1180.2348 UC / g / min), followed in descending order by variant V3 (763.6217 UC / g / min) and the minimum value was recorded in version V2 (485.5941 UC / g / min).

In the second period catalase activity shows a decrease in all variants, except V2 variant in which it intensifies (798.4831 UC / g / min).

In the third period after inoculation, , with the ageing of the fungus, recorded values in working versions are kept low (762.823 CU / g / min – for version V1 and 408.4513 UC / g / min - for V3 version), except V2 variant in which the enzyme activity continued to increase (3472.7863 UC / g / min).

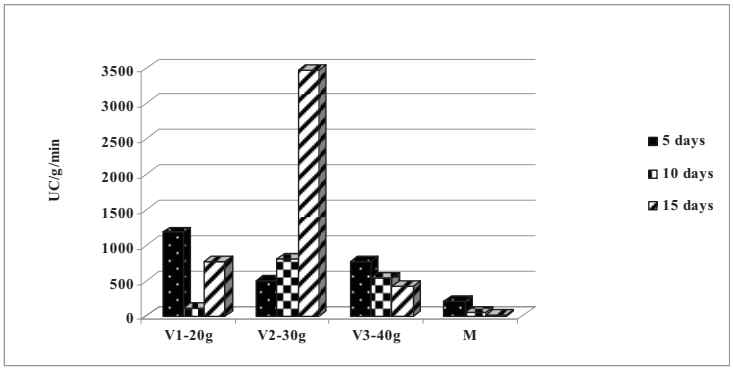

Figure 3. Catalase activity in mycelium of *Rhizopus nigricans* species grown on medium with grinded corn caryopsis

As found in figure 4 catalase activity in culture liquid at 5 days after inoculation of culture medium recorded higher values for all variants (206.2338 UC / ml / min – in the V1 version, 204.9371 UC / ml / min – in the V2 and 208.3618 UC / ml / min - for variant V3) compared with control variant (UC 74.2081 / ml / min), the results are comparable, not being able to detect significant differences depending on the concentration grinded caryopsis of the mediu.

At 10 days after inoculation of the fungus has been a significant increase in enzymatic activity in all variants containing grinded corn caryopsis (probably due to the release of the enzyme in the culture medium), except control version whose value is only slightly elevated (90.3954 UC / ml / min), values were also comparable (V1 - 1129.9807U C / ml / min, V2 - 1090.2004 UC / ml / min, UC / ml / min, V3 - 1142.303 UC / ml / min).

The ageing of fungus and depletion of nutrient soucers from the medium had led to a drastic decrease in catalase activity in the third period in all studied variants (values ranging between 27.8569 UC / ml / min in V3 and 81.1629 in V1), including control variant (13 UC / ml / min).

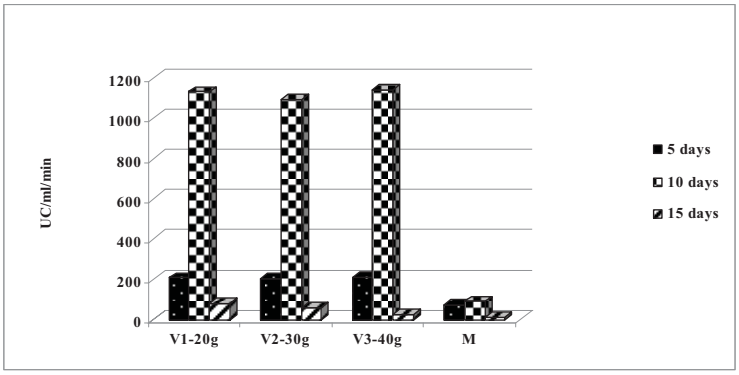

Figure 4. Catalase activity in culture liquid of *Rhizopus nigricans* species grown on medium with grinded corn caryopsis

CONCLUSIONS

After analyzing, the experimental results showed that catalase and peroxidase activity was influenced by concentration of grinded wheat caryopsis and by fungus culture age.

- Peroxidase activity in the mycelium was stimulated in all experimental variants by the introducing into the culture medium grinded corn caryopsis.
- In culture liquid peroxidase activity was stimulated in version V2 in all study periods.
- In the fungus mycelium catalase activity was stimulated in all three periods in the V2 version.
- Catalase in liquid culture was stimulated by the presence of corn caryopsis in all work options in the first two periods, and inhibited in third period, probably due to culture ageing.

REFERENCES

Aguirre, J., Rios-Momberg, M., Hewitt, D., and Hansberg, W., (2005): *Reactive oxygen species and development in microbial eukaryotes, Trends Microbiol.*, vol. 13, no. 3, pp. 111–118.

Artenie, Vl., Ungureanu E., Negura, **A.M., (**2008): *Metode de investigare a metabolismului glucidic şi lipidic – manual de lucrări practice*, Editura Pim, Iaşi, p. 97-99.
Bai, Z., Harvey, L., and McNell, B., (2003): *Oxidative stress in submerged cultures of fungi,* ***Critical Rev. Biotechnol.*****, 23(4):267-302.**
Belozerskaya, T. A., Gessler, N. N., Isakova, E. P., Deryabina, Y. I., (2006): *Neurospora crassa light signal transduction is affected by ROS*, A.N.Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia.
Cojocaru, D. C., **2009** – *Enzimologie practică*, Editura Tehnopress, p. 131-132.
Conesa, A., Punt, P. J., van den Hondel, Cees A.M.J.J., (2002): *Fungal peroxidases: molecular aspects and applications,* Journal of Biotechnology 93 143–158.
Constantinescu, O., (1974): *Metode şi tehnici în micologie*, Editura Ceres, Bucureşti, p. 105-106.
Eremin, A. N., Mikha, R. V., and Metelitsa, D. I., (2000): *Comparative kinetic characteristics of catalase of Penicillium species molds*, Prik. Biokhim. Mikrobiol., 36, 261–266.
Gessler, N.N., Aver'yanov, A. A., Belozerskaya, T. A., (2007): *Reactive oxygen species in regulation of fungal development*, Biochemestry, (Moscow), vol. 72: 1091-1109.
Gessler, N.N., Leonovich, O.A., Rabinovich, Ya.M., Rudchenko, M.N., and Belozerskaya, T.A., (2006): *Prikl. Biokhim. Mikrobiol.*, vol. 42, no. 3, pp. 354–358.
Halliwell, B., Gutteridge, J. M. C.,(2007): *Free radicals in Biology and Medicine,* Oxford University Press, 851 pages.
Hansberg, W. and Aguirre, J., (1990): *J. Theor. Biol.*,vol. 142, no. 2, pp. 287–293.
Isobe, K., Inoue, N., Takamatsu, Y., Kamada, K., and Wakao, N., (2005): *Production of Catalase by Fungi Growing at Low pH and High Temperature*, JOURNAL OF BIOSCIENCE AND BIOENGINEERING , The Society for Biotechnology, Japan Vol. 101, No. 1, 73-76.
Klotz, M.G., Klassen, G. R., and Loewen, P. C., (1997): *Phylogeneticrelationships among prokaryotic and eukaryotic catalases*, Mol. Biol. Evol., 14, 951–958.
Kritskii, M.S., (1982): *Usp. Mikrobiol.*, vol. 17, pp. 41–62.
Kurakov, A. V., Kupletskaia, M. B., Skrynnikova, E. V., and Somova, N. G., (2001): *Search for micromycetes-producers extracellular catalase and study of conditions of its synthesis. Prik. Biokhim. Mikrobio*l., 37, 67–72.
Manoliu, Al., Oprică, L. (2005): *Influenţa vitaminelor hidrosolubile asupra catalazei şi peroxidazei la specia Chaetomium globosum cultivată pe medii cu deşeuri din industria alimentară*, Simpozionul ştiinţific anual Horticultura-ştiinţă, calitate, diversitate şi armonie.
Manoliu, Al., Oprică, L. Olteanu, Z., Neacşu, I., Artenie, V., Creangă, D.E., Rusu, I., Bodal I., (2005): *Peroxidase activity in magnetically exposed cellulolytic fungi*. Journal of Magnetism and Magnetic Materials 300: e323–e326
Manoliu, Al., Oprică, L., Creangă, D. E., (2006): *The influence of the static magnetic field (SMF) on some biochemical parameters in cellulolytic fungi Chaetomium globosum and Trichoderma viridae cultivated on media supplemented with panification industrial wastes*. Rom. J. Biol. -Plant Biol., vol. 51-52, P. 25-37.
Manoliu, Al., Tutu, E., Oprică, L., Ciornea, E., Grădinariu, P., (2010): *Influence of the culture medium pH on the activity of some oxidoreductases in Monilinia laxa (Aderh.&Ruhl.) Honey parasite on plum*, Analele Stiinţifice ale Universităţii "Alexandru Ioan Cuza", Secţiunea Genetică şi Biologie Moleculară, Tom XI, Fasc.4, 41 - 46.
Moye-Rowley, S. W., (2003): *Regulation of the Transcriptional Response to Oxidative Stress in Fungi: Similarities and Differences*, Eukaryot Cell. 2003 June; 2(3): 381–389.
Qiang, Li, Harvey, L. M., McNeil, B., (2009): *Oxidative stress in industrial fungi*, Critical Reviews in Biotechnology, Vol. 29, No. 3: 199–213.
Sarikurkcu, C., Tepe, T., Karslı Semiz, D., Solak, M. H., (2010): *Evaluation of metal concentration and antioxidant activity of three edible mushroom from Mugla, Turkey*, Food and Chemical Toxicology, 48, 1230-1233
Sigler, K, Chaloupka, J., Brozmanova, J., Stadler, N., Hofer, M., (1999): *Oxidative stress in microorganisms - I. Microbial vs higher cells - damage and defenses in relation to cell aging and death*, Folia Microbiol, 1999. V. 44. P. 587-624.
Skory, C.D., Mertens, J. A., Rich, J. O., (2009): *Inhibition of Rhizopus lactate dehydrogenase by fructose 1,6-bisphosphate*, Enzyme and Microbial Technology 44 242–247.
Sokolovskii, V.Yu. and Belozerskaya, T.A., (200): *Usp. Biol. Khim.*, vol. 40, pp. 85–152.
Tanaka, C., Izumitsu, K., (2010): *Two-Component Signaling System in Filamentous Fungi and the Mode of Action of Dicarboximide and Phenylpyrrole Fungicides*, Fungides, Intech. Publishing, page 523-538.
Yoshida, Y. and Hasunuma, K., (2004): *Reactive Oxygen Species Affect Photomorphogenesis in Neurospora crassa J. Biol. Chem.*, vol. 279: 6986–6993.

1. "Alexandru Ioan Cuza" University, Iaşi
2. Institut of Biological Research, Iaşi
* tamara.barbaneagra@yahoo.com

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

MIHAELA CRISTICA[1*], ALEXANDRU MANOLIU[2], TAMARA BARBĂNEAGRĂ[1], ELENA CIORNEA[1]

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.

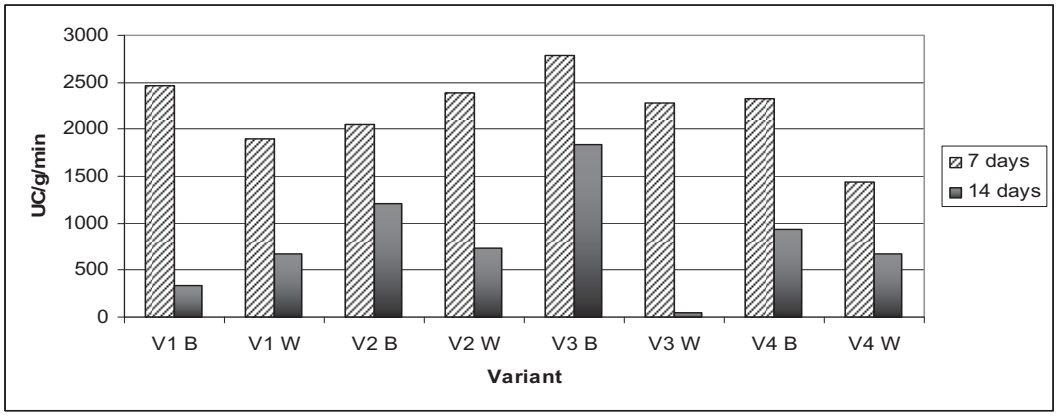

Fig.1 Catalase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws-mycelium (B-barley, W-wheat)

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.

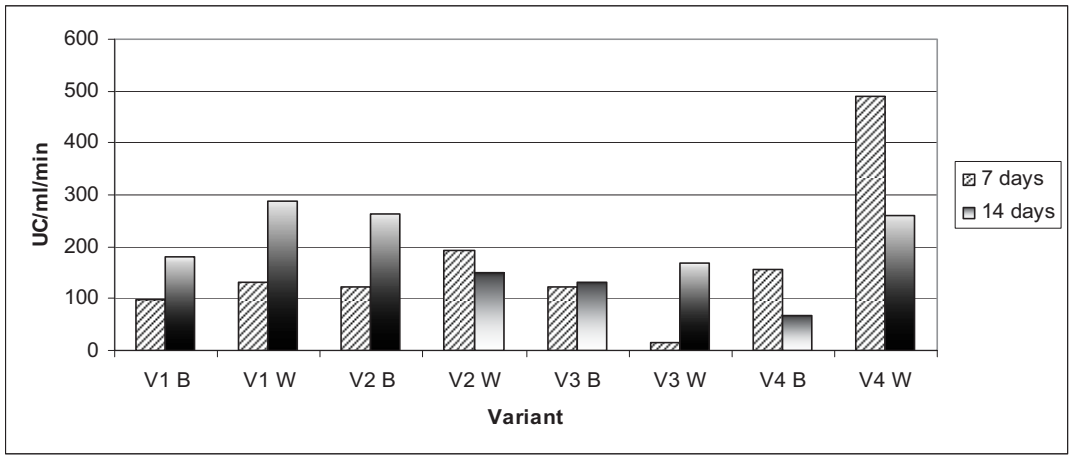

Fig. 2 Catalase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – culture liquid (B-barley, W-wheat)

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).

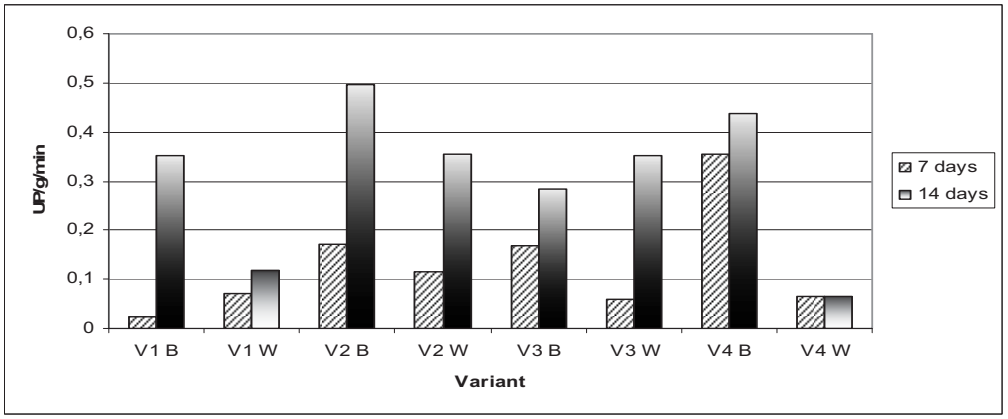

Fig.3. Peroxidase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – mycelium (B-barley, W-wheat)

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.

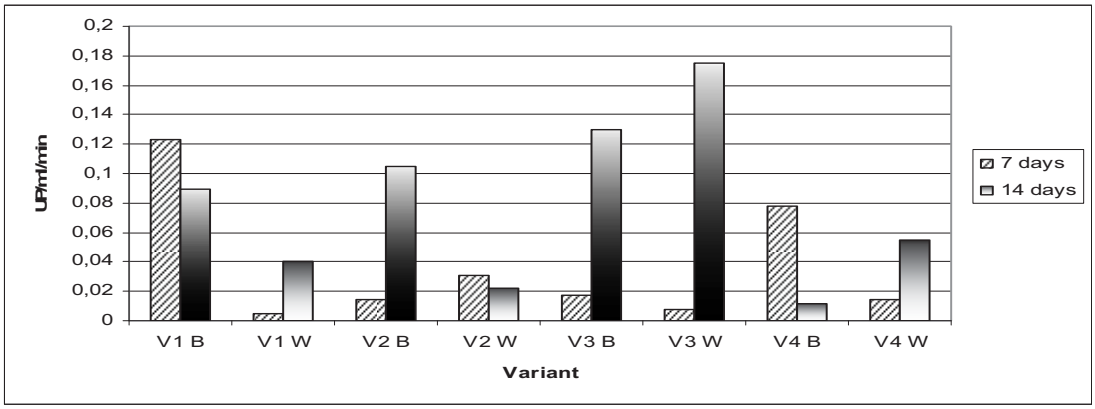

Fig.4. Peroxidase activity in fungus *Trichoderma reesei* cultivated in medium containing grinded wheat and barley straws – culture liquid (B-barley, W-wheat)

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.

REFERENCES

Antongiovanni, M., Sargentini, C., (1991), *Variability in chemical composition of straw*, Opt. Mediterr., Ser. Semin., Num. 16, 49-53

Artenie, V., Ungureanu, E., Negură, A. M., (2008): *Metode de investigare a metabolismului glucidic şi lipidic – manual de lucrări practice*. Editura Pim, 182 p.
Branco, M. R., Marinho, S., Cyrne, L., Antunes, F., (2003): *Decresease of H_2O_2 plasma membrane permeability during adaptation to H_2O_2 in Saccharomyces cerevisiae*. The Journal of Biological chemistry, Vol. 279, 6501-6506
Cojocaru, D. C., (2009) *: Enzimologie practica*. Editura Tehnopress 458 p
Constantinescu O., (1974): *Metode si tehnici în micologie*, Ed. Ceres, Bucuresti, p. 105-107.
Graham, H., and P. Aman, (1984). *A comparison between degradation of in vitro and in sacco of constituents of untreated and ammonia*
treated barley straw. Anim. Feed Sci. Technol. 10:199.
Levasseur, A., Saloheimo, M., Navaroo, D., Andberg, M., Pontarotti, P., Kruus, K., Record E., (2010), *Exploring laccase-like multicopper oxidase genes from the ascomycete Trichoderma reesei: a functional, phylogenetic and evolutionary study*, BMC Biochemistry, 11:32
Levy, E., Eyal, Z., Hochmant, A., (1992) *Purification and characterization of a catalase-peroxidase from the fungus Septoria tritici*, Archives of Biochemistry and Biophysics, Vol. 296, p 321-327
Manoliu, Al., Oprica, L. Olteanu, Z., Neacsu, I., Artenie, V., Creanga, D.E., Rusu, I., Bodal I., (2005): *Peroxidase activity in magnetically exposed cellulolytic fungi.* Journal of Magnetism and Magnetic Materials 300: e323–e326
Manoliu, Al., Oprica, L., Creanga, D.E., (2005): *Ferrofluid and cellulolytic fungi* Journal of Magnetism and Magnetic Materials 289 473–475
Manoliu, Al., Oprica, L., Creanga, D.E., (2006): *The influence of the static magnetic field (SMF) on some biochemical parameters in cellulolytic fungi Chaetomium globosum and Trichoderma viridae cultivated on medium supplemented with panification industrial wastes.* ROM. J. BIOL. -PLANT BIOL., vol. 51-52, P. 25-37.
Rosgaaed, L., Pederson S., Meyer, A.S., (2007) *Comparison of different pretreatment strategies for enzymatic hydrolis of wheat and barley straws*. Appl Biochem Biotechnol., 143(3):284-96
Saha, M., Cotta, A., (2006) *Ethanol Production from Alkaline peroxide pretreated enzymaticallz saccharified wheat straw,* Biotechnol. Prog., 22, 449-453
Turrens, J. F., (2003) *Mitochondrial formation of reactive oxygen species*. J. Physiol. 552 (2):335 344; 2003.
Veness, R.G., Evans, C., (1989) *The role of hydrogen peroxide in the degradation of crystalline cellulose by basidiomycete fungi,* Journal of General Microbiology, 135, 2799-280

1-Al. I. Cuza University, Iaşi,
2-Institute of Biological Research, Iaşi,
*cristica_mihaela@yahoo.fr