RESEARCHES REGARDING CATALASE AND PEROXIDASE ACTIVITY IN FUNGUS *RHYZOPUS NIGRICANS* GROWN ON MEDIUM WITH DIFFERENT CONCENTRATION OF GRINDED WHEAT CARYOPSIS

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Abstract

The purpose of this study was to assay catalase and peroxidase activity in the saprophytic fungus *Rhizopus nigricans*, grown on mediums containing grinded wheat caryopsis. For the conduct of the experiments, from the composition of culture medium Czapeck Dox, the carbon source - sucrose was replaced with different amounts of grinded wheat caryopsis, resulting three experimental variants: V1 = 10 g/l, V2 = 20 g/l, V3 = 30 g/l. The control variant composition remained unchanged. Measurements were made at two time intervals: 7 days and 14 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 show that there are significant differences between the two determinations and between work options. Enzyme activity is influenced by both: the age of fungus and different concentration of grinded wheat caryopsis.

Key words: *Rhizopus nigricans*, peroxidase, catalase, wheat caryopsis

Rhizopus nigricans is a fungus commonly known as black bread mold and is the most common species of *Rhizopus* genus. It frequently found in soil and vegetal products. Being an agent of decay, it produced significant damage during storage of some products and can synthesize mycotoxins. It appears frequently in warehouses when the appropriate conditions of temperature and humidity are not adequate, growing abundantly on the surface of the substrate.

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 (Belozerskaya, T.A. et all, 2006).

Some essential nutrients, together with the enzymes are participating 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), embodied in an endogenous antioxidant system, fungi are able to release exoenzime in the extracellular space to minimize the negative impact of reactive oxygen species.

In this context, the objective of this paper, based on previous research (Manoliu, Al. et al., 2005; 2006; 2010) regarding influence of some environmental factors on enzyme activity is to quantify the activity of these biochemical indicators of oxidative stress in the species *Rhizopus nigricans,* grown in laboratory conditions on medium with different concentrations of grinded wheat caryopsis, wheat representing one of most extensive cultivated cereals, due to its high nutritional value, being material for various food, animal feed and industrial raw material.

It is known that during the reduction of molecular oxygen to water by accepting four

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electrons, oxygen reactive species are generated, including hydrogen peroxide, which is not electrically charged and can run between cell compartments, its toxicity being related to hydroxyl radical formation, one of the most reactive molecular species known. Hydrogen peroxide is degraded by catalase and peroxidase, enzymes that act synergistically to protect cells.

Catalase is an enzyme able to catalyze the break down of hydrogen peroxide (H_2O_2) to water and oxygen. It is universally prevalent in nature, found in all aerobic microorganisms. Hydrogen peroxyde is the most stable of the oxygene reactive species (ROS) and is a strong nucleofilic oxidant. Reactions catalyzed by catalase are essential to life.

MATERIAL AND METHOD

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 Czapek Dox agar 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 (O. Constantinescu, 1974). The culture medium composition was modified by replacing the carbon source - sucrose, with different amounts of grinded wheat cariopses, resulting in the final three experimental variants: V1 = 10 g/l, V2 = 20 g/l, V3 = 30 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 7 days old culture of *Rhizopus nigricans*. The flasks were incubated in the thermostat, set at 28° C. Enzyme determinations were performed at two time intervals from inoculation of the fungus: 7 and 14 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

It is known that wheat grain has a very high nutritional value, containing 2.0-3.5% cellulose, 5.6-8.5% pentosans, 62-72% starch, lipids, minerals (K, Ca , Mg, Si, Na, Cu, Mb, Mn, Se, Fe, Zn (Alfthan G. and Neve J., 1996; Piergiovanni A.R. et al., 1997, Cakmak I., 2004, Peleg Z. et al., 2008), B vitamins (B1, B2, B5, B6) and vitamin PP, 10-16% proteins, prolamins, glutelins, albumins and globulins (Brooker D.B., 1992). The 30 aminoacids increases the biological value of wheat, a lesser amount being reported for lysine, tryptophan and threonine (Šramková Z., Gregová E. and Šturdík E., 2009).

Meanwhile, being heterotrophic organisms, fungi are ubiquitous, using a variety of resources to their nutrition from the living environment. Effect of carbon sources on the survival of fungi is given by changes in the activity of oxidoreductive enzymes like catalase and peroxidases (Ahmed S. and Pritchard C.G., 1970), some of catalases are coupled with the development cycle of microorganisms (Kawasaki L. et al., 1997; Michán Sh., 2002), data from the literature indicating the involvement of peroxidase in the generation of oxygen radicals in spores (Aver'yanov A. et al, 2007), linking between amino acids metabolism and activity of catalase and peroxidase (Manoliu Al. et al., 2008) and a relationship between metal uptake by fungi and oxidative enzymes activity is mentioned frequently in the literature (Ayar-Kayali H. and Tarhan L., 2005). Vitamins have also an important role in growth and development of fungi, improving the mycelium growth, germination and sporulation of spores, catalase activity is particularly stimulated by folic acid, pyridoxine and thiamine in the mycelium and culture fluid of *Chaetomium globosum* species (Manoliu Al., Oprica L., 2005).

A prime objective of our study was to determine the peroxidase activity, an enzyme that is distributed in mitochondria and in peroxisome, and catalyzes dehydrogenation of many organic compounds such as phenols and aromatic amines, hydroquinone, especially benzidine derivatives.

The results depicting peroxidase activity in the fungus mycelium is illustrated in figure 1. Thus, at 7 days after inoculation, all working variants recorded higher values compared to the control variant (0.802 UP/g/min), ranging between 1.991 UP/g/min. at version V1 (treated with 10 g/l grinded caryopsis) and 3.146 UP/g/min in version V2 (treated with 20 g/l grinded caryopsis).

With the ageing of the fungus (14 days from insemination of culture medium), there is a significantly decrease of enzymatic activity, all working variants recording higher values than the control variant, except V2 version . Thus, minimal activity was detected in the variant of treatment with 20 g of grinded wheat caryopsis (0.1935

UP/g/min.) and was comparable to untreated control variant (0.229 UP/g/min.), while the variants V1 and V3 showed 0.604 UP/g/min activity, respectively, 0.763 UP/g/min.

Further we determined peroxidase activity at 7 and 14 days after inoculation in culture liquid, knowing that fungi are able to discharge a number of compounds in the environment.

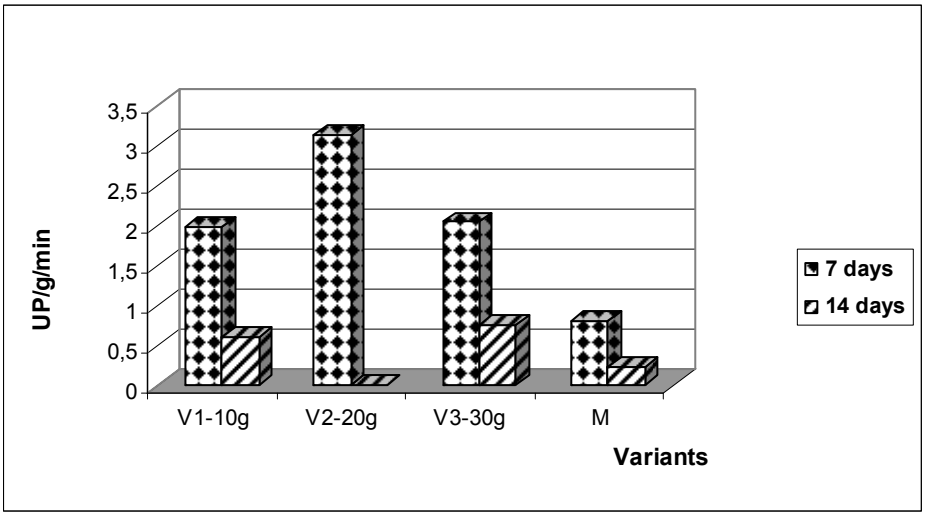

Figure 1 **Peroxidase activity in mycelium of *Rhizopus nigricans* species grown on medium with grinded wheat caryopsis**

As depicted in figure 2, at 7 days after insemination for all variants were recorded higher values compared with the control variant, peroxidase activity increased in relation to the concentration of grinded wheat caryopsis, the maximum of peroxidase activity was registered at V3 version (0.768 UP/ml/min), followed in descending order by the version with 20 g/l grinded caryopsis (0.478 UP/ml/min) and the version with 10 g/l grinded caryopsis (0.246 UP/g/min).

At 14 days after inoculation there is a significantly decrease of peroxidase activity in both experimental variants and the control variant. Only version V2 has a higher enzyme activity (0.0375 UP/ml/min) compared to the control, the other two variants (V1 and V3) with lower values compared to the control (0.023 UP/ml/min).

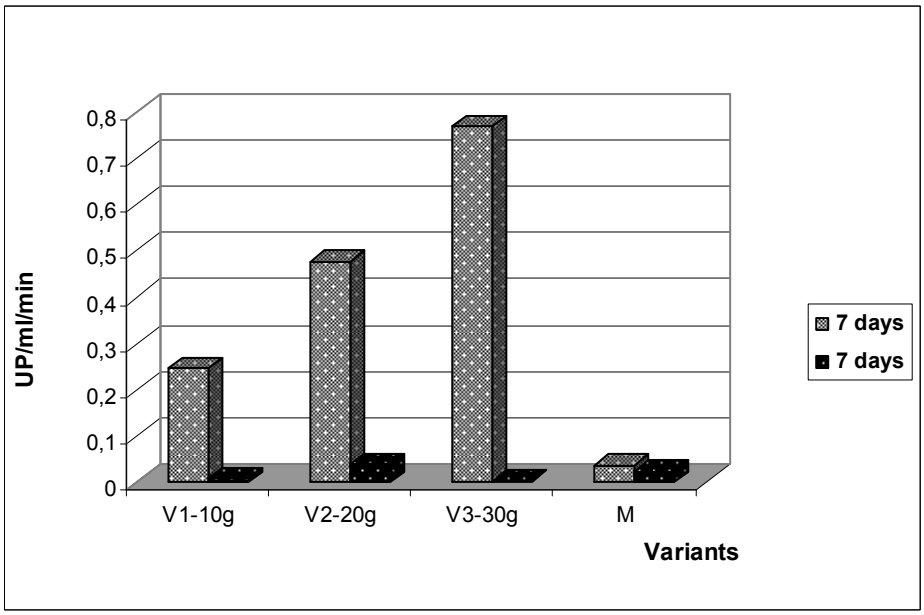

Figure 2 **Peroxidase activity in culture liquid of *Rhizopus nigricans* species grown on medium with grinded wheat caryopsis**

It is known that organisms use external power sources only after they are converted into usable forms in metabolism, mechanisms of energy conversion is related to redox processes involving transfer of electrons through a series of

intermediate carriers that make up the respiratory chain.

Although, indispensable for the life of many microorganisms, the use of oxygen in some cases can lead to the development of compounds toxic to the living cell (Ganstrom T. et al., 2002, Pinheiro R. et al., 2002), oxygen itself is a very reactive compound due its affinity for electrons. It can absorb radiation, either directly or through cellular compounds (tetrapyrrols, flavin, chlorophylls, retinoids, etc.), resulting a very reactive species that is singlet oxygen, and due to the reduction is formed hydrogen peroxide (Lledias F. et al ., 1998).

Most cells have the ability to produce and remove ROS by specific mechanisms that detect and maintain these reactive molecular species to a level as low as possible, antioxidant enzymes such as catalase, superoxide dismutase and peroxidase, as well as antioxidants such as ascorbate, tocopherol and glutathione intervening in this respect (Izava, S. et al., 1996).

Therefore the next indicator taken into study was catalase. Data on catalase activity in mycelium and culture liquid from *Rhizopus nigricans* are presented graphically in figures 3 and 4.

As shown in figure 3, for the fungal mycelium, in the first interval of studied period, catalase activity showed higher values for all variants (1014.205 UC/g/min. - for variant V1, 1046.694 UC/g/min. - for variant with 20 g/l of grinded caryopsis and 993.701 UC/g/min. – for the variant in which the medium contains a concentration of 30 g/l grinded wheat caryopsis) than the control (184.153 UC/g/min.), the results are comparable, no significant differences were detected depending on the concentration of grinded caryopsis of the medium. At 14 days after insemination of the culture medium are observed, compared to peroxidase, net differences, the first working version recording a drastic decrease in catalase activity (15.1486 UC / g / min.), while the variant V2 activity continues to grow (2819.36 UC/g/min.).

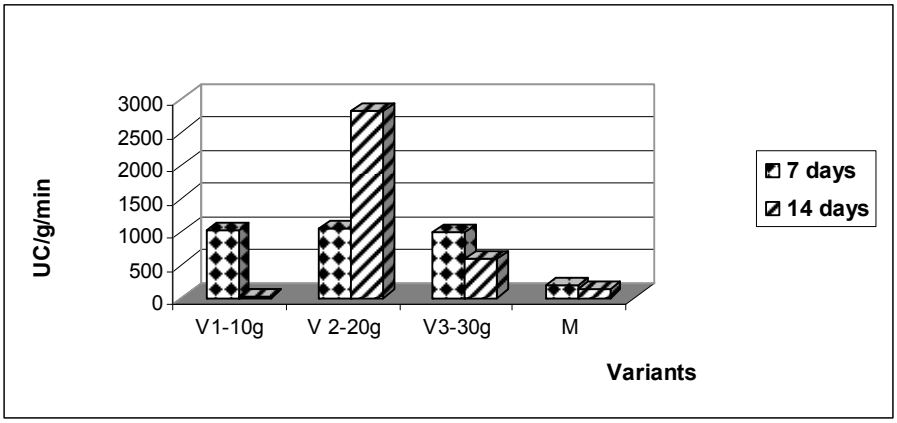

Figure 3 **Catalase activity in mycelium of *Rhizopus nigricans* species grown on medium with grinded wheat caryopsis**

Analysis of the catalase activity in culture liquid indicates changes in extracellular enzyme activity over time in all medium variants, except variant V3, in which culture ageing has not led a strong decrease of activity (99.766 CU/ml/min. at 7 days and 98.815 CU/ml/min. at 14 days after inoculation on culture medium).

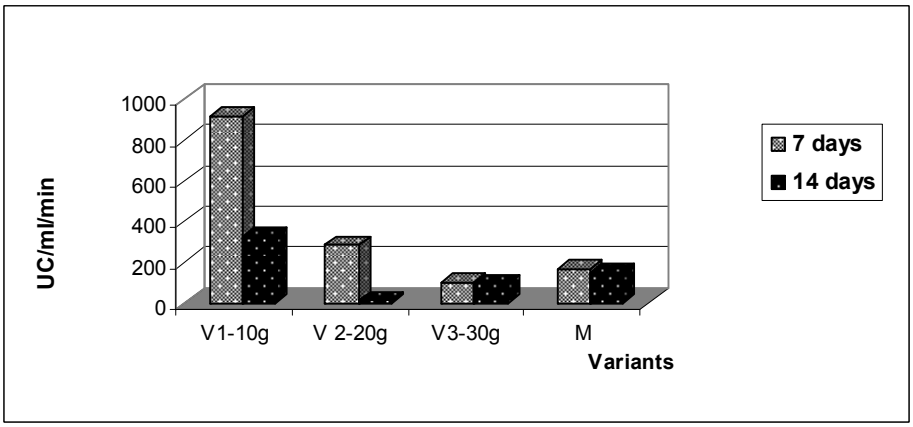

Figure 4 **Catalase activity in culture liquid of *Rhizopus nigricans* species grown on medium with grinded wheat caryopsis**

CONCLUSIONS

Our experimental results showed that catalase and peroxidase activity is influenced both by concentration of grinded wheat caryopsis and by fungus culture age.

At 7 days after inoculation the activity of peroxidase in mycelium was stimulated compared to the control, the maximum being reached in variant with 20 g/l grinded wheat caryopsis, and with the ageing of the culture (after 14 days from inoculation) the enzyme has considerably reduced the activity in all examined variants.

In the culture liquid, peroxidase activity was stimulated in the first period in all variants containing wheat caryopsis, and in the second period was inhibited, probably due to depletion of nutrients from the environment.

At 7 days after inoculation, the activity of catalase in the mycelium was stimulated in all variants containing grinded wheat caryopsis, and at 14 days after inoculation were stimulated only variants with 20 g/l and 30 g/l grinded wheat caryopsis, the variant with 10 g/l was inhibited.

In the culture liquid catalase activity was stimulated in the first period, except the variant with 30 g / l, while in the second period was stimulated only in the variant with 10 g/l wheat caryopsis.

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