RESEARCHES REGARDING SOME DEHYDROGENASES ACTIVITY IN FUNGUS *RHYZOPUS NIGRICANS* GROWN ON MEDIUM WITH DIFFERENT CONCENTRATION OF GRINDED WHEAT CARYOPSIS

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Abstract

This study followed the activity of dehydrogenases in the Krebs cycle: malate dehydrogenase, isocitrate-dehydrogenase, succinate dehydrogenase, alpha-ketoglutarate dehydrogenase, and glucose-6-phosphate dehydrogenase, an enzyme enzyme of pentose phosphate pathway in fungus *Rhizopus nigricans*. For conducting experiments, the fungus was cultivated on medium with different concentrations of grinded wheat caryopsis. From the composition of the liquid medium Czapeck Dox, carbon source (sucrose) was replaced with different amounts of grinded wheat caryopsis, resulting three variants: V1 = 1g/100ml, V2 = 2g/100ml, V3 = 3g / l and a control variant, in which the composition of medium remains unchanged. Experimental measurements were carried out at two intervals: 7 and 14 days, and were performed using fungus mycelium. The results showed the influence of fungus age and different concentrations of grinded wheat caryopsis from the culture medium composition, with significant differences between the two measurements and between the working versions.

Key words: *Rhizopus nigricans*, dehydrogenases, Krebs cycle, wheat caryopsis

The seeds, considered to be important means of production, represent the starting point of high yields with higher quality indices. Framed in phytotechnic plants group, with an extended ecological plasticity and a large food weight, wheat is one of cereals that may be contaminated by a variety of microscopic fungi during it's development. Infected seeds may determine low production with low quality by poor germination leading to uneven emergence, transmission of diseases that cause significant damage, low quality of seed, production of mycotoxins that affect the health of consumers and causing poisoning to various animals (Raicu, R., Baciu, D., 1978, Rabie, C.J. et al., 1985, Birck, N.M.N. et al., 2006).

Wheat caryopsis composition - starch, dextrins and simple carbohydrates, proteins like prolamins, glutelins, albumins and globulins, lipids, celullose, minerals such as K, Ca, Mg, Si, Na, Cu, Mb, Mn, and B vitamins (B1, B2, B5, B6), vitamin PP, E, K, H, and lower amounts of vitamin A (Starodub, V., 2008) - makes caryopsis vulnerable to attack of fungi from storage mycoflora, that affect stored seeds in improper temperature and humidity conditions.

Purchasing energy is a vital property of the microbial cell, which is achieved by releasing the chemical energy of different nutrients and transforming it through the phosphorylation in macroergic compounds. In energy metabolism, the nutrient substrate passes by oxidation and redox processes, catalyzed by enzymes, in oxidized substrate, releasing potential energy (Jelea, M., 2008).

During the citric acid cycle, which is the final part of the respiration process, dehydrogenases transfer hydrogen atoms to redox carriers NAD and FAD, that are reduced. On the inner surface of mitochondrial membrane, these reduced coenzymes are then reoxidate and oxygen is reduced to water via the electron transport chain. The energy released by electron transfer is used for ATP synthesis (Kavanagk, K., 2005).

Rhodes, R.A. et al. (1959) showed that species of *Rhizopus arrhizus* are producing large amounts of tricarboxylic acids and mainly fumaric acid when grown on mediums with low concentrations of glucose and Foster J.W. and Waksman S.A. (1939) showed the fumaric acid production in *Rhizopus nigricans*, emphasizing the influence of heavy metals on it, studies completed

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by Wegener W.A.S., and Romano A.H. (1964), Romano A.H., et al. (1969), Overman S.A. and Romano A.H. (1969).

Because *Rhizopus nigricans*, saprophytic fungus that is part of the storage micoflora, affects seeds stored under improper temperature and humidity conditions, the present study was intended to be a monitorisation of the activity of main tricarboxylic acid cycle dehydrogenases – isocitrate-dehydrogenase, α-ketolutarate-dehydrogenase, succinate-dehydrogenase, malate-dehydrogenase, and the glucose-6-phosphate dehydrogenase, an enzyme that participate in the development of pentose phosphate pathway in this species, grown in laboratory conditions.

MATERIAL AND METHOD

The fungus was isolated from germinated wheat caryopsis, which were taken from wirehouses of the Enterprise of Cereal Products from Chişinău, Republic of Moldova.

Pure culture was obtained after several subculturing on solid medium Czapeck Dox agar, on petri dishes.

To investigate the activity of Krebs cycle dehydrogenases and glucose-6-phosphate dehydrogenase, *Rhizopus nigricans* was cultivated on 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). Sucrose from the composition of the medium was replaced with different concentrations of grinded wheat caryopsis, resulting three experimental variants: V1 = 1 g/100ml, V2 = 2 g/100ml, V3 = 3 g/100ml and a control version, in which the composition of medium remained unchanged.

The three types of medium were transferred into Erlenmeyer flasks, in quantities of 100 ml in each flask. All flasks were inoculated with disks of 8 mm in diameter from 7 days old culture of *Rhizopus nigricans*. The flasks were incubated in the thermostat set at 28˚C. Measurements were made, using the fungus mycelium, at 7 and 14 days after inoculation.

Determination of dehydrogenases activity was performed using Sîsoev method, modified by Artenie Vl. (Cojocaru, D.C., 2009).

RESULTS AND DISCUSSIONS

Results on the influence of different concentrations of grinde wheat caryopsis, which were introduced into the culture medium, on the activity of malate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, alpha-ketoglutarate dehydrogenase, and glucose-6-phosphate dehydrogenase, obtained from experiments, are shown graphically in figure 1-5.

Malate dehydrogenase activity, illustrated in figure 1, recorded, in the first period of the fungus inoculation, higher values for all variants compared with control (0.0644 µg formazan/g biomass), enzymatic activity increased in relation to concentration of grinded wheat caryopsis, the maximum being reached in variant V3 (0.7507 µg formazan/g biomass), followed by V2 version, treated with 20 g/l wheat cariopse ground (0.3447 µg formazan/g biomass) and in the variant V1 was found minimum value (0.2678 µg formazan/g biomass).

At 14 days after insemination of culture medium, with the ageing of the fungus, there is a significantly decrease of enzymatic activity in all variants, except variant V3 (1.1262 µg formazan/g biomass), in which the level of enzymatic activity was amplified. All experimental variants showed higher values (0.1066 µg formazan/g biomass for version V2 and 0.0315 µg formazan/g biomass for version V1) compared to the control variant (0.0122 µg formazan/g biomass).

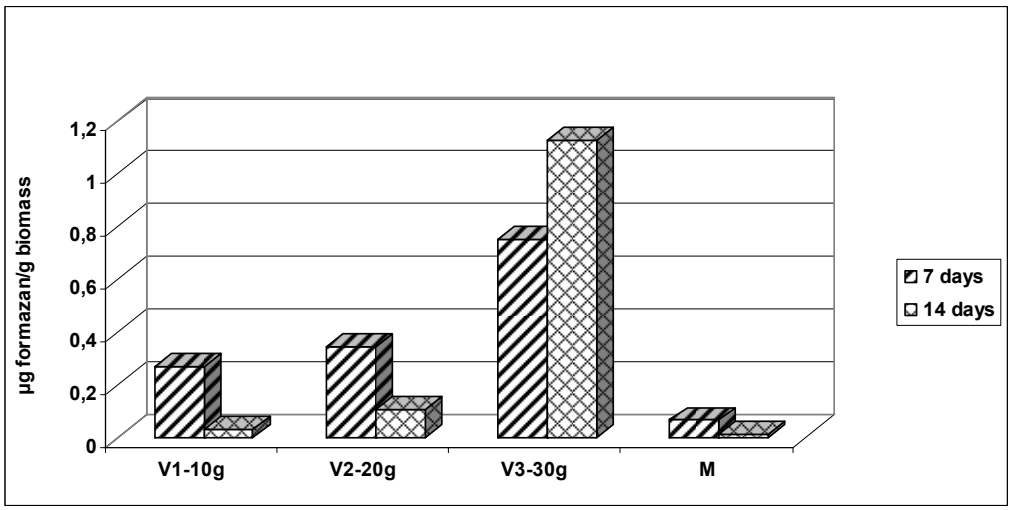

Figure 1 **Malate dehydrogenase activity in *Rhizopus nigricans* species**

As found in figure 2, isocitrate dehydrogenase activity, in the first period from the inoculation of culture medium, have much higher values in all experimental variants compared with the control variant (0.1966 µg formazan/g biomass), enzymatic activity increases in invers relation with the wheat caryopsis concentration contained in culture medium. Maximum value was recorded in the variant of treatment with 10 g/l grinded wheat caryopsis (1.9447 µg formazan/g biomass), followed by a proximate value at the V2 version with 20 g/l grinded caryopsis (1.7602 µg formazan/g biomass), the minimum value recorded in the variant with 30 g/l grinded wheat caryopsis (1.043 µg formazan/g biomass).

The ageing of the culture entailed a drastic reduction of activity of this enzyme in all variants (0.3413 µg formazan/g biomass – for the version V1 and 0.4142 µg formazan/g biomass – for the variant V2), being almost inhibited in the variant V3 (0.0314 µg formazan/g biomass). V3 version is the only variant that showed values lower than the control variant (0.1326 µg formazan/g biomass).

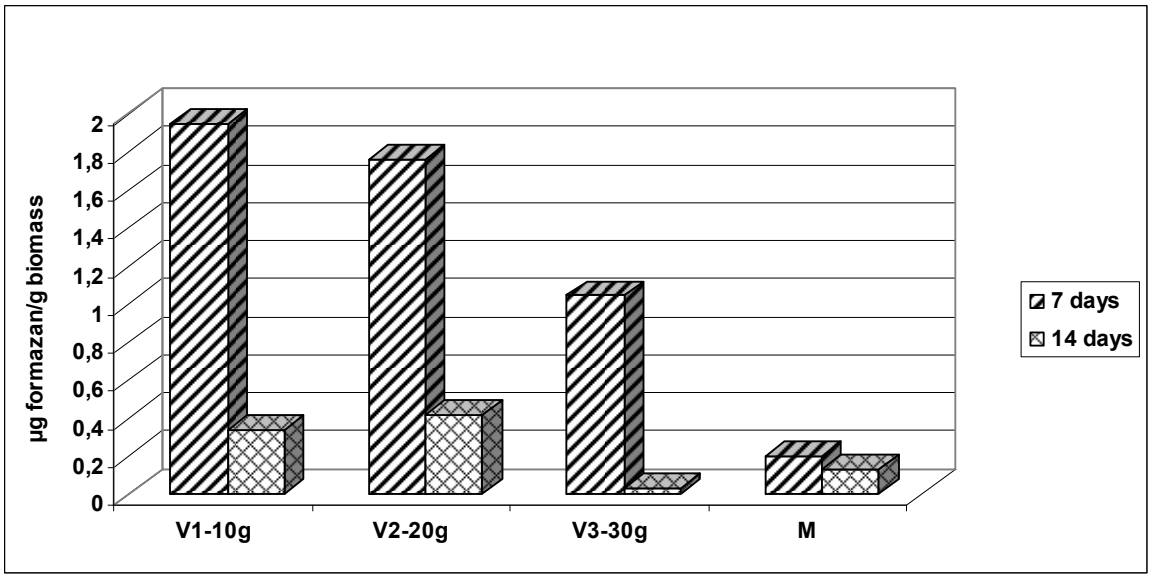

Figure 2 **Isocitrate dehydrogenase activity in *Rhizopus nigricans* species**

Data on the dynamics of succinate dehydrogenase activity in the mycelium of fungus *Rhizopus nigricans* grown on medium with different concentrations of grinded wheat caryopsis are reproduced graphically in figure 3.

The analysis of the chart reveals that at 7 days after insemination of the culture medium, all experimental variants showed higher values compared with control variant (0.0913 µg formazan/g biomass), the maximum activity occurring at version V2 (0.3061 µg formazan/g biomass), followed in descending order by variant V1 (0.1981 µg formazan / g biomass) and variant V3 (0.1754 µg formazan/g biomass).

At 14 days after inoculation succinate dehydrogenase activity has reached maximum value in the variant V3 (0.1926 µg formazan/g biomass), the rest of the variants showing rather low levels (0.0678 µg formazan/g biomass for the version with 10 g/l wheat caryopsis and 0.0739 µg formazan/g biomass for the version with 20 g/l wheat caryopsis), close to the control value (0.0457 µg formazan/g biomass).

The results of monitoring for alpha-ketoglutarate dehydrogenase activity are shown in figure 4.

In the first study period it can be seen that all medium variants, containing grinded wheat caryopsis, showed higher values compared with control variant (0.0644 µg formazan/g biomass). Maximum activity was recorded in version V2 (0.2832 µg formazan/g biomass), followed by version V3 (0.1602 µg formazan/g biomass) and variant V1 (0.0968 µg formazan/g biomass).

The ageing of fungus has led to amplification of alpha-ketoglutarate dehydrogenase activity in all variants, except control version who registred a value very close to "0" - 0.0081 µg formazan/g biomass.

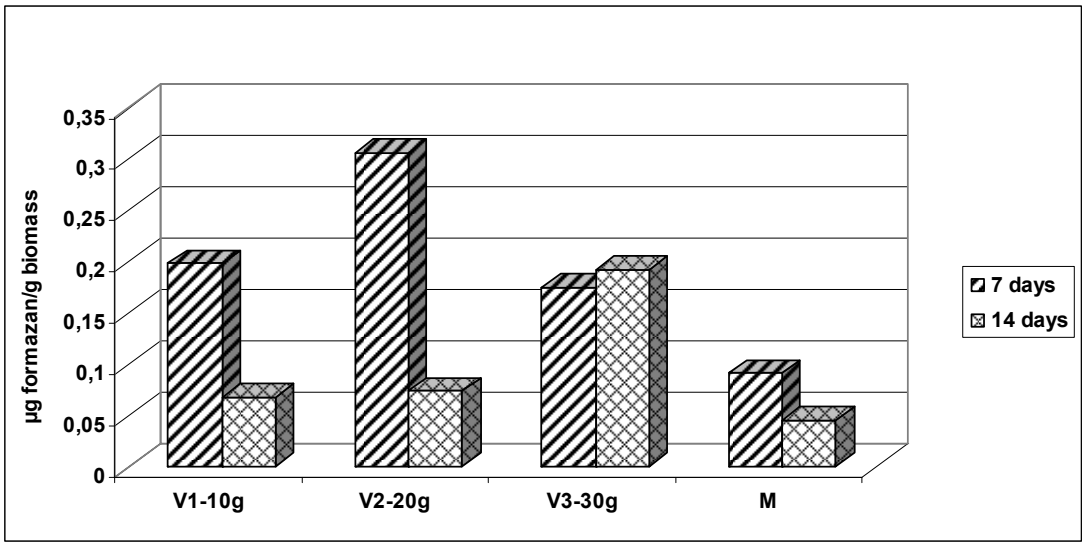

Figure 3 **Succinate dehydrogenase activity in *Rhizopus nigricans* species**

The maximum value was recorded in version V3 (0.4244 µg formazan/g biomass), followed in descending order by variants V2 (0.3208 µg formazan/g biomass) and V1 (0.2435 µg formazan/g biomass), enzymatic activity increasing in relation with the increase of grinded wheat caryopsis concentration from culture medium composition.

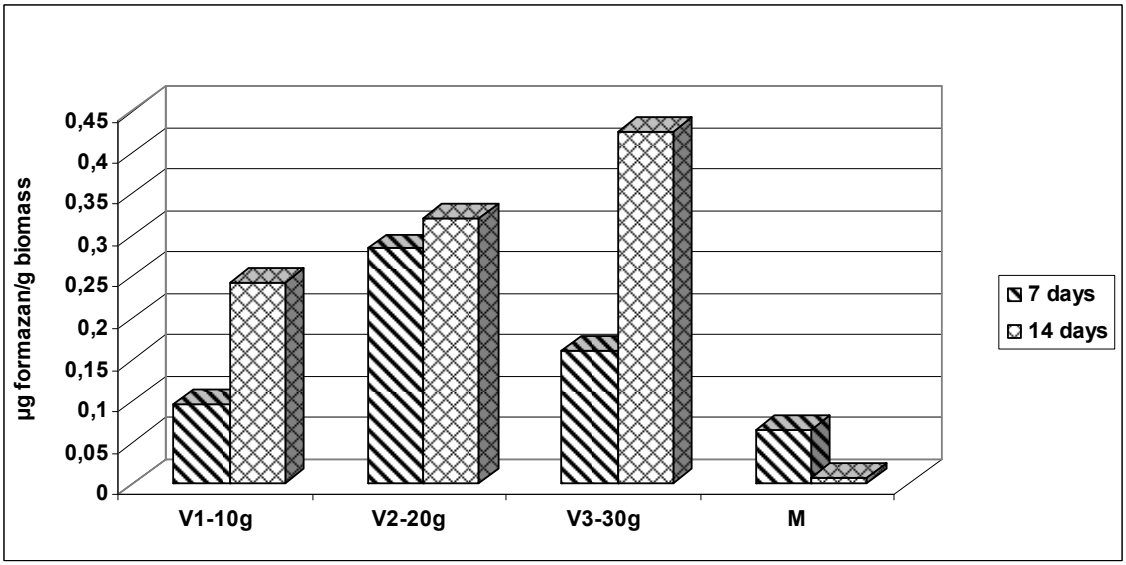

Figure 4 **Alpha-ketoglutarate dehydrogenase activity in *Rhizopus nigricans* species**

In the first period after inoculation of culture medium, glucose-6-phosphate dehydrogenase activity (*figure 4*) shows low values, close to the value of control version (0.0356 µg formazan/g biomass) in all variants (0, 0974 µg formazan/g biomass - for the variant V1, 0.0459 µg formazan/g biomass – for the variant V2 and 0.0873 µg formazan/g biomass – for the variant V3), the results are comparable, not being able to detect significant differences depending on the concentration of grinded caryopsis of the medium.

In the second period under study, enzyme activity showed higher values in all experimental variants (values ranging between 0.1144 and 0.5653 µg formazan/g biomass), including control version (0.069 µg formazan/g biomass), compared with the first determination.

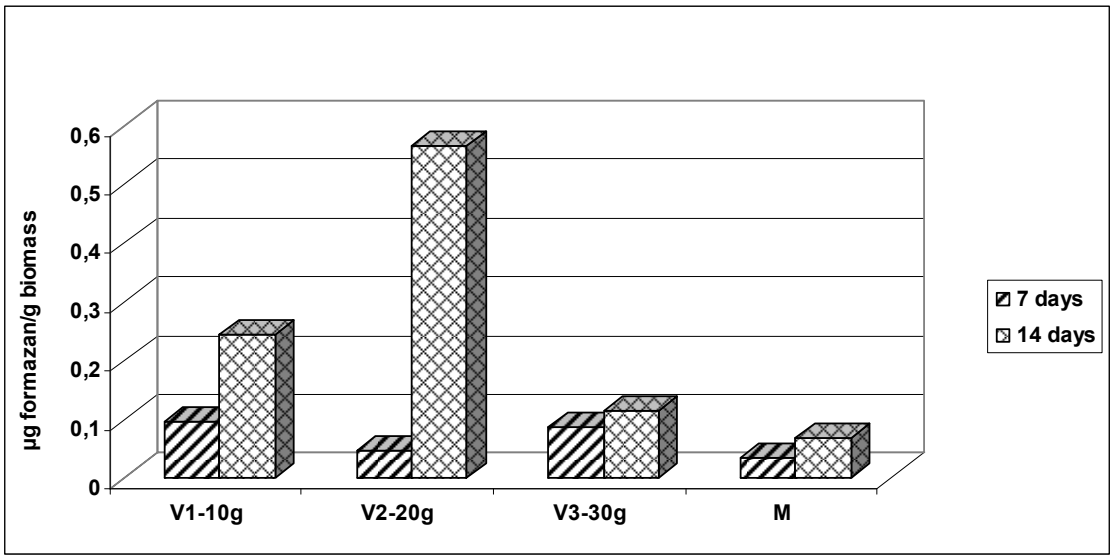

Figure 5 **Glucose-6-phosphate dehydrogenase activity in *Rhizopus nigricans* species**

CONCLUSIONS

After the analysis, the experimental data has shown that the activity of Krebs cycle dehydrogenases and glucose-6-phosphate dehydrogenase was influenced by both by concentration of grinded wheat caryopsis and by fungus culture age.

At 7 days after insemination, malate dehydrogenase activity was stimulated in variants with 20 g/l and 30 g/l grinded wheat caryopsis, while at 14 days after insemination, the enzymatic activity was stimulated in version whit 30 g/l grinded wheat caryopsis.

The first period isocitrate dehydrogenase activity was stimulated in all work versions by the grinded wheat caryopsis presence, and in the second period it was stimulated only in the variants with 10 and 20 g/l grinded wheat caryopsis.

Succinate dehydrogenase activity was stimulated in all work versions in both studied periods.

In the first and second study periods alpha-ketoglutarate dehydrogenase activity was stimulated in all variants containing grinded wheat caryopsis.

After 7 days from inoculation, the activity of glucose-6-phosphate dehydrogenase was inhibited in all experimental variants, and after 14 days it was stimulated, except variant with 30 g/l wheat caryopsis, in which culture age did not cause a significant increase in activity of this enzyme.

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