RESEARCH ON THE INFLUENCE OF H^+ IONS CONCENTRATION ON THE DYNAMICS OF THE ACTIVITIES OF CERTAIN DEHYDROGENASES OF THE KREBS CYCLE IN THE *MONILINIA LAXA* (ADERH. & RUHL.) HONEY FUNGUS PARASITIC ON PLUM TREES

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Keywords: *Monilinia laxa* (Aderh.&Ruhl.) Honey, dehydrogenases, Krebs cycle, H^+ ions concentration, pH.

Abstracts: During the process of nutrition, thus in that of their growth, microorganisms are subject to the influences of certain environmental factors that condition the microbial activity determining either the growth and reproduction, or the inhibition of activity and the inactivation of microorganisms. A well known means of expressing the H^+ ions concentration in a certain environment is the pH, an important chemical factor that is closely observed when growing ascomycetes, for any alteration of its value entails conformational alterations of their enzymes, the characteristics of the substrate, such that they can no longer interact with the active site of the enzyme or be subject to catalysis. The present study comprises the results of our research on certain oxidoreductase implied in the steps of the Krebs cycle in the *Monilinia laxa* (Aderh.&Ruhl.) Honey, a fungus that parasites the prune. The enzymatic determinations took place at 7 and 14 days from the mycelium of the fungus cultivated in Leonian media, whose pH was adjusted to values between 2.0 and 9.0 by using NaOH 1N and HCl 0,1N solutions. We registered different values of the dehydrogenasic activity, directly correlated with the physiological condition of the fungus (given its age) and with the initial pH value of the culture’s environment.

INTRODUCTION

The cellular metabolism of microorganisms in the nutrition process is influenced by a series of chemical and physical factors, among which the concentration of environmental hydrogen ions. It is well known that pH is an essential means of measuring the concentration of hydrogen ions in biological systems and that it can influence the three-dimensional structure of proteins, including the enzymes that participate in the cellular metabolism, the transport of nutrients and the electrons transfer (Dunca, S. *et al*., 2005, Cojocaru, D. C. *et al.,* 2007).

Several fungi grow on a broad range of pH values (Mehotra, R.S. and Aneja, K.R., 1990, Kawasaki, K. and Suzuki, M., 1993, Naqvi, S.A.M.H., 2004). The alterations of the external concentration of H^+ ions cause small, transitory changes in the intracitoplasmatic pH, that is around 7,6 in most filamentous fungi and whose existence is due to a homeostatic pH mechanism localized intrahifally (Bachewich, C.L. and Heath, J.B., 1997, Bagar, T. *et al*, 2009**)**. The ability of responding to the environmental pH variation in the filamentous fungi is realized by means of a mediation system comprising membranous cytoplasmatic proteins, signal transduction pathways and signal dependant pH transcription factors, acting as gene repressors or activators and their expression represents the answer of the fungal cell and constitutes a key-factor of its virulence, by intervening in the production of mycotoxins and antibiotics, and last but not least in the enzymatic activity (Peñalva, M.A. and Arst, Jr., H.N, 2002, Calcagno-Pizarelli, A. M. *et al*., 2007, Hervas-Aguilar, A. *et al*, 2010, Hua, X. *et al*, 2010)**.**

The present study wishes to be a continuation of certain research on the biology of the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus cultivated in environments with different pH values (Manoliu, Al. *et al.*, 2010); during the experiments we monitored the activities of four key enzymes of the Krebs cycle: isocitrate dehydrogenase (E.C.1.1.1.41), α – ketoglutarate dehydrogenase (1.2.4.2), succinate dehydrogenase (E.C. 1.3.99.1), malat dehydrogenase (E.C.1.1.1.37). The reasons at the root of this study are related to the following statements: the oxygen’s involvement in metabolic processes within living organisms has to do with its activation and the formation of a large number of very reactive compounds (Gessler, N.N. *et al*., 2007); the respiratory chain, whose reactions have to do with those of the citric acid cycle, is a rich source of oxygen (Turrens, J.F., 1996), the mitochondria thus becoming a vulnerable target of oxidative stress, thus affecting the functioning of the whole Krebs cycle (Hyslop, P.A. *et al*, 1988, Tretter, L. and Adam-Vizi, V., 2000), as well as the fact that the Krebs cycle enzymes are pH-sensitive (Kubicek, C.P., 1988, Papagianni, M., 2007). The central aspects of the present study consists of establishing a degree of susceptibility to various concentrations of H^+ ions of the main dehydrogenasese involved in the four stages of the tricarboxylic acids cycle and the quantification of their activity at different time intervals for the purpose of evaluating their dynamics.

MATERIALS AND METHODS

The *Monilinia laxa* (Aderh.&Ruhl.) Honey strain was isolated in the laboratory, its source being represented by fruits mummified by fungi harvested from genera of *Prunus domestica* from the Experimental Orchard of the Fruit Trees Research and Development Station in Miroslava, Iasi. The pure culture was obtained from the sporodochia previously

washed in distillate water and placed in Petri boxes with a PDA medium, and kept for 7 days in thermostat. The *in vitro* cultivation of the fungus, in submerse conditions, was made in stationary conditions, in the dark and at a constant temperature of 28º C, the fungus being placed on discs with a diameter of 0,8 mm in Erlenmayer flasks, with a Leonian environment. The various concentrations of hydrogen ions in the culture environment were obtained by means of an adequate buffer of NaOH 1N and HCl 0,1N. The pH scale varied between 2.0 and 9.0, thus obtaining 9 probes, of which one was a control probe, whose pH had not been altered. To determine the biochemical experiments, we took samples from the mycelium of the fungus at 7 and 14 days after setting the culture environment.

The method used is mainly based on the dehydrogenases' capacity to transfer hydrogen from various sub layers to the 2,3,5-triphenyltetrazolium chloride that reduces itself and passes to red trifenyl formazan. The intensity of the colour of the resulting formazan was spectrophotometrically determined by means of the Sîsoev and Krasna method (modified by Artenie). The studies indicated significant differences in the dynamics of the enzyme's activity, due on the one hand to hydrogen ions concentration in the environment, and on the other hand to the age of the mycelian culture.

RESULTS AND DISCUSSIONS

The results of the experimental determinations made during the two time frames in the mycelium of the *Monilinia laxa (*Aderh.&Ruhl.) Honey fungusare presented in Figures 1 and 2.

The rigorous analysis of these results indicates a relative proportionality of the values of the four enzymes that catalyse the main stages of the Krebs cycle, suggesting a sort of continuity and a balanced development of the reactions involved in both of the time frames.

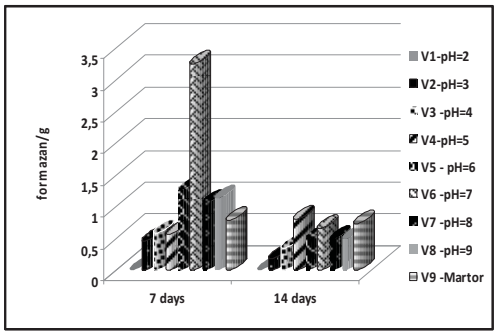

Fig.1. The activity of the isocitrate-dehydrogenase in the *Monilinia laxa* (Aderh.&Ruhl) Honey mycelium cultivated on the media with various H^+ ions concentrations

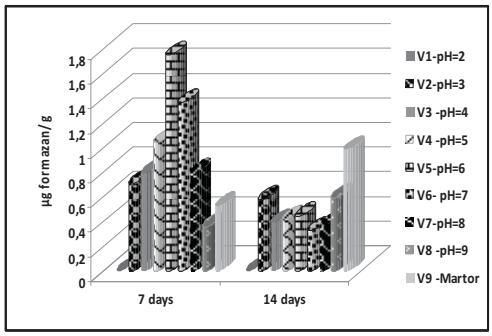

Fig.2. The activity of the α-ketoglutarate-dehydrogenase of the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus cultivated on the media with various H^+ ions concentrations

A maximum activity of the isocitrate-dehydrogenase was indicated at the day 7 stage (3,2390 µg formazan/g.mat.) in the pH -7 probe, followed by the probe with the initial pH 6, where we registered a value of 1,2601 µg formazan/g.mat.biol. The inferior limits of the endoenzyme's activity in this time frame were registered in the acid interval of the selected pH scale (pH- 2 , pH- 3 – 0,4476 µg formazan/g.mat.), with some growth on the pH- 5(0,5642 µg formazan/g.mat.), which attests to the data in the literature, which situates the optimum value of the pH for the isocitrate dehydrogenase between 7 and 8 (Cjocaru, D.C., 2009), and the inhibition point in the acid extremity of the pH scale (Adrio, J.L. and Demain, A.L., 2005). The ageing of the culture brought a relative uniformization of the activity of the isocitrate dehydrogenase manifested by a diminishing of the activity of the endoenzyme in all probes, except V2 and V3. Thus, at day 14 stage the highest activity was registered environment where the initial pH was 5 (0.7957 µg formazan/g.mat.biol.), followed by the control sample (0,7299 µg formazan/g.mat.biol.). The lowest value of the isocitrate dehydrogenases activity was registered in the pH 3 sample, i.e. probe V2 (0,1743 µg formazan/g.mat.biol.).

In the young culture, 7 days after the incubation, the optimum pH of the α-ketoglutarate-dehydrogenase in the mycelium of the fungus was registered in the V5 probe (1,7504 µg formazan/g.mat.biol.), the endogenous enzyme manifesting an intense activity both at pH 7 (1,3627 µg formazan/g.mat.biol.) and at pH 5 (1,0415 µg formazan/g.mat.biol.), which attests to the data presented in the literature and which places the optimum pH of the enzyme between 7,2 and 7,4 (Hirabayashi, T. and Harada, T., 1971), that is close to our results. Comparatively, at pH 8, pH 4 and pH 3, the α-ketoglutarate-dehydrogenase biosynthesis had lower levels at various levels and at pH 9 the oxoglutarate dehydrogenase was inhibited. The ageing of the culture brought along a diminishing of the activity of the oxoglutarate dehydrogenase in all the probes as compared to the control probe (0,9842 µg formazan/g.mat.biol.).

As for the the activity of the enzyme that catalyses the dehydrogenation of the succinic acid into fumaric acid, at the first interval of biochemical quantitative determinations it a maximum of 1,2994 µg formazan/g.mat.biol. following by the 1,1718 µg formazan/g.mat.biol. in the mycelium of the *Monilinia laxa* cultivated submersely on the medium with an initial value of the pH 3, by the 1,0942 µg formazan/g.mat.biol. in the medium with pH 5, 1,0100 µg formazan/g.mat.biol. in medium with pH 4 and is followed by a level of 0,479807 µg formazan/g.mat.biol., decelated in the initial pH 9 sample.

The running of the enzymatic tests during day 14 since the inoculation indicates a different level of activity of the succinate dehydrogenase in the acid extremity of the chosen pH scale, placing its highest point in the V4 sample (0,7865 µg formazan/g.mat.biol.), followed decreasingly by probes V6 with pH 7, V3 with pH 4 (0,7253 µg formazan/g.mat.biol. and 0,5593 µg formazan/g.mat.biol., respectively), V5 with pH 6 (0,4494 µg formazan/g.mat.biol). The activity of the endogenous enzyme in the mycelium grown on the medium with the initial pH 9 (0,2315 µg formazan/g.mat.biol.) is comparable to the one registered in the medium with pH 3 (0,2241 µg formazan/g.mat.biol), in lower levels, followed by the one in V7 with inital pH 8 (0,1953 µg formazan/g.mat.biol.).

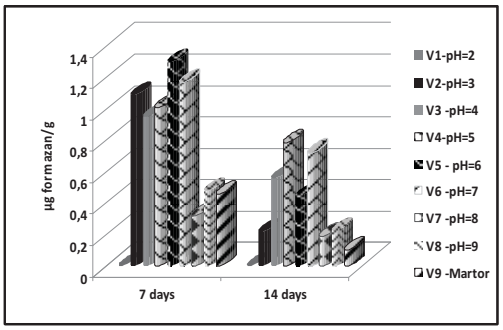

Fig.3. The activity of the succinate-dehydrogenase of the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus cultivated on the media with various H^+ ions concentrations

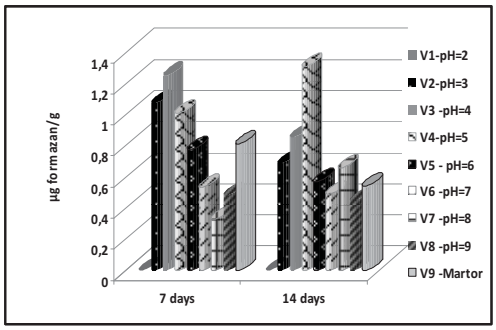

Fig.4. The activity of the malat-dehydrogenase of the *Monilinia laxa* (Aderh.&Ruhl.) Honey fungus cultivated on the media with various H^+ ions concentrations

If we reconsider the determinations from day 7, we notice the existance of an oxalacetate formation rate, catalysed by the intensive malat dehydrogenase in the acid side of the pH scale. Thus, in probe V3 (pH 4) the activity of the endogenous enzyme was 1,2528 µg formazan/g.mat.biol., and in V2 (Ph 3) and V4 (pH 5) the values are 1,0718 µg formazan/g.mat.biol. and 1,0101 µg formazan/g.mat.biol.

A classification of the modulator effect produced by the hydrogen ions concentration n the activity of the malat-dehydrogenase activity in the mature culture maintains the intense conversion rate of the malat in oxaloacetic acid in the acid part of the current experimental pH scale, except its extremity (pH 2), where the enzyme activity is null. Thus, in the V2 sample (pH 3) the regeneration of the oxalacetate rendered by the activity of the endoenzyme reached a level of 1,0718 µg formazan/g.mat.biol., in V4 (initial pH 5) a level of ,0101 µg formazan/g.mat.biol. but the maximum activity was registered in V3 (initial pH 4), i.e. 1,2528 µg formazan/g.mat.biol.

The quantification of the enzymatic activity registered in the two time frames highlighted a series of dynamic changes in the mitochondrial activity, confirming its role in the Krebs cycle, in the conditions of interdependency with the hydrogen ions concentration from the environment, as well as its modulator effect on preserving the redox balance and the involvement of the mitochondria in the antioxidant defensive.

CONCLUSIONS

The investigations run for the analysis of the modulator role of the hydrogen ions concentration on the activity of the enzymes that catalyze the main stages of the Krebs cycle in the mycelium of the *Monilinia laxa* species, have highlighted the following aspects:

- Seven days after the incubation, the activity of the isocitrate dehydrogenase was stimulated by the pH 7, pH 6, pH9 and pH 8, while the α- ketoglutarate dehydrogenase increased the performance of the reaction it catalyzed in the media with pH 6, pH 7, pH 5, pH 8, pH 4 and pH 3. The optimum pH for the succinate dehydrogenase in the mycelium of the fungus was decelated in the probe with pH 6, the enzyme being intensified in initial pH 7 medium, in pH 5 as well pH

4 and pH 3, while the malate dehydrogenase was influenced positively in the media with acid pH, i.e. pH 4, pH 3 and pH 5.

- Fourteen days after inoculating the culture media, the activity of the isocitrate dehydrogenase was faintly intensified in the environments with pH values of 5, while the activity of the α- ketoglutarate dehydrogenase was inhibited in all the probes. In the probes with pH values of 5, 7, 4, 6, 9, 3 and 8, succinate dehydrogenase had an intense activity in this time frame, and malat dehydrogenase manifested an increased activity at an optimum pH of 5, closely followed by pH 4, pH3, pH 8 and pH 6.

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STUDIES CONCERNING THE INFLUENCE OF SOME AMINO ACIDS ON THE DYNAMICS OF KREBS CYCLE DEHYDROGENASES ACTIVITY AT *MONILINIA LAXA* (ADERH.& RUHL.) HONEY PARASITE ON PLUM TREES

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Keywords: *Monilinia laxa,* isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase.

Abstract. The amino acids are metabolised via the enyzmatic reactions of the Krebs cycle, the central mechanism for metabolism in the cell that is generating energy for production of adenosine triphosphate (ATP) molecules. For this reason, in the present paper, the dynamics of the isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase is investigated in mycelium of submerged cultivated strain of *Monilinia laxa* fungus and the analysis of the results concerning the influence of some amino acids as alanine, glutamic acid, aspartic acid, asparagine, cystine, cysteine, histidine, methionine, phenylalanine, valine, leucine, lysine and serine on the Krebs cycle dehydrogenases shows that the profile of the variation curves are dependent by the type of amino acid introduced in culture medium and fungus age.

INTRODUCTION

The filamentous fungi provide their structure and functions (which involve the maintenance of biochemical and functional equilibrium) with a continuous energy input from the outside environments. Numerous data from literature describe a strong relationship between amino acids and the physiological functions of the fungal cell and nitrogen is considered to be essential for the biosynthesis of cellular molecular complexes (Isaac, S., 1992). Most fungal species cannot use inorganic nitrogen sources as well as organic compounds that contain nitrogen (proteins and amino acids) for growth, a wide range of fungi require amino acids for growth, being able to use it better in the presence of carbon sources. Amino acids degradation occurs via oxidation, with oxygen consumption, an biological process improperly called biological oxidation that takes place during the tricarboxylic acid cycle, Krebs cycle being a central point of the metabolism, linked to processes that are part of energy production (Cojocaru, D.C. and Sandu, M., 2004) and in which the carbon skeleton resulting from desamination or transamination is converted into a series of metabolic intermediates (Artenie, Vl., 1991). Amino acids such as alanine, cysteine, cystine, glycocolului, serine, threonine and hydroxyproline, usually enter the citric acid cycle via pyruvate (Lentner, C. quoted by Cojocaru, D.C. and Sandu, M., 2007), through acetyl-CoA. This too is the point of junction with the Krebs cycle of amino acids such as phenylalanine, tyrosine, leucine, tryptophan and lysine, which first forms acetacetil-CoA and phenylalanine and tyrosine are the amino acids that are coupling the Krebs cycle made through fumarate (Kang, J., 2008), while methionine, valine and isoleucine enter the tricarboxylic acid cycle at the level of succinil-CoA (Karp, G., 2009).

Other amino acids such as arginine, proline, histidine and glutamine enter the Krebs cycle by forming in advance, as an intermediary, glutamic acid, which passes through transamination to α-ketoglutarate that represents the point of connection with the citric acid cycle. Biosynthesis of intermediates containing carbon in the Krebs cycle must take into account the metabolism needs of the cell and the energy needs, many biomolecules synthesizing their hydrocarbon skeleton on the account of some intermediaries of the tricarboxylic acid cycle, which enshrines this cycle as a path with amphiboly fingerprint (catabolic and anabolic).

The metabolism of amino acids by fungi and their use in building of protein blocks, enzymes, RNA and DNA is mostly in lag phase, and the number resulting from cell biosynthesis is the maximum in the exponential growth phase, their amount decreasing with the age of the culture, all these procedures being closely linked to the Krebs cycle (Gottlieb, D. and Van Etten, J.L. 1964).

MATERIALS AND MEHODS

The biological material used in this study, represented by ascomycetous *Monilinia laxa* (Aderh. & Ruhl.) Honey was isolated from mummified fruits collected from of *Prunus domestica* varieties from the experimental orchard for Fruit Research and Development Miroslava, Iasi County. The *in vitro* cultivation of the fungus was made using the Leonian medium, shared in Erlenmeyer flasks, in which were added over 0.125 mg of the following amino acids: glutamic acid, aspartic acid, alanine, asparagine, cystine, cysteine, histidine, methionine, phenylalanine, valine , leucine, lysine and serine, working with a control sample without amino acids. The 14 medium were seeded with slices cut from a culture of *Monilinia laxa* (Aderh. & Ruhl.) Honey aged for 7 days and incubated in an thermostat. The experimental measurements performed at 7, respectively, 14 days, were made from the fungus mycelium for each treatment variant three parallel determinations were made, the enzyme activity, followed in dynamics, was determined using Sîsoev and Krasna spectrophotometric method (Cojocaru, D.C., 2009).

RESULTS AND DISSCUTIONS

A detailed analysis of the results obtained from experimental measurements performed in the mycelium of *Monilinia laxa* fungus at 7, respectively, 14 days and graphically presented in Figures 1 and 2, allowed a conclusive framework on which it can be certainty stated that the activity of dehydrogenases enrolled in the study had variations in both time intervals, depending on the type of amino acid added to the culture medium, a correlation with the age of culture mycelia was also found.

So, the isocitrate dehydrogenase during the maturation of the mycelium of *Monilinia laxa* species had an ascending trend over time, modulated by amino acids as methionine, leucine and serine (from 1.7573 µg formazan / g mat. at 1.9778 µg formazan / g mat., from 0.7606 µg formazan / g mat. up to 1.8732 µg formazan / g mat. for serine and from 0.9416 µg formazan / g mat. to 1.3913 µg formazan / g mat. for leucine).

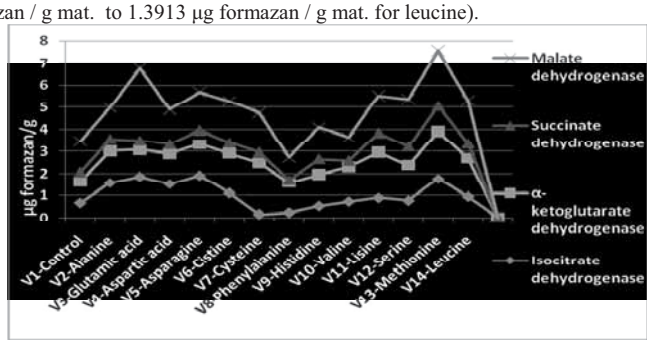

Fig. 1 – The amino acids influence on dehydrogenases of the Krebs cycle in a 7 days old mycelium from *Monilinia laxa* fungus

This suggests that in those cultures catabolic rate is high, respiration is performed at high levels and that's because on the one hand, it is necessary to convert isocitrate to oxoglutarate and, on the other hand, the need for α - ketoglutarate synthesis, essential for other amino acids biosynthesis (possibly among others, methionine, which may derive from e by synthesis "de novo", taking into account the high rates of enzyme biosynthesis in both time intervals).

It is known that by maturation and aging of the mycelia cultures the activity of isocitrate dehydrogenase lowers or cease altogether and it can be concluded that the remaining experimental variants mediums supplemented with amino acids - asparagine (from 1.9072 µg formazan / g mat at 0.9950 µg formazan / g mat.), alanine (from 1.5713 µg formazan / g mat. to 0.8419 µg formazan / g mat), acid glutamic (1.8631 µg formazan / g mat to 0.8350 µg formazan / g mat.) aspartic acid (1.5201 µg formazan / g mat to 0.8549 µg formazan / g mat.), cystine (1.1044 µg formazan / g mat.to 0.7007 µg formazan / g mat.) and valine (0. µg formazan / g mat to 0 , 4854 µg formazan / g mat.) the metabolic activity became slower, as amino acids consumption for various building elements (purine and pyrimidine bases, other enzymes, enzyme cofactors as - thiamine pyrophosphate for example, necessary for the enzyme activity in the Krebs cycle, to fill the needs of the cell (such as active transport of substances) with energy, for other amino acid production or, when the culture came out from trophophase and went into idiophase, for basic functions and for the structures of any secondary metabolism products). Although the values were smaller than those present in version control for other amino acids the endoenzyme dynamic had a upward trend.

Therefore, in the medium with phenylalanine, the enzyme activity increased from to 0.23400 µg formazan / g mat. to 1.0381 µg formazan / g mat, in that with additional cysteine rised from the 0.1540 µg formazan / g mat. to 0.7697 µg formazan / g mat. and histidine supplementation of the culture medium was assisted by a rise isocitrate dehydrogenase from 0.5234 µg formazan / g mat to 0.8365 µg formazan / g mat.

The α-ketoglutarate dehydrogenase activity at 7 days after inoculation compared with isocitrate dehydrogenase activity for the same time, had significant increases in all medium variants of supplemented with amino acids, compared with the version control that had the value of 0.9757 µg formazan / g mat. This confirms the role of α - ketoglutarate dehydrogenase in the detoxification of reactive oxygen species, but also amino acids role in α-ketoglutarate homeostasis, that seemed to be allocated for oxoglutarate production during oxidative stress. Examining of α-ketoglutarate dehydrogenase dynamics shows differences in the amplitude variation curves for oxidoreductase activity at both time intervals depending on the type of amino acid introduced into the culture medium.

Hence, a relatively modest increase had the enzyme activity under the stimulatory action of cysteine - from 2.3224 µg formazan / g mat. to 2.5052 µg formazan / g mat., of cystine from 1.8062 µg formazan / g mat. to 2.2851 µg formazan / g mat., while histidine approximately doubled the oxidoreductase activity in time - from 1.4258 µg formazan / g mat. to 2.3478 µg formazan / g mat, the behaviour also applied to alanine - from 1.4467 µg formazan / g mat. to 2.0885 µg formazan / g mat. and glutamic acid - from 1.2114 µg formazan / g mat. to 2.0419 µg formazan / g mat. The

oxoglutarate dehydrogenase activity showed a slight descent, which coincided with the presence of serine in the culture medium (from 1.6094 µg formazan / g mat. up to 1.9293 µg formazan / g mat.), and of leucine (1.7502 µg formazan / g mat at 1.9261 µg formazan / g mat.), the medium without amino acids (control variant) had the same enzymatic behaviour through time (from 0.9757 µg formazan / g mat to 1.8477 µg formazan / g mat.). Asparagine influenced less the oxoglutarate dehydrogenase activity, varying between the two time intervals from 1.4216 µg formazan / g mat. to 1.4800 µg formazan / g mat. By contrast, the presence of aspartic acid in the submerged culture of *Monilinia laxa* was followed by a slight decrease in oxidoreductase activity over time - from 1.3845 µg formazan / g mat. to 1.2546 µg formazan / g mat., the endoenzyme had a decreasing amplitude in the case of medium variants with phenylalanine (from 1.3457 µg formazan / g mat. up to 1.2668 µg formazan / g mat valine (from 1.5759 µg formazan / g mat.to 0.9920 µg formazan / g mat. lysine (from 2.0674 µg formazan / g mat to 1.1754 µg formazan / g mat.), methionine (2.1559 µg formazan / g mat to 1.5225 µg formazan / g mat. The influence of these amino acids on the enzyme in the mycelium after 14 days of incubation reflected in a much reduced level compared to the first interval and suggests the end of the primary metabolic cycle of events and the start of the next stage.

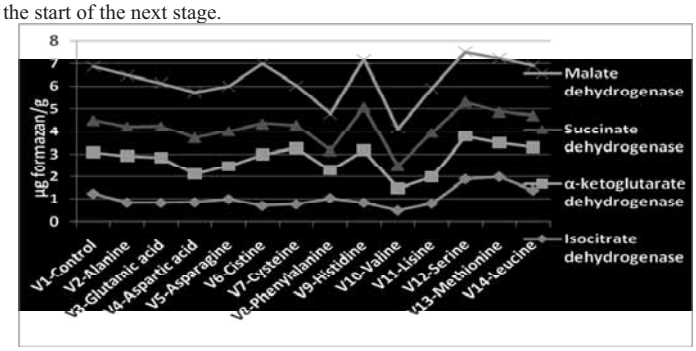

Fig 2 – The amino acids influence on dehydrogenases of the Krebs cycle in a 14 days old mycelium from *Monilinia laxa* fungus

A quantification of the activity of the enzyme that catalyses succinate dehydrogenation in the fungus mycelium, represented graphically shows both continuity and balanced development of the Krebs cycle at this level, but also some significant differences in the activity of succinate dehydrogenase, that has some turns imposed by the age of the culture mycelia and by the type of amino acid on which the culture medium was supplemented.

A careful analysis indicates that the reaction catalysed by the oxidoreductase increased through time for all medium variants supplemented with amino acids, even if they showed some inhibitory effects in the mycelium of *Monilinia laxa* species. Given the fact that, while the fungus is getting older, the activity of α- ketoglutarate dehydrogenase and malate dehydrogenase slowers, while the activity of isocitrate dehydrogenase is stopped, succinate dehydrogenase is the only enzyme whose activity remains high due to its location in the mitochondrial membrane, which lasts as long as there are parts of it, even if the mitochondria begins to conduct the programs related to the cell apoptosis. Under these conditions, the dynamics of succinate dehydrogenase activity shaped when amino acids are used as benchmarks, is upward, the control variant shows an increase from 0.4250 µg formazan / g mat. to 1.4334 µg formazan / g mat., while the culture enriched with alanine, the oxidoreductase has an increase from 0.5467 µg formazan / g mat. to 1.3014 µg formazan / g mat. and in that supplemented with glutamic acid, from 0.4215 µg formazan / g mat to 1.3711 µg formazan / g mat.

During the course of the experimental program, the medium variable containing aspartic acid showed an increase in the respiratory rate and metabolism, the succinate dehydrogenase activity increased from 0.4360 µg formazan / g mat. to 1.6300 µg formazan / g mat., and asparagine, an amide of the aspartic acid acted as an inducer, the biosynthesis levels and oxidoreductase activity increased from 0.6354 µg formazan / g mat. to 1.5640 µg formazan / g mat. Thiol amino acids as cystine and cysteine stimulated the activity of the enzyme that catalyses the third step of the citric acid cycle, its limits going up from 0.4852 µg formazan / g mat. to 1.3955 µg formazan / g mat. respectively and from 0.4799 µg formazan / g mat. to 1.0347 µg formazan / g mat. Although in both quantitative determinations intervals phenylalanine had an inhibitory effect on succinate dehydrogenase, it increased as quantity from 0.1127 µg formazan / g mat. to 0.8769 µg formazan / g mat.

Large variations were found in the medium with the histidine (from 0.7117 µg formazan / g mat to 1.9043 µg formazan / g mat., valine (from 0.2900 µg formazan / g mat to 1013 µg formazan / g mat. and lysine (from 0.8869 µg formazan / g mat at 2.0003 µg formazan / g mat.). Through time, the submerged cultivated mycelium of *Monilinia laxa* fungus in association with methionine activated the succinate dehydrogenase progressively, its limits are marked with 1.1370 µg formazan / g mat., respectively 1.3843 µg formazan / g mat., while the enzyme levels in the culture enriched with leucine jumped from 0.6712 µg formazan / g mat. to 1.3843 µg formazan / g mat.

The malate oxidation with restored oxalacetate, the reaction that ends the Krebs cycle is catalyzed by malate dehydrogenase. What draws the attention to malate dehydrogenase activity after 7 days of incubation is that it is higher than that of succinate dehydrogenase in the same time period, suggesting that a part of the catalyzes substrate by this oxidoreductase enteres the cycle Krebs through other metabolic pathways (eg, in the cultures with glutamic acid or methionine, the difference might be due to a high intensity function of glicoxylate cycle which from the specific isocitric acid leads to glyoxylic acid formation, which by condensation with acetyl-CoA formes malic acid that enteres in the reaction catalysed by malate dehydrogenase). Examination of malate dehydrogenase activity dynamics model at *Monilinia laxa* points out two aspects: the first takes into account the stage in which the organism consumes partially or totally all amino acids from the medium, using them as carbon source and as indicators for oxidoreductase production, the malate dehydrogenase behaviour taking an ascending route for most samples and a downward allure for media supplemented with glutamic acid and methionine. And the second aspect is the fact that, beyond the matching behaviour succinate dehydrogenase for the medium variants with histidine, valine and phenylalanine, even if they had intensification of enzyme activity over time, the inhibitory effect remained present in both time intervals.

The singularity of the enzyme in case of glutamic acid at 7 days after inoculation has seen a fall in the activity throgh 14 days, the values passed from 3.2808 µg formazan / g mat. to 1.8987 µg formazan / g mat. and the presence of methionine in the culture medium was followed by a decrease in malate dehydrogenase activity from 2.5142 µg formazan / g mat. Biol. to 2.3599 µg formazan / g mat. Biol., while the existence of cysteine was followed by a minor decrease (from 1.7957 µg formazan / g mat. to 1.7143 µg formazan / g mat).

The biochemical tests revealed, however, intensification of biosynthesis of oxidoreductase and enzymatic activity results correlate very well with the observations on this behaviour in the case of some amino acids in both time periods. So, malate dehydrogenase activity in the variant without amino acids sources increased from 1.4105 µg formazan / g mat. to 2.3286 µg formazan / g mat., and for cystine from 1.8896 µg formazan / g mat. to 2.5988 µg formazan / g mat. Progressive enzymatic activity in both asparagine and aspartic acid ranged from 1.7286 µg formazan / g mat. to 1.9605 µg formazan / g mat. respectively, from 1.5414 µg formazan / g mat. to 1.9866 µg formazan / g mat.

As time passed, the malate dehydrogenase activity values went from 2.1495 µg formazan / g mat to 2.2176 µg formazan / g mat. under the influence of serine, from 1.9493 µg formazan / g mat to 2.1633 µg formazan / g mat under leucine induction, from 1.4133 µg formazan / g mat. to 2.2368 µg formazan / g mat. The same positive influence was also reflected in the case of lysine, the variation of growth went from 1.6968 µg formazan / g mat to 1.9281 µg formazan / g mat. We could also see from of graphical data analysis that in the case of phenylalanine, histidine and valine the induced activity of the endoenzyme was negative in both periods, despite the advancement operational rates for that stage of the Krebs cycle.

Thus, the value variation was from 1.0602 µg formazan / g mat to 1.9281 µg formazan / g mat in the circumstances of valine present in the submerged culture, from 1.0541 µg formazan / g mat. to 1.6204 µg formazan / g mat. in the case of phenylalanine and from 1.4339 µg formazan / g mat. to 2.0908 µg formazan / g mat. in the mycelium with additional histidine.

CONCLUSIONS

In the young mycelium, aged for 7 days, the isocitrate dehydrogenase activity was stimulated by asparagine, glutamic acid, methionine, alanine, aspartic acid, cystine, leucine, lysine, serine and valine. All amino acid induced the high biosynthesis of α-ketoglutarate dehydrogenase, and the succinate dehydrogenase activity was stimulated by methionine, lysine, serine, histidine, leucine, asparagine, alanine, cystine, cysteine and asparatic acid, while the activity of malate dehydrogenase was stimulated only by glutamic acid, methionine, serine, leucine, cystine, cysteine, asparagine, lysine, aspartic acid, histidine and alanine.

In the mature culture, aged for 14 days, the isocitrate dehydrogenase activity was stimulated by methionine, serine and leucine, the α-ketoglutarate dehydrogenase by cysteine, histidine, cystine, alanine, glutamic acid, serine, and leucine, the succinate dehydrogenase activity by lysine, histidine, asparatic acid, asparagine, serine and the malate dehydrogenase activity by cystine and methionine.

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