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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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