EVOLUTION OF CATALASE ACTIVITY DURING NYSTATIN BIOSYNTHESIS

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Abstract: The research studies focused on the dynamics of catalase during nystatin biosynthesis by *Streptomyces noursei*. The catalase activity was determined by growing a pure culture of *Streptomyces noursei* from the strain collection owned by the company S.C. Antibiotice Iasi on biosynthesis medium. The test was performed on two experimental models of biosynthesis, one using sunflower oil, while the other soybean oil as basic nutrients. Special attention was paid to the connection between the evolution of the biomass and the level of catalase activity.

INTRODUCTION

The biological processes, extremely complex by their nature and rapidity, involve an extensive number of different biochemical reactions catalyzed by an appropriate number of enzymes. The enzymes are true research instruments for the metabolism, allowing the detection of a metabolic pathway and the identification of the regulation and control mechanisms characteristic to it (Cristea Popa, E., Popescu, A., Trutia, E., Dinu, V., 1991). To know the metabolic pathways of a living organism means to possess a useful instrument promoting the induction of changes that allow the selective biosynthesis of a product, which can be produced and used on industrial scale. In the case of enzymes isolated from microorganisms, an important part is played by the composition of the growth medium. The speed of the biosynthesis of the bacterial enzymes is highly influenced by the presence in the growth medium of the substrates or final products of the metabolic pathway in which they are involved (Dinu, V., Trutia, E., Cristea Popa, E., Popescu, A., 1996). Thus, some enzymes which catalyze reactions within a metabolic pathway are not normally synthesized being induced by the presence of substrates or substrate analogues in the growth medium. The effect of the presence or absence of a chemical substance in the growth medium on the biochemical structure and biological behavior of the microorganism grown is called adaptation, and it was the adaptive enzyme systems that led to the formulation of the hypothesis of enzyme induction and repression (Hervé-Grépine, T.V. *et al.* 2008).

MATERIALS AND METHODS

Nystatin biosynthesis was carried out by submerged cultivation of a *Streptomyces noursei* culture on specific laboratory media. At the first stage, the vegetative culture was obtained, which served to inoculate the biosynthesis medium (previously distributed in thirteen 500-ml Erlenmeyer flasks, 100 ml of medium/flask), using 1.5 ml of culture per flask. The flasks were stored on a rotary shaker (245 rpm) at 28^{o}C for 312 hours. Established quantities of sunflower oil/soybean oil and 50% dextrose solution were added during the biosynthesis process. The catalase activity was determined every 24 hours using the iodometric titration (Artenie, V., Tănase, E., 1981).

RESULTS AND DISCUSSIONS

Microorganisms, like many other organisms, need energy for biosyntheses, growth and multiplication. Experience has shown that under normal conditions of microorganism cultivation in liquid media at a neutral pH and relatively low temperatures, only energy-generating reactions can take place spontaneously (Dunca, S., Ailiesei, O., 2004).

All the other reactions, including the biosynthesis ones, which require energy consumption, are blocked and cannot take place unless coupled with energy-generating reactions.

Microorganisms, like other biological systems, are unable to use the entire amount of energy of a system. At a given temperature, only a fraction of this energy is released, called the free energy of the system, which expresses the maximum amount of energy of the system potentially available to do useful work.

More recent research studies have demonstrated that in spite of the fact that the molecular oxygen is essential for the life of many organisms, its use may result - in certain circumstances - in the generation of compounds highly toxic for the living cells.

The nature of the toxic effects exerted by the aerobiosis on some microorganisms was explained by Fridovich (1978), who demonstrated both the major role of the superoxide radical

(which forms easily, is highly stable and extremely toxic), which acts as an agent of oxygen toxicity, and the function of superoxide dismutases as essential components of the biological reactions of defense against that effect. He showed that the complete reduction of an oxygen molecule to water requires the intervention of 4 electrons and that the sequential, univalent pathway for complete reduction of oxygen involves for certain the generation of several intermediate compounds, such as the superoxide anion radical, hydrogen peroxide, and hydroxyl radical, which are too reactive to be tolerated by the biological systems.

Their first reaction of defense is provided by the enzymes, which transform them into harmless products by catalytic conversion. The hydrogen peroxide is removed by catalases, by conversion to H_2O and O_2, or by peroxidases, which reduce it to water using a number of reducers contained by the cell. The superoxide ion is eliminated by superoxide dismutases, which catalyze the conversion to H_2O_2 and O_2 (Dumitru, I. F., Iordăchescu, D., 1980).

The presence of catalase is an additional protection mechanism against H_2O_2, which is a less toxic by-product of the superoxide ion.

The tests performed to determine the activity of the catalase in penicillin, tetracycline and oxytetracycline-producing strains showed a number of interesting connections between the enzymatic activity and the level of the antibiotic biosynthesis.

Artenie, V. *et al*. (1977) noticed that in the penicillin-producing organism*, Penicillium chrysogenum*, catalase activity runs approximately parallel to the antibiotic biosynthesis curve, while in the tetracycline-producing organism, the enzyme activity exhibits a peak at 48 hours and then decreases.

The objective of our research studies was to examine the dynamics of catalase during the biosynthesis of nystatin. The assay to detect catalase activity included the development of two experimental models, namely the biosynthesis of nystatin using sunflower oil additions during the process, and the biosynthesis of nystatin using soybean oil additions during the process. Special consideration was given to the relationship between the evolution of the biomass and the level of catalase activity. The values of the catalase activity and biomass determined in the two experiments are indicated in Table 1.

In the nystatin biosynthesis model using inputs of sunflower oil, catalase activity reached a maximum at the beginning of the trophophase, i.e. 9.6 (c.u./100 g), and remained constant in the first part of the idiophase with levels between 0.64 – 0.44 (c.u./100 g), when the biomass increased from 28% to 76%. By the end of the biosynthesis, catalase activity increased; thus, from 220 hours to 308 hours, its values ranged between 0.96 to 3.00 (c.u./100 g), while the biomass decreased from 72% to 64%.

The evolution of catalase activity in the experimental model 1 is graphically represented in Figure. 1.

In the nystatin biosynthesis model using inputs of soybean oil, catalase activity exhibited slight variations during both the trophophase and the idiophase.

Thus, until 110 hours, catalase activity exhibited levels between 1.48 – 0.64 (c.u./100 g), while the biomass increased from 30% to 62%; after that age to the end of the biosynthesis process, it had slight fluctuations between 0.24 – 1.28 (c.u./100 g) correlated also with the variation in the level of the biomass, which ranged between 60% - 71%. At the end of the process, catalase activity was 2.0 (c.u./100 g) corresponding to a biomass of 65%.

The evolution of catalase activity in the experimental model 2 is graphically represented in Figure 2.

Table 1. Catalase activity and level of biomass during the nystatin biosynthesis

Age (hours)	*Experimental model 1*		*Experimental model 2*	
	Catalase activity (c.u./100 g)	*Biomass (%)*	*Catalase activity (c.u./100 g)*	*Biomass (%)*
22	9,60	28	1,20	30
44	0,64	39	1,48	40
66	0,60	45	0,37	48
88	0,53	61	0,34	53
110	0,48	68	0,64	62
132	0,40	70	1,28	60
154	0,44	74	1,00	65
176	0,44	76	0,88	70
198	0,52	72	1,08	68
220	0,96	70	0,52	71
242	1,20	70	0,24	70
264	1,80	68	0,36	70
286	2,00	65	0,32	70
308	3,00	64	2,00	65

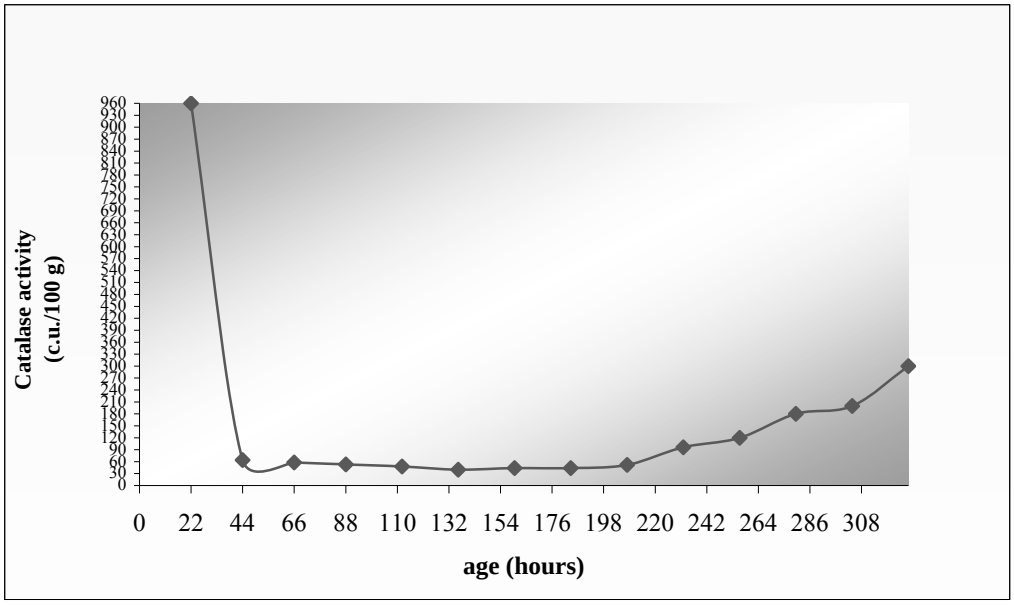

Figure 1. Catalase activity during nystatin biosynthesis using sunflower oil inputs

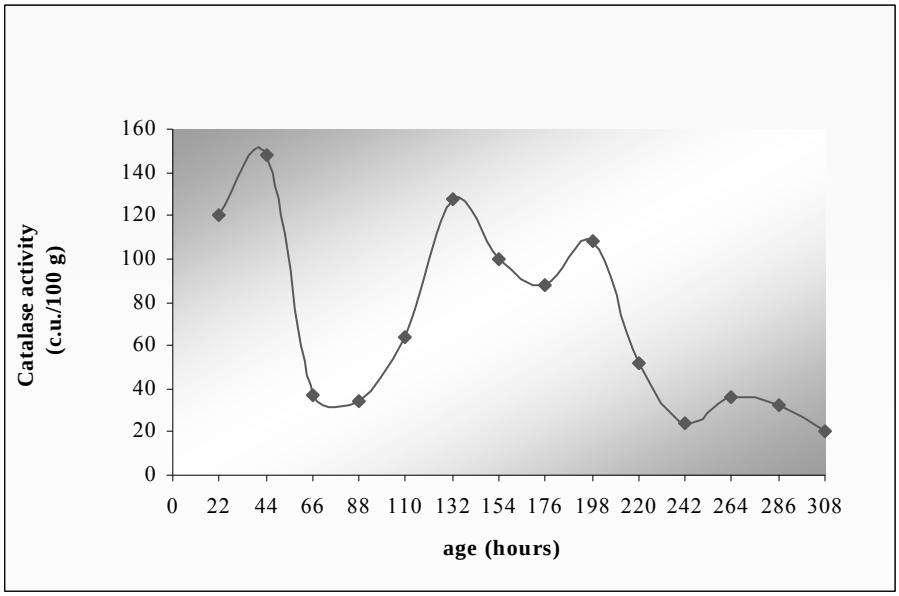

Figure 2. Catalase activity during nystatin biosynthesis using soybean oil inputs

CONCLUSIONS

During the biosynthesis of nystatin with inputs of sunflower oil, catalase activity reaches the maximum level at the beginning of the process, after which it exhibits low and constant values.

Catalase activity has low, fluctuating levels during the process of nystatin biosynthesis using soybean oil inputs.

During both biosynthesis processes, no matter the nature of the vegetable oil used as primary nutrient, the level of catalase activity is correlated with the value of the biomass, i.e. when the biomass decreases, catalase activity increases and vice versa. At the end of the nystatin biosynthesis, irrespective of the nature of the vegetable oil added, catalase activity enhances because of the accumulation of the end products of metabolism.

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