# THE SEARCHING OF ACTIVE CATALASE PRODUCERS AMONG THE MICROSCOPIC FUNGI

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**Abstract.** The screening among 158 fungal strains from different taxonomic groups - 128 new soil strains, from plant roots and 30 NCNM strains was carried out on the criterion of catalase synthesis. It was demonstrated that some new strains were more active than those from NCNM. All tested strains from the *Penicillium* and *Aspergillus* (108) genus had catalase activity and 26 representatives of other strains did not. *Penicillium funiculosum* strain, isolated from selected soils of Moldova, was selected as a potential catalase producer. This strain had a catalase activity of 244 units / ml. The optimal parameters for crop cultivation were: temperature of $28^0$C, the cultivation period - 6 days, the pH value of the nutritional medium – 6.6.

**Keywords:** *strains, taxonomic groups, micromycetes, extracellular catalase.*

## INTRODUCTION

Catalase is one of the main enzymes which destroy the active forms of oxygen. It is one of the main primary antioxidant of the defense system, which catalyzes decomposition of hydrogen peroxide to water.

Catalase has found its application in various biotechnological processes. For example it is used in textile industry in textile processing for quick removing of residual hidrogen peroxide from textiles after peroxide bleaching before the operations of drying and printing, otherwise the residual of peroxide can destroy the dyestuff on textiles, change the colour spectrum. Such an operation becomes more effective and environmentally friendly [6, 15]. For contact linz desinfection is used hydrogen peroxide, but catalase is used for peroxide neutralization. Catalase may be used in solution and in tablets forms. Tablets lower the concentration of peroxide during the first 20 minutes up to 1%, the further reduction of neutralization finishing in two hours [12].

In food industry catalase is applied for removing of residual amount of $H_2O_2$ after the processes of cold sterilization of beer, drinses, dairy produce. Catalase is widely used in medicine thanks to its ability to reduce the action of free-radical particles in a person's organism. Catalase is widely used in medicine as an antioxidant in different pathologies, in diagnostical centres, and also for polyenzime antioxidant drugs obtaining in the form of water – soluble conjugates with polymers or soimmobilized proteins on carriers. Such drugs are necessary for purification of biological liquids from $O_2$ and $H_2O_2$, for many diseases and especially for radiotherapy in different pathologies [10, 14, 23].

The enzyme is applied in scientific research, in environmental monitoring, in biosensoring technologies for determination of hydrogen peroxide and etanol number containing [3].

These days catalase is intensively being investigated in different scientific – research centres in Japan [11], USA [7], France [24], England [8], China [9], Bulgaria [22], Russia [13], Ucraine [20], Belorusia [18], Netherlands [25], etc.

Theoretical and practical importance of making up to date biotechnology to produce this enzyme preparation – catalase. In industrial scale catalase is produced from mammalian liver and fungus *Aspergillus niger*, which produce intracellular enzymes, it allows to simplify the technology of enzyme preparation [7, 19]. As the price of enzyme preparation is rather high, it is very important to find perspective producers of intracellular catalase and new ways to intensify the process of biosynthesis.

The aim of this work is to search perspective producers of intracellular catalase among micromycetes to study the conditions of their formation.

## MATERIALS AND METHODS

The objects of the investigations were 158 strains of micromycetes, 128 of these strains were isolated from the soil of Moldova and from the roots of plants, 30 strains were taken from the National Collection of Nonpathogenic Microorganisms (NCNM).

For the micromycetes isolation were used the following mediums: malt-agar, Czapek, Raystrik were used.

Strains identification was effectuated by determinants recommended for the specific taxonomic group of microscopic fungi [1, 2].

Micromicetes were grown in Erlenmeyer flask of volume 250 ml in 50 ml medium with the following composition: % $KNO_3$-0.5; glucose – 4.0; $NaH_2PO_4$ – 0.15; $KH_2PO_4$ – 0.1; $MgSO_4x7H_2O$ – 0.5; $FeSO_4x7H_2O$ – 0.001 yeast extract – 0.1, pH – 6.3, on a shaker (160 rpm) by the temperature $28^0$C during 6 days and nights [13].

Catalase activity was determined in the culture fluid by titration [17]. As a unit activity was considered the enzyme quantity that splits 1 mkm hidrogen peroxyde (0.034 mkg) per 1 min and was expressed in units/ml (U/ml) cultural liquid, and also in units / mg (U/mg) biomass ( the productivity ability of the mycelium fungus).

Culture fluid was separated from the biomass by filtration through paper filters.

## RESULTS

The fungi have some features, which provide their wide distribution in biocenoses (ecosystem). The most important of them are the filamentous structures of the thallus, high speed of growth and reproduction, high metabolic activity, which is manifested in a wide range of environmental factors: temperature, humidity, light, medium acidity etc. and also great biological and genetic variability, which allow the fungus to adapt to the changing conditions of habitat and to new nutrient substrata [16, 27].

While searching producers of intracelular catalase, 158 strains of fungi were tested. 48 of them belonged to the species *Aspergillus*, 70 - to *Penicillium*, 40 - to different taxonomic groups (*Alternaria, Trichoderma, Fusarium, Rhizopus* and others).

It was stated that 132 strains posses intracellular catalase.

It proved that freshly isolated strains synthesize the intracellular catalase more active than the strains, which were kept on agar medium in NCNM for a long time.

The activity of the intracellular catalase in the studied strains varied from species to species and among the representatives of the same species as well. An intracellular catalase was found in all the strains of the *Aspergillus* and *Penicillium* species, but catalase was not synthesized from 40 strains of 26 representatives of other taxonomic groups.

The Fig. 1 and Fig. 2 represent the results of catalase determination in the most active representatives of the *Aspergillus* and *Penicillium* species.

It is seen that the tested strains of *Penicillium* species have a more active intracellular catalase than the strains of the *Aspergillus* species. Eight strains of the species *Penicillium* have the intracellular catalase with the activity more than 100 U/ml, to them belong two strains from NCNM *Penicillium expansum* NCNM (125 U/ml) and *Penicillium verrucosum* NCNM (140 U/ml), and 5 isolates – were isolated from the soil (*Penicillium piceum* 4, *Penicillium funiculosum* 6, *Penicillium expansum* 18, *Penicillium frequentans* 19, *Penicillium janthinelum* 102).

The activity of the intracellular catalase in such isolates varies within 105 – 244 U/ml, it is higher than in the strains from the collection.

The testing of intracellular catalase activity in 5 most active strains in the condition of cultivation allowed to isolate the most active strain- *Penicillium funiculosum* 6.

It is known that enzymes biosynthesis depends on the physiological state of microorganisms in the nutrient medium and also from some physical and chemical factors (duration of cultivation, medium acidity, temperature, aeration, humidity, etc.).

The growth of a microorganism is possible only within certain factors, but for different microorganism groups these limits are often not the same [4, 21].

Optimization of the parameters of the deep cultivation of the studied micromycetes was performed in stages. On each stage the optimal meaning of the previous factor was taken into consideration.

On the first stage the optimal time of the cultivation for receiving maximum of catalase activity was defined.

Based on the received data shown on the Figure 3, maximum of the biosynthesis of the exracellular catalase was established on the sixth days of the cultivation. On the picture you can see that the activity of the catalase is growing begining from the fourth day and on the seventh day the catalase biosynthesis is slowly reducing.

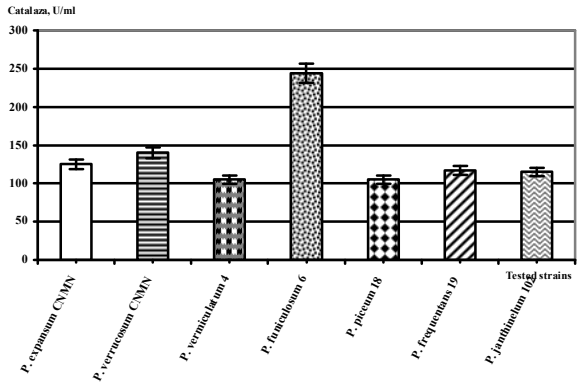


**Figure 1.** The catalase activity of the active strains of the *Penicillium* species.

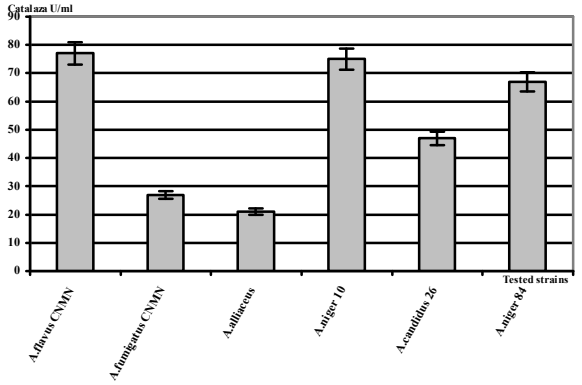


**Figure 2.** The catalase activity of the active strains of the *Aspergillus* species.

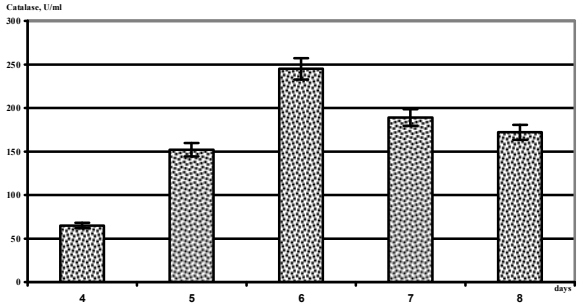


**Figure 3**. The activity of catalase *Penicillium funiculosum* 6 in dependance on the time cultivation.

Thus, the experiments proved that the optimal time for *Penicillium funiculosum* 6 and its maximum catalase biosynthesis takes place on the sixth day.

It is known from other scientific works that the temperature of growing is a very important factor which acts both on the intensity and on the direction of biosynthesis of secondary metabolities including enzymes. As a rule, the temperature is not optimal for the growth, it can be optimal for enzymes biosynthesis. The optimal temperature for enzymes biosynthesis for many strains is $26^0$C, but for fungi growing the temperature is $28^0$C [5, 21, 26].

The next step was the optimization of the temperature within the deep cultivation of the *Penicillium funiculosum* 6. The action of different temperature (20 - $22^0$C, 24 - $26^0$C, 28 - $30^0$C, 34 -$36^0$C) was studied.

On the Figure 4 the dynamics of data catalase biosynthesis in the studied strains at different temperatures are presented.

It was found out that while growing the fungus *Penicillium funiculosum* 6 the cultivation temperature is changing greatly and affects the catalase biosynthesis. Catalase biosynthesis is little active when a strain has the temperature 20-$22^0$C. We noticed a great increasing of enzymes biosynthesis up to 2.5 times, when the temperature increases to 24-$30^0$C. When the temperature increases up to (34 - $36^0$C) we saw a noticeable reduction of this enzyme formation.

On the Figure 4 it is seen that the best temperature for the catalase biosynthesis is the temperature $28^0$C-$30^0$C (catalase activity is in the range of 254 U/ml).

The medium acidity has a great importance for the microgerm growth. Microscopic fungi are very sensitive for the extreme meanings of pH – 2.0 - 9.0. Most of the fungi grow better in slightly acid medium (pH 5 - 6). Scientific works mark that the well-known catalase producers *Penicillium piceum, Penicillium vitae* are cultivated at pH medium 6.2 – 6.6 [4, 5, 21, 26].

In the next series of experiments we studied the influence of the medium level pH 6.0 – 7.5 on the cultivation activity. The changing of pH in the limit 6.0 – 7.5 didn't influence much on the cultivation growth. It was noticed the apparent stimulation of catalase synthesis at pH medium 6.6 (Fig. 5).

The obtained results reveal that *Penicillium funiculosum* 6 strain cultivation at 6.6 pH medium value leads to the acceleration of the extracelular catalase biosynthesis process, contributing to the appearance of its maximal activity. So, on the basis of the obtained results can be concluded that the *Penicillium funiculosum* catalase biosynthesis, its direction and intensity is under the control of different factors, which have a great importance for the primary metabolism.

## DISCUSSION

For the enzymatic compounds obtaining at an industrial scale are used diverse micromycetes strains which produce extracellular enzymes that simplify considerably the compounds separation and purification processes, that finally lead to the product total cost decrease.

According to the literature data, from the micromycetes that produce extracellular catalase, the most active strains are those from the *Penicillium* genus [13, 16]. The obtained results in this study also demonstrated that from the 158 tested strains the most active were those from the *Penicillium* genus.

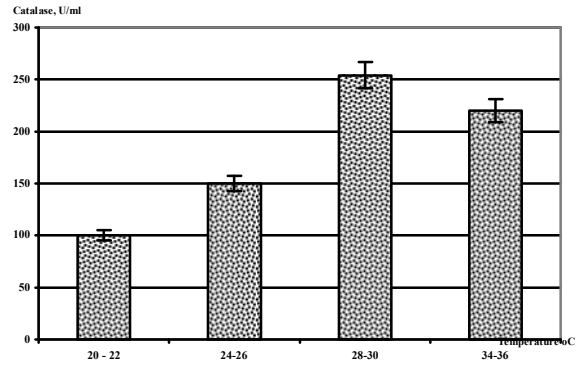


**Figure 4.** The action of the temperature cultivation on the catalase activity of the *Penicillium funiculosum* 6.

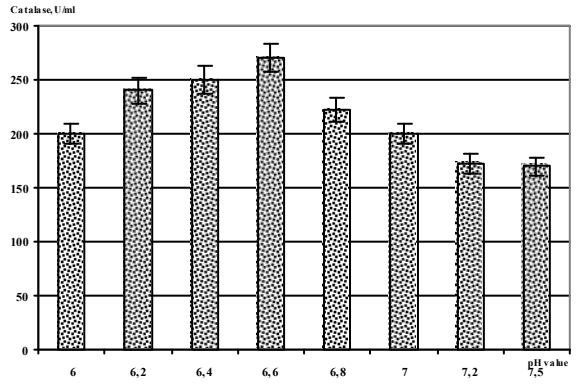


**Figure 5.** The influence pH cultivation medium on catalasa activity *Penicillium funiculosum* 6.

The obtained results showed that a perspective catalase producer for subsequent research was selected the *Penicillium funiculosum* strain that possess a superior activity in comparison with the rest of the studied strains.

The dynamic study of the catalase activity of the *Penicillium funiculosum* strain has demonstrated that the maximum value of it is manifesting at the 6-th cultivation day.

The cultivation medium factors (temperature, pH) exert a differentiated regulating effect on the growth and the catalase biosynthetic processes of the *Penicillium funiculosum* strain. The maximum catalase activity manifests at the $28^o$C temperature and 6.6 initial pH value. The optimization of the submerse cultivation conditions has lead to the increase with 10% of the catalase activity.

Thus, as the result of our research was selected the *Penicillium funiculosum* strain as a perspective

autochthonous catalase producer and were optimised the conditions for its submerse cultivation.

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