CATALASE ACTIVITY IN ARCTIC MICROFUNGI GROWN AT DIFFERENT TEMPERATURES

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The total of 98 strains of moulds were isolated from soils collected in arctic tundra (Spitzbergen). Among these strains *Penicillium cyclopium* 1, the most effective for production of catalase, was selected by the method of test-tube microculture. The time course of growth and catalase production by this strain showed the intracellular activity of this enzyme to be about 3-fold higher than its extracellular level. Some properties of crude catalase preparation, isolated from postculture liquids by lyophilization, were also examined. Catalase activity showed its maximum at 15 °C, indicating adaptation of the enzyme to lower temperatures of the arctic environment.

Keywords: Catalase – psychrotroph – screening – arctic fungi – *Penicillium cyclopium*

INTRODUCTION

Catalase [(CAT) EC 1.11.1.6] catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and free oxygen. Commercial sources of CAT are mammalian liver and *A. niger*. Catalases from various sources are utilized in numerous industrial applications such as food or textile processing to remove hydrogen peroxide, used for the "cold" pasteurization of milk with hydrogen peroxide or bleaching [1, 9, 13]. Catalase from *Aspergillus* is useful because of its relative heat-resistance [12]. In plants, catalase scavenges H_2O_2, which is generated during the photorespiration and β-oxidation of fatty acids. It also plays an important role in detoxification of active oxygen species generated by various types of environmental stress [18]. For industrial-scale production, the extracellular production of enzymes is more desirable than extracting intracellular enzymes. However, catalases located in the cytoplasm and peroxisomes are not usually excreted from the cell.

Despite their numerous potentials of application in fundamental and applied fields, microorganisms isolated from cold environments have received little attention, especially in comparison to thermophiles. Their advantages, as well as both

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their ecological and economical values, may not yet have been realized sufficiently. Nevertheless, in the past decade an increasing interest of biotechnologists in these extreme organisms could be observed [2, 10, 11]. Cold-adapted (psychrophilic and psychrotrophic) microorganisms are distinguished from mesophiles by their ability to grow at low temperatures. Under such conditions apart from other physiological characteristics-psychrophiles and psychrotrophs have lower metabolic rates and higher catalytic efficiencies than mesophiles. Cold-adapted microorganisms have a considerable potential in biotechnological application, such as waste treatment at ambient temperatures, enzymology, food industry and medical applications [10, 11]. Application of cold-adapted microorganisms offers numerous advantages: high microbial growth rates, as well as high enzymatic activities and catalytic efficiencies in the temperature range of 0–20 °C prevents the risk of microbial contamination (especially in continuous systems), may shorten process times and allows to reduce expensive heating/cooling systems, which is a considerable progress towards the solution of saving of energy.

There have appeared a number of reports concerned hydrolytic enzymes production by psychrophilic microorganisms [2, 5, 15, 17]; however to our knowledge, no investigations have been performed on catalase.

We report the catalase activities of arctic fungal isolates at different growth temperatures, as the first step toward characterization of cold-adapted enzymes.

MATERIALS AND METHODS

Microorganisms and media

Fungi were isolated from soils in the Calypsostrandy situated in southern Bellsund region (lat 77°33'N long 14°30' E), Wedel Jarlsberg Land (West Spitsbergen). Composition of Martin medium used for screening of microorganisms was as follows: glucose, 1%; peptone, 0.5%; K_2HPO_4 , 0.1%; $MgSO_4 \times 7H_2O$, 0.05%; rose bangal (sterilized separately) 0.003%. After sterilization 0.003% of streptomycin was added.

The composition of basal medium (BM) used for catalase production was as follows: glucose, 8.0%; peptone , 0.3%; $NaNO_3$, 0.05%; KH_2PO_4 , 0.1%; $CaCO_3$ (sterilized separately), 3.5%.

Taxonomic characterization of fungi

Identification of isolated moulds strains was achieved on the basis of their micro- and macromorphological features, expressed in microcultures, and on agar plates containing known diagnostic media for moulds, by the method of Domsch et al. [4] and Onions and Brady [14].

Isolation of catalase producing microorganisms

Isolation was completed by means of a dilution plate method. Portions of 1.0 g of representative soil were placed in a dilution tube containing 9 ml of sterile distilled water, and shaken for 15 min. Serial 10-fold dilutions were made and surface inoculated onto agar plates of Martin medium plates were incubated for 8-10 days at 15 and 30 °C. Fungi were isolated from plates that contained no more than 50 well separated colonies.

Selection of strains for catalase activity

The first selection of catalase active strains was done on the basis of the magnitude diffusion zone of the enzyme (mm, in diameter) secreted into agar medium by growing colonies (plate method *a*) [6]. As similar diffusion technique alternately used for catalase formed by culture supernatants were agar plates (2%, 20 ml) containing in 0.1 M McIlvaine buffer pH 6.8 (g/l): o-dianisidine, 0.1; H_2O_2, 0.034. In this approach (plate method *b*), the liquids (0.05 ml) were dropped into a well (8 mm diameter) previously cut out in agar medium.

The plates were developed with peroxidase (500 U/mg, horseradish, Boehringer-Mannheim, Vienna, Austria), 0.03 in 0.1 McIlvaine buffer pH 6.8. During the enzymatic reaction (6 h at 15°C) the colourless zones on a brown background were formed.

The strains showing the greatest diffusion areas were further and more accurately examined by test-tube cultivation (22 mm diameter, 12 ml medium) on a rotary shaker (220 rev/min). After 72 h incubation at 15 °C and centrifugation (5 min at 3000 g) the catalase activity was determined in post-culture liquids by plate method *b*.

The strains selected on the basis of the above described criteria were incubated in 500 ml conical flasks, each containing 100 ml of medium. The media were inoculated with 2 ml spore suspensions (about 2×10^6 spores) and cultured for 4 days as above. Then the mycelium was separated by filtration through Miracloth quick filtration material (Chicopee Mills, Inc., New York, USA) and catalase activity was measured in the filtrate.

Disruption of mycelium by homogenization

Homogenization was performed in glass Universal Lab. Aid (type MPW-309, Poland) homogenizer with a glass pestle operated at about 7000 rev min^{-1}. A ratio of 3 ml 0.1 M McIlvaine buffer, pH 5.0 to 2.0 g wet weight was applied, followed by twenty passes of the pestle. Intracellular catalase was extracted from the homogenized mycelium with 97 ml of this buffer.

Isolation of the crude enzyme preparate

Crude catalase preparation was isolated from the postculture liquid by gel filtration on Sephadex G-25 (26 × 9 cm column, 10 mM McIlvaine elution buffer pH 5) and lyophilized after dialysis against deionized water.

Analytical procedure

The extra- and intracellular (measured as cell-free extracts) catalase activity was measured spectrophotometrically by observing the decrease in light absorption at 525 nm during decomposition of H_2O_2 by the enzyme [7]. One unit (U) of catalase activity was defined as amount of the enzyme catalysing decomposition of 1 μmol hydrogen peroxide at 15 °C under these assay conditions. The protein content in the medium and postculture liquid was determined by the method of Schacterle and Pollack [19]. After cultivation, the mycelium dry weight was determined by washing and drying it at 105 °C. Fermentations were performed in 3 replicate culture flasks, and analyses were carried out in duplicate. The data given here are means of the measurements. Mean standard error of the enzyme estimate was ±0.23 and ranged from ±0.003 to ±0.32 U ml^{-1}.

pH and temperature assays

The influence of pH and temperature on enzyme activity, as well as its thermostability, were investigated in 0.1 M McIlvaine buffer. Thermostability was assayed by 1 h exposure of the enzyme preparation at various temperatures.

RESULTS AND DISCUSSION

The total of 98 strains of moulds were isolated from tundra soils and examined for extracellular catalase activity by plate method *a*. Among these strains, forty-five showed extracellular catalase activity (45.9%). Twelve of them were grown at 4 and 30 °C, eighty-six at 4 °C and none only at 30 °C. Twelve strains showing the highest catalase activity were used for further experiments. These included genera *Chrysosporium, Cladosporium, Cylindrocarpon, Mortierella, Penicillium* and *Sclerotium,* as judged from microscopic observation. All these isolates grew very well at 4, 20 and 30 °C, indicating that they were psychrotrophic. These cultures were evaluated for their catalase activity by plate method *b*. The results of the experiments are summarized in Table 1, showing that the best catalase producers were strains *Penicilliun cyclopium* Westling, *Chrysosporium pannorum* (Link) Hughes and *Cylindrocarpon magnusianum* (Sacc.) Wollenw. Since the isolates were obtained

from representative samples in the environment, we can presume that catalase producing microorganisms are well distributed in arctic tundra soil.

The ability of the most active strain *Penicillium cyclopium* 1 to synthesize extra- and intracellular catalase was evaluated by its cultivation at different temperatures in the submerged culture. The results of these series of experiments are summarized in Table 2. They confirm a great predisposition of *Penicillium cyclopium* 1 to synthesize catalase at different cultivation temperatures. Extra- and intracellular catalase activity grew almost simultaneously to reach the highest value after 8 days at 15 °C

Table 1
Preliminary evaluation of catalase activity of moulds grown in test tubes on basal medium

Organism	Number of strains tested	Catalase activity (mm)	
		E	I
Chrysosporium pannosum	1	20	17
Cladosporium herbarum	1	0	13
Cylindrocarpon magnusianum	2	18–20	17–18
Mortierella alpina	1	0	19
Mortierella minutissima	2	14–16	19–20
Penicillium chrysogenum	1	16	17
Penicillium cyclopium	1	30	25
Penicillium islandicum	2	12–14	16–18
Sclerotium sp.	1	0	13

E – extracellular
I – intracellular
Relative activity was expressed in diameter of enzyme diffusion zone in mm formed on agar plates (plate method *b*)

Table 2
Time course of extra- and intracellular catalase production by *Penicillium cyclopium* 1 incubated at different temperatures

Time (days)	Catalase activity (U ml^{-1}) at cultivation temperature (°C)									
	4		15		20		25		30	
	E	I	E	I	E	I	E	I	E	I
2	0.4	0	3.1	27.3	12.6	71.5	22.1	69.4	30.5	86.2
4	1.3	0	28.4	82.0	33.6	86.2	36.5	86.2	55.2	88.8
6	3.7	0	46.3	168.3	52.6	199.8	49.4	142.0	47.3	152.5
8	11.5	15.8	69.4	142.0	61.0	184.0	46.3	136.7	44.1	144.0

E – extracellular
I – intracellular

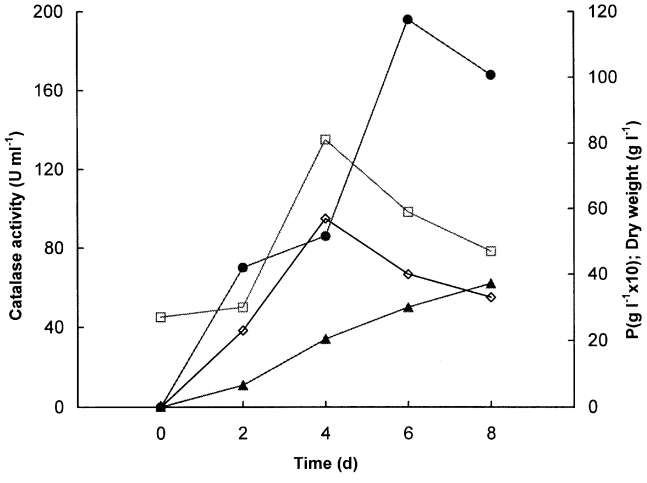

Fig. 1. Dynamics of catalase production by the most active strain *Penicillium cyclopium* 1 during 8 days of submerged culture at 20 °C. ▲, extracellular catalase activity; M, intracellular catalase activity; , protein (P); ◇, dry weight of *P. cyclopium* 1 mycelium

and 6 days at 20 °C, respectively. It is worth to note that the increase in temperature from 4 to 30 °C resulted a in significant increase in extracellular catalase activity after 2–4 day cultivation, but the decrease occurred after 6–8 days at temperature 25–30 °C. At low temperature (4 °C), catalase production by *Penicillium cyclopium* 1 was also effective, suggesting a truly adaptation to grow and hydrogen peroxide degradation in the cold.

The dynamics of extra- and intracellular catalase biosynthesis by the most active strain *Penicillium cyclopium* 1 is presented in Fig. 1. As it can be seen in the diagrams, a rapid increase in extra- and intracellular catalase activity of this strain is accompanied by that in dry weight of the mycelium and protein content. In contrast to the continuous increase in intra- and extracellular catalase activity – up to the sixth or eight days, respectively – the protein amounts and dry weight decreased dramatically from the fourth day. It is difficult to explain clairly this phenomenon as the autolysis of mycelium and hydrolysis of nonenzymatic protein by intracellular proteases might have occurred, though catalases modified by glycosylation could have been more resistant to enzymatic degradation by proteases. Intra- and extracellular catalase activity grew almost simultaneously to reach the highest value, after 6 and 8 days, respectively. Intracellular enzyme activity was about three times higher in comparison with the extracellular fraction of the enzyme during all culture stages and temperature cultivation. A similar ratio of intra- and extracellular catalase activity was found in mesophilic strain *A. niger* AM-11 [7]. Bradner et al. [2] found that antarctic isolates of microfungi studied had showed general growth characteristics and hydrolase production resembling to those of mesophilic species. However, some

strains and enzymes showed adaptation to elevated and/or colder temperatures indicating the development of some means to deal with environmental stress generated by non-mesophilic growth temperatures. A relatively good growth and chitinase activity were displayed by *Penicillium* and *Trichoderma* isolates at 10 °C as well as protease activity in *Trichoderma* sp. [2].

As far as we know, reports on catalase activity of psychrotrophic fungi have not yet been published. Therefore, our results in this respect can be compared with the data concerning mesophilic fungi. The activities of extra- and intracellular catalase produced by *Penicillium cyclopium* 1 were within limited values reported for mesophiles, such as *Alternaria alternata* [3] or significantly higher (about 4.6-fold for extra- and over 3-fold for intracellular enzyme) in comparison with the enzyme synthetized by the mutant of *A. niger* [6].

In further experiments, the influence of pH and temperature on the enzyme activity, as well as thermostability were investigated. The optimum pH for the activity of catalase secreted into the medium by *Penicillium cyclopium* 1 was at the level of 7.0, which is in agreement with earlier studies on catalase from genus *Penicillium* [8, 16]. Maximal activity was achieved at temperatures, ca 15°C. The enzyme is relatively heat resistant. Its activity decreased by 17% after 60 min heating at 45 °C, and further increase in temperature to 60 °C resulted only in partial inactivation of the enzyme (by 60%). The enzyme was inactivated after 60 min at 80 °C (data not shown). Literature reports data indicate that catalase of *Penicillium* exhibits its maximal activity at temperatures 35–40 °C. The enzyme is relatively heat resistant at 40 °C, while higher temperatures cause its rapid inactivation [8].

The results reported here point to the possibility of obtaining an active producer of catalase from psychrotrophic microorganisms. The main disadvantage concerns a longer cultivation time of this fungus, which can be easy avoided by using an immobilized mycelium of *Penicillium cyclopium* 1 in continuous culture.

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