

GLUCOSE OXIDASE ACTIVITY IN STRAINS OF *ASPERGILLUS NIGER* AND *PENICILLIUM FUNICULOSUM*

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Glucose oxidase and catalase activities of *Aspergillus niger* A7 and *Penicillium funiculosum* PS and their respective mutants MN181 and MN238 were studied. *A. niger* strains showed intracellular glucose oxidase activity while *P. funiculosum* showed both intra- and extracellular activity. Besides, the glucose oxidase and catalase activities were more pronounced in *P. funiculosum* than in *A. niger*.

INTRODUCTION

Glucose oxidase was first isolated from mycelia of *Aspergillus niger* and *Penicillium glaucum* by Muller in 1928. Later he studied the properties of the enzyme from *P. glaucum* (Muller, 1936). In view of wide industrial application of the enzyme studies were carried out on the glucose oxidase activity of different species of *Penicillium* (Coulthard *et al.*, 1945; Bodmann and Walter, 1965; Kusai, 1960; Nakamatsu *et al.*, 1975), *Aspergillus* (Munk *et al.*, 1963; Paskova and Munk, 1963a, b) and also of *Pullularia pullulans* (Su *et al.*, 1977; Liu *et al.*, 1978). At present, the enzyme is commercially prepared from *A. niger*, *P. notatum*, *P. glaucum*, *P. chrysogenum* (Fox, 1974; Blain, 1975) and *P. amagasakiense* (Nakamatsu *et al.*, 1975).

By way of mutagenic treatment, two fungal strains were developed in this laboratory producing high yield of calcium gluconate from glucose. In this fermentation process, glucose is oxidised to gluconic acid by the activity of glucose oxidase and catalase enzymes. It may be reasonably expected that these two strains might have very strong glucose oxidase activity and this prompted us to study these enzymatic activities of the strains in the interest of their possible exploitation for commercial production. The present communication describes observation of the same.

MATERIALS AND METHODS

Strains :

The wild strains *Aspergillus niger* A 7 and *Penicillium funiculosum* P 5 and their two respective mutants MN 181 and MN 238 were used.

Media and cultivation :

500 ml Erlenmeyer flasks, each containing 75 ml of fermentation medium (for *A. niger*, g/l, glucose, 150 ; NH_4Cl , 1.2 ; KH_2PO_4 , 0.20 ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 and CaCO_3 , 37.5 and for *P. funiculosum*, g/l : glucose, 150 ; $(\text{NH}_4)_2\text{HPO}_4$, 1.0 ; KH_2PO_4 , 0.50 ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.15 and CaCO_3 , 37.5) were inoculated in a rotary shaker (250 rpm) at 28°C for 96 hrs.

Enzyme assay :

To study extracellular glucose oxidase activity, centrifuged culture filtrates were used as the crude enzyme.

For intracellular enzyme activity harvested mycelia were crushed with 0.1 M phosphate buffer (pH 6.8). Following centrifugation at 1500 g for 10 min, the supernatant was taken out as the intracellular enzyme extract.

All operations for the enzyme extract were carried out at 4°C.

Glucose oxidase activity was assayed using glucose oxidase-peroxidase reagent according to the method described in Worthington Biochemical Corporation, Freehold, New Jersey (1966). One unit of glucose oxidase activity was defined as that amount of enzyme causing a change in absorbance (400 nm) of 1.0/min under the assay condition followed. This unit was found to be approximately equivalent to 0.79 of the commercial unit, which was defined as equal to that amount of activity taking up $10 \mu\text{l O}_2$ per minute in a Warburg Manometric assay under specified conditions (Scott, 1953). Specific activity was expressed as O. D. change/min/mg protein.

Catalase activity was determined by the method of Rendina (1971). The enzyme activity was expressed as the $\mu\text{moles of H}_2\text{O}_2$ decomposed by catalase in five minutes at 0°C. The specific activity was expressed in $\mu\text{moles H}_2\text{O}_2$ decomposed/min/mg protein.

Estimation of protein :

Protein content was estimated following the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Glucose oxidase and catalase activities of all the four strains are presented in Table 1. It reveals that in *A. niger* glucose oxidase activity was only intracellular corroborating the observations of others (Muller, 1928 ; Blain, 1975). On the other hand *P. funiculosum* showed both intra ; and extracellular activity although intracellular activity was more pronounced than extracellular activity.

It should be noted that from a commercial view point glucose oxidase preparations should contain appreciable catalase activity as this enzyme is advantageous for many of the industrial applications of glucose oxidase. Hence, the quality of

TABLE 1. Glucose oxidase and catalase activity of the strains of *A. niger* and *P. funiculosum*

Strains	Sources of enzymes	Specific activity	
		Glucose Oxidase	Catalase
		OD Change/min/ mg protein	μ moles H_2O_2 de- composed/min/mg protein
<i>A. niger</i>			
A7 (Wild)	Cell free extract	1.16	9.25
	Culture filtrate	—	—
MN 181 (Mutant)	Cell free extract	2.13	11.09
	Culture filtrate	—	—
<i>P. funiculosum</i>			
P5 (Wild)	Cell free extract	2.15	10.70
	Culture filtrate	1.71	9.20
MN 238 (Mutant)	Cell free extract	3.52	17.33
	Culture filtrate	2.98	12.02

glucose oxidase preparation is obviously dependent upon the amount of catalase enzyme coupled with it. As indicated in Table 1, the strains produced appreciable catalase to be coupled with their glucose oxidase enzyme.

However, all the strains were cultivated in a fermentation condition which was optimal for gluconic acid production and it may not represent the parameters for optimal glucose oxidase yield. Indeed, it is reasonably expected that if systematic studies are carried out on the cultural conditions of the strains for glucose oxidase production, one can possibly find the parameters suitable for commercial glucose oxidase production. In that case these mutants MN 181 and MN 238 would be highly appreciated in microbial industry for their use not only in calcium gluconate production but also in the production of glucose oxidase enzyme. Work is in progress in this direction.

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