# GLUCOSE OXIDASE PRODUCTION BY *CLADOSPORIUM*, *ALTERNARIA*, AND *ASPERGILLUS* FROM STORED GRAINS

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## الملخص

خلال هذه الدراسة تم عزل خمسين نوع من الفطريات المنتجة لإنزيم جلوكوز أوكسيديز من الحبوب المخزونة. ثمان وأربعون من العزلات كانت من نوع أسبرجيلوس نيجر، واحدة ألترناريا، وواحدة كلادوسبوريوم. إنتاج جلوكوز أوكسيديز من النوعين الأخيرين لم يسبق مشاهدته من قبل.

تراوح نشاط الجلوكوز أوكسيديز في مستخلص الخلايا مابين 0.003 ، 1.04 وحدة لكل جرام ميسيليوم ولم يلاحظ هناك أي نشاط يذكر للجلوكوز أوكسيديز خارج الخلايا.

## Abstract

Fifty glucose oxidase producing fungi were isolated from various stored grains: The isolates were 48 *Aspergillus niger*, one *Alternaria* species and one *Cladosporium* species. Production of glucose oxidase by the latter two species has not been previously reported.

Glucose oxidase activity in cell extracts of the organisms ranged from 0.003 to 1.04 units/g mycelia. There was no detectable extracellular activity of glucose oxidase.

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## INTRODUCTION

Glucose oxidase (GOX) (β-D-glucose: oxygen 1-oxidoreductase, EC 1.1.3.4.) from *Aspergillus niger* catalyzes the oxidation of β-D-glucose by molecular oxygen to give D-Glucono-δ-lactone and hydrogen peroxide (Creuger and Creuger, 1990).

GOX is of considerable industrial importance. It is widely applied for the determination of glucose in body fluids and for removing residual glucose or oxygen from foods and beverages. Furthermore, GOX-producing molds such as *Aspergillus* and *Penicillium* species are used for the biological production of gluconic acid (Lockwood, 1975; Schmid and Karube, 1988).

**The present study describes the isolation and screening procedure for various GOX producing fungi from stored grains.**

## MATERIALS AND METHODS

### Isolation of fungi

A sheet of sterile filter paper placed in a petri dish was moistened with sterile distilled water. The grains (bean, maize, wheat, and barely) were placed separately on the filter and the plate was incubated at room temperature (20°C) for five days. Growing fungi were transferred to slants of potato dextrose agar (Oxoid), incubated at 30°C for 2 to 3 days and stored at 4°C. Isolated fungi were identified by colony morphology and

light microscopy examination as described by Domsch *et al.* (1980).

**Screening for GOX production**

*Aspergillus niger* NRRL-3 was used as the reference strain. Preliminary screening of the isolates for GOX production was done as described by Fiedurek *et al.* (1986). The assay agar plates contained: glucose (1%), O-dianisidine (0.01%), peroxidase (200 u/mg) (0.003%), agar (1.5%), 0.1 M phosphate buffer, pH 7.0 and 0.7 M KCl. A brown zone around the growing colonies after 18 hours at 37°C was considered as an indication for GOX production. The zone diameter was measured to quantify the enzyme against a commercial preparation of GOX (27,500 u/g, Sigma) applied to wells (6 mm in diameter, cut in the agar plates) as the standard.

**Preparation of cell-free extracts**

Flasks (250 ml) containing 50 ml Czapek-Dox (Oxoid) liquid medium was inoculated with $10^7$ conidia, and inoculated at 37°C for 72 hours with vigorous shaking. The mycelia were collected by filtration, washed and resuspended in 0.2 M Tris-phosphate buffer, pH 7.0 (5 ml/g mycelia). The mycelia were homogenized using a glass-glass homogenizer. The homogenate was then centrifuged at 15,000 X g for one h at

4°C and the supernatant was collected.

### Spectrophotometric assay

The GOX activity was assayed in both cell-free extracts and post-culture liquid following the procedure of Fiedurek *et al.* (1986). GOX activity was calculated from a calibration curve constructed by using the commercial GOX.

## RESULTS AND DISCUSSION

Out of 140 screened mold isolates, fifty proved to be GOX producers by the employed agar-diffusion method. The GOX-producing molds were identified as *Aspergillus niger* (48), *Alternaria* species (1), and *Cladosporium* species (1).

GOX activity was detected only in the cell-free extract of the producer strains. The extracts exhibited a GOX activity ranging from 0.003 to 1.04 units/g mycelia. The post-culture fluid, however, did not show any GOX activity.

A number of studies pointing out to the nature of GOX production in certain molds are available: for instance in different species of *Penicillium* the enzyme could be extracellular (Kusai *et al.*, 1960) or intracellular (Rando *et al.*, 1997), whereas in *Aspergillus niger* it is intracellular (Pazur, 1966; Van Dijken *et al.*, 1980; and Sharif & Alaeddinoglu, 1992).

Changing the culture conditions, however, may affect the nature of the enzyme production. This has been reported by Mischak *et al.* (1985) who could by a pH shift induce the extracellular excretion of the enzyme from *Aspergillus niger*. In the current study, the results of Mischak *et al.* could not be reproduced. This could be due to differences, such as permeability characteristics, in the strain which they used in their study.

Under the assay conditions applied, and as shown in the bar diagram in Figure 1 below, some *A. niger* isolates (e.g., *A. niger* N-4, 0.41 u/g mycelia), *Alternaria* (0.43 u/g), and *Cladosporium* (1.04 ulg) exhibited higher enzyme activity than *A. niger* NRRL-3 (0.13 u/g), the latter is being used for large scale production of GOX or gluconic acid in industry.

In molds, GOX production was so far confined only to *A. niger* and certain species of *Penicillium*. This is the first report describing the production of GOX by *Cladosporium* and *Alternaria* species, both of which exhibited considerably high enzyme activity and can be used for the commercial production of the enzyme. Further work is needed for the fine characterization of these two species.

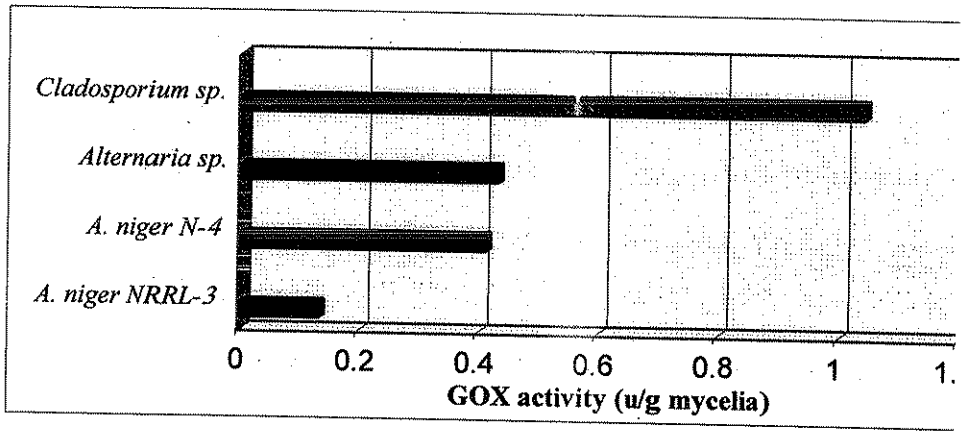


Figure 1. A bar diagram illustrating the highest spectrophotometrically-measured GOX activities observed in the cell-free extract of A. niger NRRL-3 (the reference strain), A. niger N-4, Alternaria sp. and Cladosporium sp. Results are indicated as enzyme units per gram mycelia of the corresponding fungi.

While screening the isolates for GOX production, it was found out that the diffusion zone diameters correlated linearly with the logarithm of GOX concentration (results are not shown). By using this simple agar diffusion method it is possible to rapidly detect and quantify GOX production in large number of fungal isolates. Although this method is easy to perform, the relationship between zone diameter and GOX concentration is linear only in the range of 0.16 to 1.6 enzyme units. The spectrophotometric assay, however, is more sensitive, especially at lower GOX concentrations i.e., below 0.16 units. Still, the agar diffusion assay can be applied for the

preliminary quantification of GOX production.

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