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## Isolation and Screening of Amylolytic and Pectinolytic Fungi from Soil

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## Abstract

Eleven fungal species namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium* spp., *Alternaria alternata*, *Fusarium pallidoroseum*, *Fusarium oxysporum*, *Aspergillus fumigatus*, *Rhizopus stolonifera*, *Fusarium moniliforme* and *Cephalosporiopsis* sp. were isolated. Out of these species, first 7 were amylolytic and last 4 were pectinolytic. These species were employed for the production of amylases and pectinases respectively. Among the amylolytic species the glucoamylase activity (IU/ml) in surface cultures was found in the order; 17.4, 15.20, 14.0, 9.6, 5.0, 4.50, and 4.00 for *A. niger*, *A. flavus*, *A. terreus*, *Penicillium* sp., *F. pallidoroseum*, *F. oxysporum* and *Alternaria alternata* respectively and glucoamylase activity in submerged cultures was found for the same fungal strains in the order 10.00, 9.00, 6.80, 6.20, 5.0, 3.88 and 3.50 units/ml respectively. Among the pectinolytic fungi the pectinase activity was found only in the surface culture in the order; 15.30, 12.00, 10.20, and 8.00 units/ml for *Rhizopus stolonifera*, *Aspergillus fumigatus*, *Fusarium moniliforme* and *Cephalosporiopsis* sp. respectively.

## Introduction

Fungi perform a number of industrial processes involving fermentation (Alexopoulos and Mims, 1979) by the production of enzymes or metabolites. Among the various enzymes produced by fungi, amylases are widely used in food processing and baking industries due to their biocompatibility which had led the USFDA to approve their use in food processing industries (Shah *et al.*, 1991). Besides amylases, microbial enzymes catalyzing the degradation of pectic polysaccharides play an important role in foods and food processing. As far as food industry is concerned, pectolytic enzymes primarily from fungal sources play a key role in fruit juice technology by degrading pectins and thus clarifying the juices (Alana *et al.*, 1989). Keeping in mind all these facts, the efforts were made to isolate and screen the amylolytic and pectinolytic fungi from the soil.

## Materials and Methods

Soil samples from different areas of canal (NIAB) were collected. All the samples were mixed thoroughly and divided into three portions. One portion was amended with starch, other with pectin and one unamended. All the three portions were incubated at 30°C for 24 hours. Warcup (1950) isolation method was used and samples were plated on culture medium containing various inorganic salts in addition to corn starch and citrus pectin as carbon source respectively. Unamended soil sample was plated on PDA. The isolation plates were made after 1, 2, 3, 6, 9 days of incubation. Culture plates were incubated at 30°C and growth observations were made from time to time. Appearance of species was recorded by their presence or absence on any of the duplicate plates. Pure cultures were obtained by subculturing on PDA slants or petri plates. After obtaining the pure cultures of various species, screening of the amylolytic and pectinolytic enzymes produced by the fungal species was carried out. For screening of amylolytic fungi, corn starch was used in submerged cultures and

wheat bran in surface cultures. For pectinolytic fungi pectin was used as substrate for growth in submerged culture. Bernfeld (1955) enzyme assay method was carried out for amylases. For pectinolytic fungi, pectin was used as substrate for growth in submerged culture. For pectinase enzyme assay method of Gomes *et al.* (1992) was used. Screened species were partially identified by observing them under the microscope and carrying out their free hand drawings with the help of authentic available literature. Especially the work by Booth (1971), Ellis (1971) Barron (1972) Raper and Fennell, (1965), Mirza *et al.* (1983) and Paul (1989) proved useful during identification.

## Results and Discussion

Eleven fungal species were isolated during this investigation (Table 1). Out of these species, 7 were amylolytic and 4 were pectinolytic.

Table 1: Glucoamylase Activity (units /ml).

Fungal Strain	Enzyme Activity (Units/ml)
<i>Surface Cultures</i>	
<i>Aspergillus niger</i>	17.40
<i>A. flavus</i>	15.20
<i>A. terreus</i>	14.00
<i>Penicillium</i> sp.	9.60
<i>Fusarium pallidoroseum</i>	5.00
<i>F. oxysporum</i>	4.50
<i>Alternaria alternata</i>	4.00
<i>Submerged cultures</i>	
<i>Aspergillus niger</i>	10.00
<i>A. flavus</i>	9.60
<i>A. terreus</i>	6.80
<i>Penicillium</i> sp.	6.20
<i>Fusarium pallidoroseum</i>	5.00
<i>F. oxysporum</i>	3.88
<i>Alternaria alternata</i>	3.50

Table 2: Pectinase activity (units/ml)

Fungal Strain	Enzyme activity (units/ml)
<i>Submerged cultures</i>	
<i>Rhizopus stolonifera</i>	15.30
<i>Aspergillus fumigatus</i>	12.00
<i>Fusarium moniliforme</i>	10.20
<i>Cephalosporiopsis Sp.</i>	08.00

Glucoamylase activity in (IU/ml) surface cultures was found in the order; 17.4, 15.20, 14.0, 9.6, 5.0, 4.50 and 4.00 for *A. niger*, *A. flavus*, *A. terreus*, *Penicillium sp.*, *F. pallidoroseum*, *F. oxysporum* and *Alternaria alternata* respectively. While glucoamylase activity in submerged cultures was found in the following order for the same fungal strains: 10.00, 9.6, 6.8, 6.2, 5.0, 3.88 and 3.50 units/ml respectively (Table 1). It is obvious from above experiments that solid substrate fermentation is a better method of enzyme production than submerged fermentation. More enzyme concentration is obtained in solid state fermentation as compared to that of submerged fermentation. It is found (Shah *et al.*, 1991) that surface fermentation is more economical procedure for glucoamylase production than that of submerged fermentation.

Among the pectinolytic fungi, *Rhizopus stolonifera* was found to be the best pectic enzyme producer (15.3 units/ml), 2nd efficient fungal strain was *Aspergillus fumigatus* (12 units/ml). *Fusarium moniliforme* and *Cephalosporiopsis sp.* also gave good results (10.2 and 8.0 units/ml) respectively (Table 2).

Similar results were observed for pectinase from *Aspergillus niger* (Zetelaki-Horvath and Thien, 1985) and for PL from *Penicillium expansum* (Spalding and Abdul-Baki, 1972).

It was concluded that enzyme production may be enhanced if parameters for enzyme production were optimized.

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