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Isolation and selection of *Aspergillus* species for hyper-production of polygalacturonases

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Abstract: Pectinase is an enzyme that breaks down pectin, a polysaccharide found in plant cell walls. Commonly referred to as pectic enzymes, they include pectolyase, pectozyme and polygalacturonase. Polygalacturonases (PG) are pectinolytic enzymes that have biotechnological, functional and biological applications in food processing, fruit ripening and plant-fungus interactions. The aim of the study was to isolate the fungi from different vegetative/fruit wastes and screen them on the basis of enzyme activity. In the current study, 10 fungal strains were isolated from moldy vegetables and fruit samples collected from different vegetated fields. Among these isolates, 5 strains were selected and identified on the basis of morphological and enzymatic assays of *Aspergillus* species. One single strain was selected at 0.5%, 1.0%, 2.0% and 2.5% pectin enzyme activity. Strain "A" showed the maximum enzyme activity among the 5 selected strains, i.e. 1170 U/ml/min. This native hyper-producing strain of *Aspergillus niger* can be used for polygalacturonase production in several biotechnological industries.

Keywords: *Aspergillus* spp, pectinolytic activity, polygalacturonase, submerged fermentation.
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INTRODUCTION

Pectinases are a group of enzymes that depolymerize the pectin by hydrolysis and trans-elimination as well as by de-esterification reactions that hydrolyse the ester bond between carboxyl and methyl groups of pectin[1]. Pectin is a complex polysaccharide found in the cell wall of higher plants and cementing material for the cellulose network. Pectinases account for 10% of global industrial enzymes produced and their market is increasing day by day[2].

Pectinases are widely used in industrial processing of fruits and vegetables because they reduce the viscosity of juices and facilitate extraction, maceration, liquefaction and clarification processes[3].

Polygalacturonases are pectic enzymes that hydrolyze pectic substances into their monomeric units[4]. They are depolymerizing enzymes that cleave glycosidic bonds of pectins by means of hydrolysis[5]. Polygalacturonases are formed in the majority of plant tissues particularly in ripening fruits. Even various plant pathogenic and saprophytic microorganisms produce polygalacturonases[6]. The critical role of these enzymes in the degradation of the host middle lamella and cell walls, leading to plant tissues merceration and cellular death had been documented [7-8].

Aspergillus niger strains are widely used in several fermentation processes for the production of pectic enzymes. The synthesis of pectinases is induced by pectin or some of its derivatives[9]. The cell growth, sporulation and production of the enzymes can be affected by the composition of the medium and fermentation conditions[10, 11.]

This study was aimed to identify and select the fungal isolate on the basis of maximum proteolytic activity by *Aspergillus* spp. in submerged fermentation.

MATERIALS AND METHODS

Morphological screening of organism

Numerous fungal strains were isolated from the rotten vegetables and soil samples. These samples were collected from different vegetative fields of Karachi, Pakistan. The isolates were purified and screened for pectinolytic activity. Potassium-iodide solution was used to observe the clear zone around the colonies [12]. Five strains were selected on the basis of maximum zone of hydrolysis. These strains were further analyzed and identified on the basis of morphological and enzymatic assay of *Aspergillus* species[13].

Fermentation medium for the isolation of pectinase producing Aspergillus niger spp.

Submerged fermentation was carried out using the medium comprised of 1.0% pectin, 2.5% glucose, 0.5% yeast extract, 0.1% peptone, 0.4% KH_2PO_4, 0.4% $(NH_4)_2SO_4$, 0.2% $MgSO_4.7H_2O$. In addition, 2.4% agar was added to solidify the medium wherever required. The pH was adjusted to 5.5.

Culture maintenance

Isolated fungal strains were maintained on Czapek-Dox Agar slants at 4ºC.

Microorganism inoculum

Sterile pectin broth medium (5.0ml) was inoculated with a fungal growing culture and incubated at 30ºC for 3 days.

Production of enzyme

Total 5.0ml of the inoculum was transferred in 250-ml Erlenmeyer's flask containing 45.0ml sterile pectin broth medium and then incubated at 30^0C for different time intervals. Cells were harvested by centrifugation at 4000 rpm for 30 minutes at 4^0C and the clear supernatant containing extracellular polygalacturonase enzyme was preserved at-18^0C for the enzyme activity assay.

Estimation of reducing sugar

The reducing sugar was determined by 3,5-Dinitrosalicylic acid (DNS) method[14].

Enzyme assay

The enzyme assay was analyzed by using citrus pectin as a substrate and galacturonic acid monohydrate as the standard. The amount of reducing sugar released as galacturonic acid was analyzed by 3, 5-dinitrosalicylic acid method[14]. One unit of pectinase activity is defined as the amount of enzyme required to release 1 μM of galacturonic acid per minute under standard assay conditions.

RESULTS AND DICUSSION

Purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures (Figure 1). Fungal isolates were characterized as *Aspergillus niger*. Culture slides were observed at various magnifications at 10x, 40x, and 100x under electron microscopy (Figure 2).

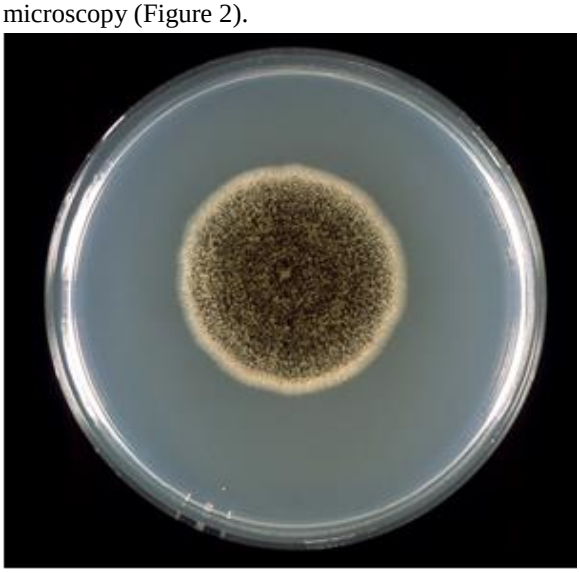

Figure 1: Isolated colonies of *Aspergillus niger* on pectin agar plate.

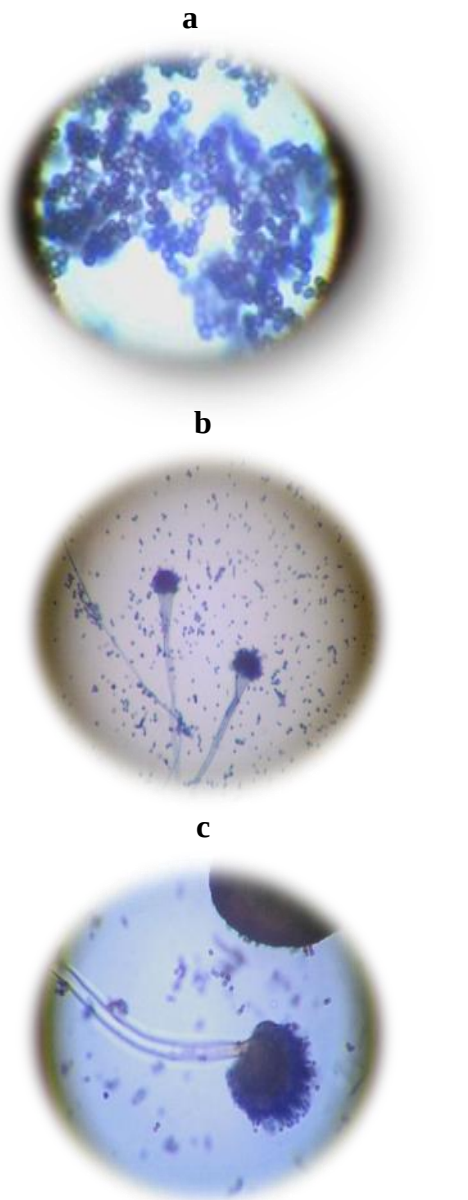

Figure 2: Microscopic slides of isolated *A. niger* observed at (**a**) 10x, (**b**) 40x, and (**c**) 100x magnifications.

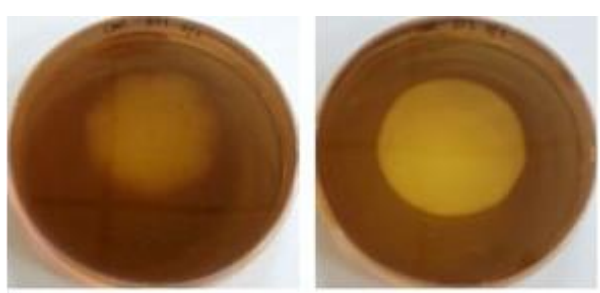

Figure 3: Isolated strain of *A. niger* showed largest clear zone of pectinolytic activity around the colonies, after staining with iodine-potassium iodide solution.

Table 1: Enzyme Activity of 10 selected fungal strains (A to J) observed on day 3, 4, 5, 6, and 7of incubation at 30^0C. Among all of them, strain A showed higher concentration of reducing sugar on day 5.

Strains	Enzyme Activity (U/ml/min)				
	Day 3	**Day 4**	**Day 5**	**Day 6**	**Day 7**
A	649	694	**1170**	869	669
B	587	421	**966**	800	667
C	298	64	529	529	449
D	311	466	220	120	90
E	290	470	629	429	229
F	44	93	510	310	210
G	376	456	**787**	587	387
H	138	485	619	319	119
I	416	534	**832**	732	632
J	396	493	**655**	455	355

Selected fungal isolates were grown on pectin agar plate for screening purpose and pectinolytic activity was observed using plate assay method. The isolated strains showed clear zone around the colonies, after staining with iodine-potassium iodide solution. The results indicated that among these isolated strains, fungal strain A showed maximum pectinolytic activity on pectin agar plate (Figure 3). Similar results have also been reported earlier[12].

The enzyme activity of 10 selected fungal strains (A to J) were determined on day 3, 4, 5, 6 and 7 of incubation at 30^0C. Among them, strain A showed the maximum production of enzyme on day 5 as compared to day 3 and 4. The activity was found to be decreased on day 6 and 7 (Table 1). Similar results were also reported[15].

Furthermore, this study was specified for the selection of 5 strains (A, B, G, I, J) on the basis of enzyme activity *vs*. variation in substrate concentration (0.5%, 1.0%, 2.0% and 2.5% pectin) as shown in Figure 4. Strain A showed the highest production of enzyme at 1% substrate concentration.

Many species of fungi are capable of degrading pectin by producing pectic enzymes. The fungal isolates of fruit pulp wastes were also found to produce pectinases. Production of pectin enzymes by fungi such as *Alternaria*, *Cladosporium*, *Colletotrichum*, *Mucor*, *Penicillium*, and *Trichoderma* was confirmed[16,17].

The fungus *Aspergillus niger* CSTRF, when grown on mineral medium containing pectin (Sigma) produced polygalacturonase. The production of the PG into the culture filtrates of the fungus confirmed the hydrolysis of pectin leading to the production of PG. The hydrolysis of pectin in the culture medium of *A. repens* had also been reported earlier[18].

The production of PG by *Aspergillus*, *Fuasrium*, *Penicillium*, *Thermoascus*, *Lentinus* species on various substrates during solid substrate fermentation and submerged fermentation[19] are strong evidences of the hydrolysis of pectin and pectin containing materials for the growth of the fungi[20].

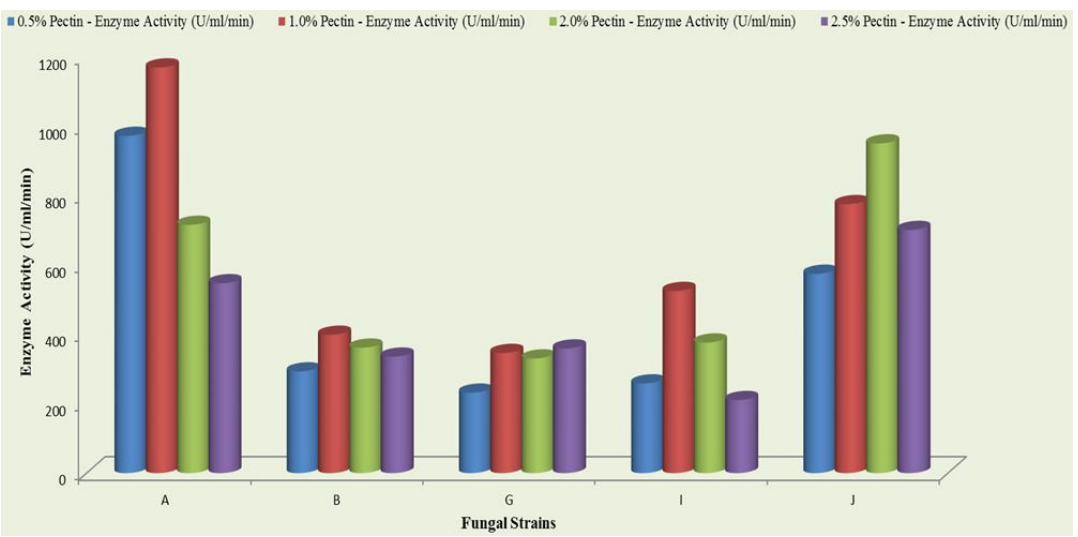

Figure 4: Selection of fungal strain on the basis of enzyme activity *vs*. substrate concentration.

CONCLUSION

Newly isolated strain of *Aspergillus niger* was studied from native sources for the hyper-production of polygalacturonase that can be used in various industrial applications.

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