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Strain Improvement through Mutation to Enhance Pectinase Yield from *Aspergillus niger* and Molecular Characterization Of *Polygalactouronase* Gene

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Abstract

Strain improvement is an important tool in commercial development of microbial fermentation processes for hyper production of enzymes. *Aspergillus niger* was isolated and identified as best pectinase producer through submerged fermentation and the same culture was subjected to mutate through physical (UV irradiation at 254 nm) and chemical (ethidium bromide, ethyl methyl sulphonate and sodium azide) mutation for enhancement of pectic acid-degrading enzymes (pectinase). Mutated *A.niger*UV radiation exposure for 60 mins yields 260 nkat of pectinase activity when compared with wild strain yielded 235.06nkat. A mutant of *A.niger* showed higher enzyme activity on treatment with ethyl methyl sulphonate (2mg/ml for 60 mins) followed ethidium bromide (6mg/ml for 60mins) treatment found to be 265 nkat and 340 nkat of pectinase activity respectively. The pectinase producer which is susceptive to sodium azide (0.1% for 30min) yields 180 nKat pectinase activity. The combined UV and EtBr treatment (60min, 6mg/ml) yielded mutant with 1.69 fold enhanced polygalacturonase production compared with wild strain. The structure of poly galactouronase gene (*pga*I) was investigated and the amino acid sequence encoding enzyme was found to have 99% similarity with other *A.niger* isolates.

Key words: *Aspergillus niger*, mutation, ethidium bromide, sodium azide, *pga*I gene sequencing.

INTRODUCTION

For commercial production of enzyme, filamentous fungi are more commonly employed than yeast and bacteria [1]. Saprophytic and plant pathogenic fungi produces various enzymes for degrading plant cell wall component, which majorly comprises pectinase, the enzyme involved in the degradation of pectin. Pectinase are the group of enzymes involved in Pectin degradation; a polysaccharide substrate found in the cell wall and middle lamella of plants [2]. Pectins are high molecular weight, negatively charged, acidic, complex glycosidic macromolecules that are present in the Plant kingdom. Most pectic enzyme are used in the fruit processing industry. Pectic enzymes alone account for about one quarter of the world's food enzyme production [3]. The most common application of pectinase is in the food industry to extract and clarify fruit and beverages [4,5]. Pectinases are also used in industrial processes, such as in ramie fiber degumming, oil extraction, coffee and tea fermentation, and industrial wastewater treatment [6,7].

Production of Pectinase has been largely focused in *Aspergillus niger*[8]. *A.niger* is a work horse of industrial microbiology that has been subjected to different types of mutagenesis for enhanced production of pectinase. Enzymes produced from the fungi *Aspergillus*, are generally regarded as safe (GRAS) [9]. Various fruit and vegetable processing waste have been employed for the production of pectinases [10]. Due to the fact that, agricultural residues are attractive due to its low cost and abundant availability, the ability of *A. niger* isolates are tested for the production of polygalacturonase by the utilization of citrus fruit peel as substrate. Strain mutation, induction and screening techniques can improve microbes for pectinase production by using different mutagens such as nitrous acid, diethyl sulfate (DES), and ethyl methyl sulfonate. When fungi are grown with mutagens at sub-lethal concentrations, the rate of enzyme production often increases. The treatment of spores and vegetative mycelium is recognized as an effective means for generating mutants [11,12]. Exposure of fungal cells to UV have increased pectinase yield [13]. Polygalacturonase (PGA) is one of the major members of pectinases which cleaves α-1,4-glycosidic of D-galacturonic acid in pectin and it is classified into endo- and exo-polygalacturonase on the basis of the way of eliminating galacturonic acid. Seven different *pga* genes present have been already characterized [14,15]

In this paper, various mutational studies are reported with an aim to improve the enzymatic yield and molecular characterization of *pgaI* gene from soil fungi *Aspergillus niger*. Its Nucleic acid and amino acid homology are studied through sequence alignment with existing data base of *A.niger*.

MATERIALS AND METHODS

Microorganism

The high yield pectinase producer *Aspergillus niger* culture was isolated from soil collected from Porur vegetable market) and was maintained at 4°C on potato dextrose agar slants (HiMedia, India).

Fermentation media

Submerged fermentation medium of 100 ml was prepared with Wheat bran 20% and Orange peel 10%, pH 5.5 (Adjusted using acetic acid) and sterilized at 15 psi (121°C). The spore suspension (10^7 spores.ml^{-1}) was inoculated and incubated at RT for 8 days under 200 rpm agitation. The culture medium (5 ml) was centrifuged (10,000 g, 10 min, at 30°C) and supernatant was collected for further analysis.

Mutation by physical agents

UV mutagenesis

The cell suspensions of different dilutions were prepared 10^{-6},10^{-7},10^{-8}. The induction of mutation was carried out by exposure to UV germicidal lamp (256 nm) kept at a distance of 50 cm for time intervals ranging from 10–90min, [16,17]. After irradiation, the plates were wrapped in aluminum foil and were kept in dark. 0.1ml of cell suspension was inoculated into Potato dextrose agar plates. About 45 single cell colonies were isolated from those plates and screened for pectinase activity. The fungal isolate which has maximum zone of clearance (UV9) was inoculated in production medium and incubated at RT for 7 days.