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RESEARCH ARTICLE

Screening of *Aspergillus niger* Strains for Pectinolytic Activity by Solid State Fermentation

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Abstract

Various strains of *Aspergillus niger* isolated from soil, spoiled fruits and vegetables were screened for pectinase production. Amongst 102 strains isolated, 28 strains were positive for pectinase production on pectinase assay plates. Strains showing large clear zones in the plate assay were used for enzyme production by solid state fermentation. Depending upon the zone of clearance around the colony they were classified as good pectinase producers (0.5-1 cm); if the halos were <0.5 cm they were considered poor pectinase producers, while non-pectinolytic strains showed no zone of clearance. The positive pectinase producers were subjected to solid state fermentation using citrus fruit peel as substrate. Among 28 strains tested, the *Aspergillus niger* strain PSV23 showed maximum polygalacturonase activity (5.411 IU/mL) and emerged as a potential strain for the production of pectinase from citrus fruit peel.

Keywords: *Aspergillus niger*, pectinase, zone of clearance, solid state fermentation, citrus fruit peel.

Introduction

Pectin is an important component of middle lamella and primary cell wall of higher plants. Pectinases are mainly used in the food industry to clarify fruit juices and other beverages. These are high molecular weight polysaccharides primarily made up of α(1−4) linked D-galacturonic acid residues with a small number of rhamnose residues in the main chain and arabinose, galactose and xylose on its side chain (Deul and Stutz, 1958; Singh *et al.,*1999; Kapoor *et al.,* 2000; Lang and Do-Renberg, 2000).

Pectinases are widely used for biotechnological applications in food industry (fruit juice extraction, coffee and tea fermentation, oil extraction, improvement of chromaticity and stability of red wines), textile, paper and pulp industries (Cao *et al*., 1995). Pectinase production has been reported from bacteria including Actinomycetes (Bruhlmann *et al.,* 1994; Elegado *et al.,* 1999; Beg *et al.,* 2000), Yeasts (Huang and Mahoney, 1999) and Fungi (Hawksworth *et al.,* 1983; Gummadi and Panda, 2003). However, for the industrial production of pectinases, *Aspergillus niger* strains are used exclusively (Marcia *et al.,* 1999). The enzyme preparations used in the food industry are of fungal origin because fungi are potent producers of pectic enzymes and the optimal pH of the fungal enzymes are very close to the pH of many fruit juices, which ranges from pH 3-5.5. Such preparations are not suited for the production of vegetable purees or other preparations in which pH values are close to neutral. Therefore, the commercial pectinase production is still dominated mainly by *Aspergillus niger* strains.

But these enzymes suffer from limitations like low temperature stability. Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It is of special interest in processes where the crude fermented product may be used directly as the enzyme source. This system offers numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipments, etc (Pandey *et al.,* 1999). Hence, an attempt has been made to screen the potential *Aspergillus niger* strains for pectinase production by solid state fermentation.

Materials and methods

Chemicals: Mineral salts, Pectin, CTAB and 2,6 Di-nitrosalicylic acid used in the study were of analytical grade procured from Hi-media Laboratories, Mumbai, India.

Isolation of A. niger strains: The fungal strains were isolated from spoiled fruits, vegetables and soil samples. The isolation medium consisted of 1% pectin; 0.14% $(NH_4)_2SO_4$; 0.20% K_2HPO_4; 0.02% $MgSO_4.7H_20$; 0.10% nutrient solution (5 mg/L $FeSo_4.7H_2O$; 1.6 mg/L $MnSO_4.H_2O$; 1.4 mg/L $ZnSO_4.7H_2O$; 2.0 mg/L $CoCl_2$) and 20 g/L agar, pH 5. The samples were diluted in sterile distilled water and then inoculated onto the earlier prepared medium and incubated at 30°C for 24-72 h (Hawksworth *et al.,* 1983).

All the morphologically distinct colonies were purified by repeated streaking on pectin medium. Identification of genus was based on morphological and biochemical characteristics and was maintained on PDA slopes as stock cultures. The *Aspergillus niger* strain MTCC 3323 procured from IMTECH, Chandigarh was used as a positive control during the study.

Plate assay of depolymerized pectin: The medium was the same used for isolation of cultures, supplemented with 2% agar. Pure cultures were inoculated on the medium used for isolation and incubated at 30°C for 24-48 h. After the colonies reached approximately 3 mm, 1% hexadecyl tri-methyl ammonium bromide was added to detect clear zones (Aneja, 2005).

Production of pectic enzymes by solid-state fermentation: Strains showing large clear zones in the plate assay were used for enzyme production by solid state fermentation. Citrus fruit peel was collected from nearby juice vendors and dried in hot air oven overnight to remove available moisture. The fermentation studies were carried out in 250 mL Erlenmeyer flasks containing 25 g of previously dried citrus fruit peel and was adjusted to 70% moisture level with sterile distilled water and then inoculated with 2 mL of spore suspension (10^6 spores/mL) and were incubated at 30°C. After every 24 h intervals, 1 g of the fermented substrate was withdrawn and the enzyme was extracted in 10 mL of 0.2 M citrate buffer (pH 5) and filtered through Whatmann filter paper No 1. The filtrate was then centrifuged and supernatant was used to evaluate the polygalacturonase activity. The fermentation was carried out for 120 h till the enzyme activity decreased.

Assay of polygalacturonase activity: Polygalacturonase activity was determined by measuring the release of reducing groups using the di-nitrosalicylic acid reagent by DNS assay (Miller, 1959). The reaction mixture containing 0.8 mL of 1% pectin in 0.2 M citrate buffer (pH 5) and 0.2 mL of culture supernatant was incubated at 40°C for 10 min. Then the reaction was stopped by the addition of 2 mL of DNS reagent and the optical density was measured at 540 nm. The enzyme activity (IU) was defined as 1μ mole of galacturonic acid released per mL, per min.

Results

A total of 82 *Aspergillus niger* strains were isolated from spoiled fruits, vegetables and soil samples on the medium containing pectin as the sole carbon source (Table 1). These strains were further tested for pectin hydrolysis by plate assay at pH 5. Clear zone around the colony indicated pectin degradation. Depending upon the zone of clearance around the colony they were classified good pectinase producers (0.5-1 cm); if the halos were <0.5 cm they were considered poor pectinase producers, while non-pectinolytic strains showed no zone of clearance.

Table 1. *Aspergillus niger* strains isolated for pectinase activity.

Source	No. of isolates
Spoiled fruits	
a) Banana	08
b) Citrus	20
c) Apple	02
d) Pomegranate	16
Spoiled vegetables	06
Soil	30
Total	82

Table 2. Polygalacturonase activity of *Aspergillus niger* strains under SSF.

Strains	Enzyme activity (IU/mL)
PSV1	4.871
PSV3	3.675
PSV5	4.657
PSV7	5.112
PSV8	3.452
PSV9	5.213
PSV11	5.321
PSV15	4.897
PSV21	5.165
PSV23	**5.411**
PSV26	4.014
PSV35	4.157
PSV47	4.213
PSV56	4.213
PSV68	3.254
PSV72	3.654
PSV77	2.654
PSV82	3.456
Control MTCC 3323	**5.320**

Among the 82 strains, 18 strains were positive for pectinase production (Table 2). *Aspergillus niger* strains demonstrating high pectinolytic activity on pectin plate assay were grown on dried citrus fruit peel as substrate for polygalacturonase production under SSF. The results revealed that, *A. niger* strain PSV23 showed maximum polyglacturonase activity (5.411 IU/mL) compared to control MTCC3323 strain (5.320 IU/mL) subjected to solid state fermentation studies with citrus fruit peel as substrate (Table 2).

Discussion

In recent years, considerable interest has been paid for the use of microorganisms in industrial fermentation processes. To maximize any production process by an isolated organism the basic need is to have preliminary information on growth conditions and its associated fermentation characteristics. There is an increased interest in the utilization of fungi for the production of acid pectinases which has wide application in the fruit processing industry. Due to the fact that, agricultural residues are attractive due to its low cost and abundant availability, the ability of *A. niger* isolates was tested for the production of polygalacturonase by the utilization of citrus fruit peel as substrate.

The present study suggested that citrus peel were found to be the best substrate for polygalacturonase production by *A. niger* strain PSV23. Similar studies were also carried out where orange bagasse gave higher yields of polygalacturonase by *Penicillium viridicatum* RFC3 (Silva *et al.,* 2002). Orange peel, a waste generated from orange juice and canning industry has been successfully used for the production of pectinase by *A. carneus* NRC1 (El-Sheek *et al.,* 2009). This waste is generated after the extraction of juice is available in high quantity from fruit processing industries, but has a limitation of availability in only particular season. It's dumping in nature causes pollution problems; hence its eco-friendly utilization is essential which tempted us to use this agro-waste for pectinase production by SSF (Afifi and Foaad, 2002). The exploitation of pectinases, mainly exo-polygalacturonase have been well established in variety of fruit juice and wine processing industries to increase the juice yield, clarification, promoting antioxidant formation and juice concentrate production. The addition of such exogenous enzyme also allows more specific degradation which is necessary to give a characteristic smooth texture, colour and increases level of reducing sugar (Vlugt *et al.,* 2000).

Conclusion

The potential of pectinases solely as a food enzyme is well known in biotechnological industries because of their numerous applications. The production of these enzymes from agrowastes by fungal strains in SSF systems not only is cost-effective but can also offer several process merits. Citrus fruit peel in the present context is largely available these days is also an environmental hazard, can serve as a substrate for the production of pectinases. The results also prove that SSF could offer advantages over Smf for this purpose. *Aspergillus niger* PSV23 can be used for further scaling-up process for pectinase production by SSF.

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