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Short communication

Mango peel as substrate for production of extra cellular polygalacturonase from *Aspergillus fumigatus*

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Indian mango processing industry is facing problem in disposal of mango peel which constitutes approx. 15-20 per cent of total fruit weight. The peel is usually not utilized for any specific purpose rather it is either gifted away for cattle feed or is dumped in open area to add to environmental pollution. Polygalacturonase (EC 3.2.1.15), a principal components of pectinase, hydrolyzes α-1-4-galacturonide bonds in pectin. It is widely used for extraction of juices, clarification of wines, etc. In India, bulk of this enzyme used by food processing industries is imported and hence, an immediate attention is required to produce it in the country. The cost of enzyme production can be significantly reduced if low value substrates like fruit processing wastes are used as substrate for its production (Martin *et al.*, 8). Mango peel is generally termed as total waste. If a factory is processing 5 tonnes of mangoes per hour say working of 8 h a day, about 6 tonnes of peel would be available as waste. The peel is rich in pectin and its amount varies from 10.9-19.0 per cent on dry weight basis among different cultivars (Tandon and Garg, 10). Therefore, experiments were conducted to determine the potential of using mango peel as substrate for polygalacturonase (PG) production using a pectinolytic microbial isolate.

Ripe peel obtained after processing of fruit was dried and powdered and then used for PG production. Pectinolytic microorganisms were isolated from decomposing mango peel, citrus peel, degrading guava and other pectin rich wastes and purified by sub culturing on MP-5 and MP-7 medium (Speck, 9). Preliminary screening of forty three isolates was carried out for PG production by plate assay method (Hankin and Anagnostakis, 2). Twenty cultures showing high potency index were subjected to secondary screening on the basis of galacturonic acid released in the medium under submerged fermentation conditions.

The fungal isolate showing maximum enzyme activity, isolated from degrading guava, identified as *Aspergillus fumigatus* Fresenius MTCC 8234, by Microbial Type Culture Collection (MTCC), Chandigarh, India, was taken up for studies. The fungus was maintained on Potato Dextrose Agar slants. The effect of parameters like substrate concentration, aeration, nitrogen and phosphorus addition, incubation temperature and pH on PG production was studied. Partial purified enzyme was used to study the enzyme kinetics. For optimization of substrate concentration for the production of PG, mango peel suspensions (1-10% w/v) were prepared and pH was adjusted to 5.0. The effect of pH on PG production was studied between pH 4.0-8.0 using 5 per cent mango peel suspensions. For temperature optimization, 5 per cent mango peel suspension (pH 5.0) was inoculated at different temperatures ranging from 30 to 50°C. All experiments were conducted in triplicate and the mean values are reported. For enzyme kinetic study, polygalacturonic acid (0.3- 3.0 % w/v in 50 mM phosphate buffer, pH 5.0), pH (4.0-8.0), temperatures (20-70°C) were used as variables. For solid-state fermentation, sterilized mango peel (10 g) having $(NH_4)_2SO_4$ (1.0 g), pH 5.0 and moisture 70 per cent was inoculated with 10^6 fungal spores and incubated at 30 ± 2°C for 5 days. The crude enzyme was extracted by adding 0.2 M acetate buffer (pH 5.0) followed by filtration and centrifugation at 14000 g for 15 min. The supernatant was used for enzyme assay.

Submerged fermentation was done using sterilized mango peel suspension (5% w/v) along with $(NH_4)_2SO_4$ (0.05% w/v) was inoculated with 10^6 fungal spores and incubated at 30 ± 2°C for 5 days under stationary or stirred (200 rpm) conditions. The cell free content was obtained by filtration followed by centrifugation. The resultant clear extract was assayed for PG activity. For PG assay, reaction mixture containing 0.8 ml of one per cent (w/v) polygalacturonic acid in 50 mM phosphate buffer (pH 5.0) and 0.2 ml of the enzyme solution was incubated at 30°C for 30 min. PG activity was quantified by measuring the amount of reducing sugars released in the medium (Miller, 5). One unit of PG is the amount of enzyme that produced one ìmol of galacturonic acid min^{-1} ml^{-1} under assay conditions. The production of PG is expressed as unit/g substrate. Protein in the supernatant was measured by the method

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of Lowry *et al*. (4). For partial purification, enzyme in the culture filtrate was precipitated by centrifugation at 14000 rpm using cold acetone and then dried under vacuum to eliminate any acetone residue. The partially purified enzyme after precipitation was loaded on SDS-gel as per method described by Laemmli (3), with modification for better resolution of protein. Twelve per cent separating and 5 per cent stacking gel was prepared and a molecular weight standard of 3500-205000 Da was used. Commercial pectinase (E. Merck, Germany) was used as control. After primary and secondary screening, out of forty three microbial isolates, *Aspergillus fumigatus* Fresenius MTCC 8234 exhibited maximum (39.05 u/g) PG activity (Fig. 1) and fermentation conditions were optimized using this culture. Phutela *et al*. (6) used a thermophilic *Aspergillus fumigatus* strain for PG production on wheat bran as substrate. Dried orange peel powder served as a very good inducer of PG by *Aspergillus niger* (Nighojkar *et al*., 7).There are reports of pectic enzyme production by various strains of *Aspergillus* in different synthetic media (Singh and Rao, 8).

The results on substrate concentration revealed that among five concentrations of mango peel, *viz.* 1, 3, 5, 7 and 10 per cent, highest PG production (41.96 u/g) was observed at 5 per cent concentration (Fig. 2). Kaur *et al.* (4) reported 2 per cent (w/v) pectin isolated from citrus peel for maximum PG production. Addition of nitrogen as ammonium sulphate (1.5% w/w of substrate) had a positive effect (42.72 u/g), while addition of phosphorus (0.5% w/w of substrate) as O-phosphoric acid had a negative effect on PG production (Fig. 3). The observation is in concurrence with those of Phutela *et al.* (6) also observed stimulatory synthesis of PG due to nitrogen addition. Our earlier findings have also shown that mango peel is a poor source of protein and hence addition of nitrogen is required to support the fungal growth (Garg *et al.,* 1). The maximum PG production (43.24 u/g) was observed at 30^{0}C. However, it could be produced at 50°C also (Fig. 4). Higher PG (43.14 u/g) production was observed at pH 5.0 (Fig. 5). Either increase or decrease in pH beyond this value, decreased enzyme production. Aerated conditions

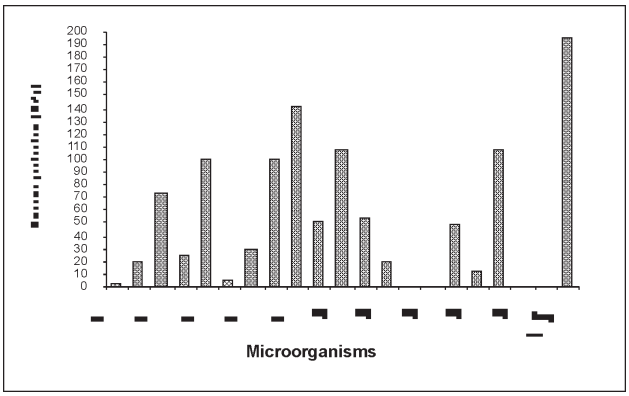

Fig. 1. Screening of microorganisms for polygalacturonase activity.

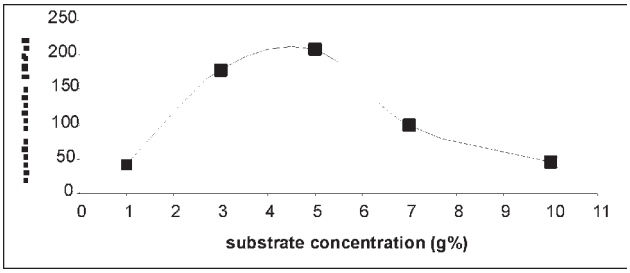

Fig. 2. Effect of substrate concentration on polygalacturonase production using *A. fumigatus*.

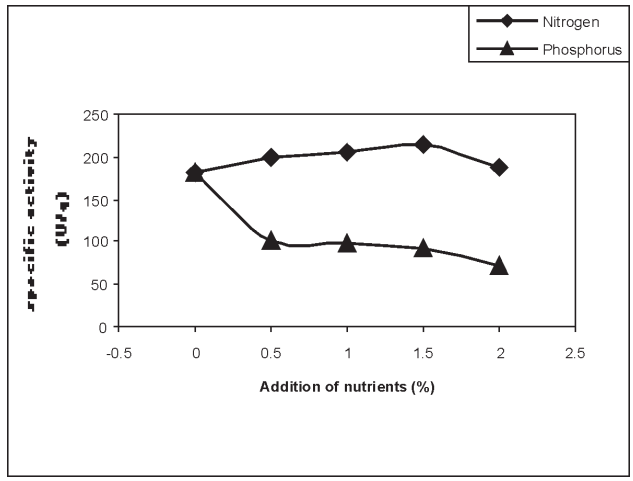

Fig. 3. Effect of nutrient addition on polygalacturonase production using *A. fumigatus*.

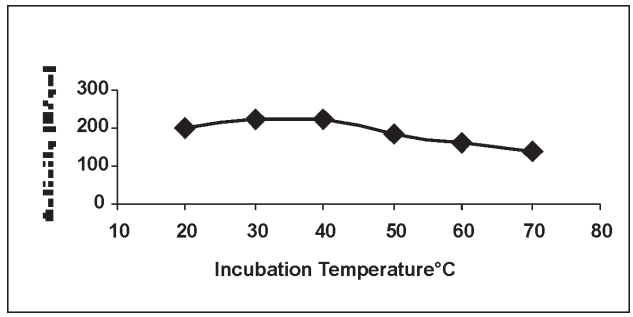

Fig. 4. Effect of incubation temperature on Polygalacturonase production using *A. fumigatus*.

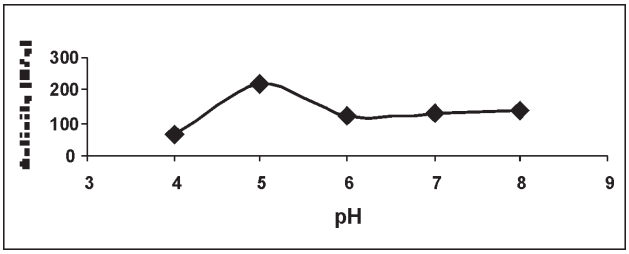

Fig. 5. Effect of pH on polygalacturonase production using *A. fumigatus*.

resulted in higher PG production (54.91 u/g) compared to static conditions (44.31 u/g). Solid state fermentation produced PG (54.9 u/g) with enzyme activity of 245.05 u/mg. Km and Vmax for PG were 6.135 mg ml^{-1} and 0.121 mM min^{-1} (Fig. 6). Determination of effective reaction pH and temperature reflected maximum PG activity at 5 pH (107.58 u/mg) and temperature 30°C (178.03 u/mg). After acetone precipitation, the protein sample was loaded on as SDS-gel. Pure pectinase (E. Merck, Germany) was loaded as control in another well. Bands of molecular weight of 23 and 46 kDa were observed in culture filtrate, which corresponded to those of pure pectinase (Fig. 7). The PG activity and gel electrophoresis results suggested that extracellular protein had pectinase activity. Existence of more than one band from mango peel growth medium, suggests the existence of other enzymes, apart from PG.

It could be concluded from the study that mango peel could serve a potential substrate for fermentative production of PG using *A. fumigatus*.

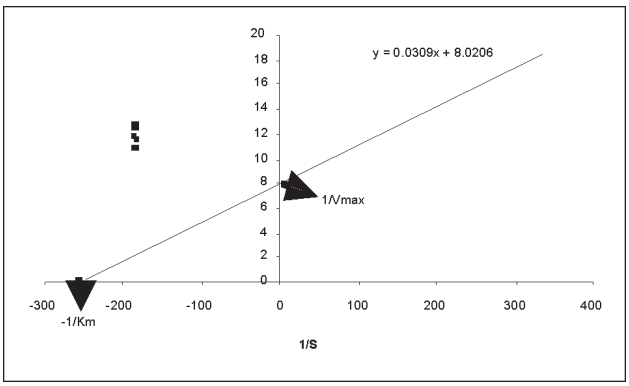

Fig. 6. Effect of substrate concentration on activity of polygalacturonase from *A. fumigatus* (Lineweaver - Burk plot).

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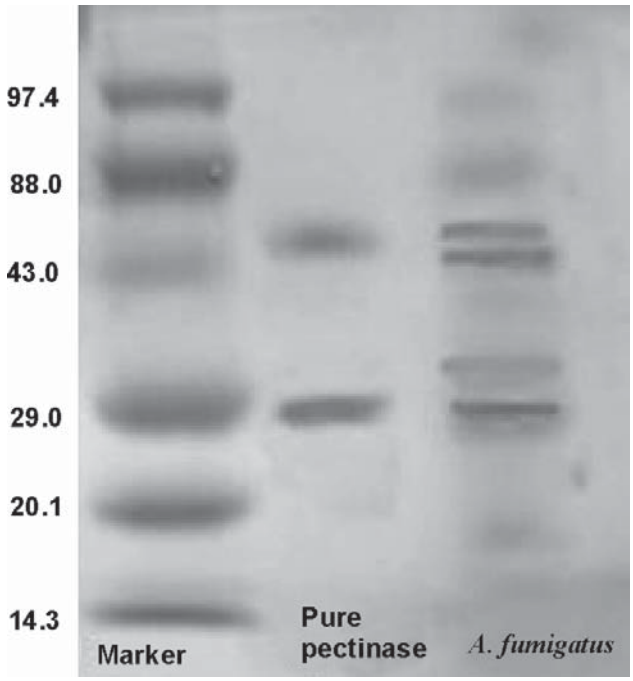

Fig. 7. Bands (KDa) of polygalacturonase from *A. fumigatus* corresponding to commercial pectinase.

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