International Journal of Advanced Multidisciplinary Research (IJAMR)
ISSN: 2393-8870
www.ijarm.com

Research Article
Effect of Sucrose on Microbial Pectin Esterase and Pectate Lyate activity

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Keywords

Fruit peel wastes,
Pectinase,
Bacteria,
Fungi,
Pectin esterase,
Pectate lyase and
Sucrose.

Abstract

Pectinases are the group of enzymes, which cause degradation of pectin that are chain molecules with a rhamnogalacturonan backbone, associated with other polymers and carbohydrates. These pectinases have wide applications in fruit juice industry and wine industry. In the present study, the effect of sucrose on microbial pectin esterase and pectin lyase activity was investigated. Pectionlytic bacterial and fungal cultures were isolated from fruit peel wastes. Bacterial cultures were identified as *Bacillus* sp. and *Pseudomonas* sp. and the fungal cultures were identified as *Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum.* All the bacterial and fungal cultures were found to produce appreciable level of pectinolytic enzyme *viz*., pectin esterase and pectate lyase. Regarding different concentrations of sucrose, at 0.4 percent sucrose, the bacterial isolate *Bacillus* sp. produced highest pectin esterase whereas, the isolate *Bacillus* sp. showed maximum pectin esterase activity in glucose medium. All the fungal isolates showed maximum activity of pectin esterase at 0.4 percent sucrose. Maximum pectate lyase activity was recorded by *Aspergillus niger* in medium with 0.4 per cent sucrose.

Introduction

Pectinase are the group of enzymes that catalyze the breakdown of pectin containing substances. These enzymes are produced by plants and microbes and are not synthesized by animal cells (Forgarty and Kelly, 2003; Pandey *et al*., 1999). At present, a majority of commercial enzymes are obtained by employing fungal cultures. Pectic enzymes have been classified based on different criteria (Bateman and Millar, 2013; Rexova Benkova and Markovic, 2006). The recent classification of pectinolytic enzymes is based on the method proposed by Fogarty and Kelly (2003). Basically, there exist three types of pectic enzymes *viz*., pectinesterases, which remove methoxyl residues from pectin, a range of depolymerizing enzymes (pectinase), and protopectinase, which solubilizes protopectin to form pectin (Sakai *et al*., 2003). Pectinases are classified according to their mode of secretion as extracellular and intracellular pectinases. An extracellular enzyme is excreted (secreted) outside the cell into the medium in which that cell is living. Extracellular enzymes usually convert large substrate molecules (i.e. food for the cell or organism) into smaller molecules that can then be more easily transported into the cell, whereas an intracellular enzyme operates within the confines of the cell membrane. Membrane proteins remain attached in some way to the cell membrane. Both intracellular and extracellular pectinases are classified on the mode of their attack on the galacturonan part of pectin molecules (Hankin and Anagnostakis, 2005). Pectinases are frequently used in fruit and vegetable industry, and pectin is also employed widely in food industry. Peel oil finds many useful applications in both food and pharmaceutical industry. It is good for the skin Citrus solvent is a biodegradable solvent occurring in nature as the main component of citrus peel oil. Citrus Solvents have pleasant aroma, & FDAGRAS rating ("generally recognized as safe") makes it suitable to be used as solvent, citrus solvent can replace a wide variety of products, including mineral spirits, methyl ethyl ketone, acetone, toluene, glycol ethers, and of course fluorinated and chlorinated organic solvents. Dietary fibers are the most recent value added product and are used as means of roughage (Geetha *et al*., 2012; Saranraj and Naidu, 2014).

Materials and Methods

Collection and preparation of fruit peels

The disposed fruit peels like Orange peel, Citrus peel and Potato peel were collected from the Annamalai University canteen, Annamalai Nagar. The collected peels were sorted out manually based on their fine texture and rigidity. The collected peels were minced to pieces and were

hot air oven dried at 55°C until constant weight was achieved. The dried peel was diminuted in a Ball mill and they were clarified in a sieve shaker. The peel fractions from the mesh size 12 were used for the extraction in the Soxhelet extractor.

Isolation of pectinolytic bacteria and fungi

Pectin degrading fungi and bacteria were isolated from the fruit peel wastes. For isolation using sterile pestle and mortar fruit peels were ground and made into slurry using sterile distilled water. An aliquot of this sample was serially diluted with dilution blanks and plated on Hankin's medium containing one percent pectin (Aneja, 1996) for bacteria and modified Czapek's medium with 1% pectin and 1 ml of 1000 ppm streptomycin sulphate for fungi. After incubation, the isolated bacteria were purified by streak plate technique and the fungal cultures were purified by single hyphal tip method and maintained in pectin Czapek's agar slopes under refrigerated conditions.

Characterization of bacterial and fungal isolates

The isolated bacterial cultures were characterized based on morphological tests such as shape and Gram reaction. In addition to morphological characters, the isolated bacterial cultures were cultivated in selective media and certain biochemical tests were also performed. Purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures.

Assay of pectinolytic enzymes

Pectin esterase

Pectin esterase activity can be measured either by measuring the amount of methanol released or increase in free carboxyl group by titration using pH meter (Tolboys and Busch, 2000). For assaying pectin esterase activity, 20 ml of one percent pectin dissolved in 0.15 M NaCl (pH 7.0) and four ml of crude enzyme extract were taken in a beaker and incubated for one hour. After incubation, the solution was titrated against 0.02 N NaOH to reach the pH 7.0 using phenolphthalein as indicator. The heated crude enzyme extract was used as control. Pectin esterase activity was calculated by using the following formula:

$$\text{Pectin esterase activity} = V_s - V_b \text{ (Normality of NaOH)} \times 100/V_t$$

Where , V_s - Volume of NaOH used to titrate sample (ml), V_b - Volume of NaOH used to titrate blank (ml), V - Volume of incubation mixture (ml), t - Reaction time (hours). Pectin esterase activity is expressed as milli equivalents of NaOH consumed min^{-1} ml^{-1} of crude enzyme extract under the assay conditions.

Pectate lyase

Pectate lyase activity of the isolated cultures was assayed by measuring the increase in absorbance at 232 nm due to the production of unsaturated bonds during the depolymerization of galacturonic acid (Kapat *et al*., 2008). The reaction mixture was prepared with 2.5ml of 0.5 percent sodium pectate dissolved in 50 mM Tris HCl (Sigma) buffer and 1mM calcium chloride and the activity was measured at 232 nm. The crude enzyme extract heated at high temperature was kept as control. One unit of pectate lyase activity was defined as the amount of enzyme that caused absorbance at 232 nm to increase at a rate of 0.1 OD min^{-1} ml^{-1} of crude enzyme extract under the assay conditions.

Effect of different sucrose concentrations on pectinase activity

To find out the effect of different sucrose sources on pectinase enzymes activity, media with the best level of pectin (1 per cent) were prepared for bacteria and fungi along with sucrose each at four different levels (0.1, 0.2, 0.3 and 0.4 per cent) and incubated at room temperature in a rotary shaker at 100 rpm and the extracts were used for enzymes assay.

Results and Discussion

Pectinases are group of enzymes that attack pectin and depolymerise it by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 1998). These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network (Thakur *et al*, 1997). Pectinases accounts for 10% of global industrial enzymes produced and their market is increasing day by day (Stutzenberger, 2002).

Two pectinolytic bacteria and three fungal cultures were isolated from fruit wastes. The bacterial isolates were characterized based on Gram staining, plating on selective media and several biochemical reactions. Purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures.

Bacterial isolates were identified as *Bacillus* and *Pseudomonas*, while the fungal isolates were characterized as *Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum.* The results of the isolates confirmed the findings of Rolz *et al*. (2011) and Roussos *et al*. (2010) who reported that pectinolytic bacteria were the important

fraction of the microbial population on whole fruit wastes and pulped fruits.

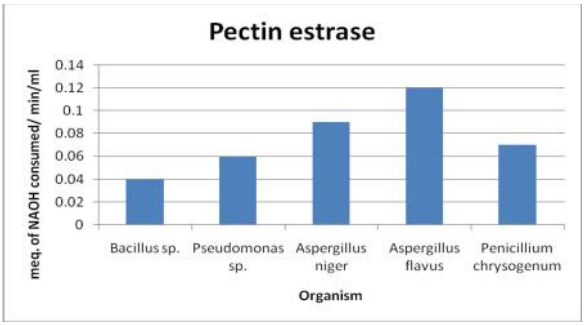

Figure - 1: Pectin esterase activity of the cultures from fruit peeling waste

Pectinolytic organisms isolated from fruit peel wastes were found to produce an array of pectinolytic enzymes collectively called as pectinases. All the bacterial cultures recorded significant quantities of pectinolytic enzymes such as pectin esterase and pectate lyase. Higher levels of pectinase activities were expressed by the bacterial isolate *Pseudomonas* sp. Chatterjee *et al*. (2011) also reported that several bacteria like *Erwinia* sp., *Pseudomonas* sp., *Bacillus* sp., were able to produce pectinases. The result of the present study is also in confirmation with the findings of Liao *et al*. (2009). 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 by Isshiki *et al*. (1997 32) and Kapat *et al*. (1998).

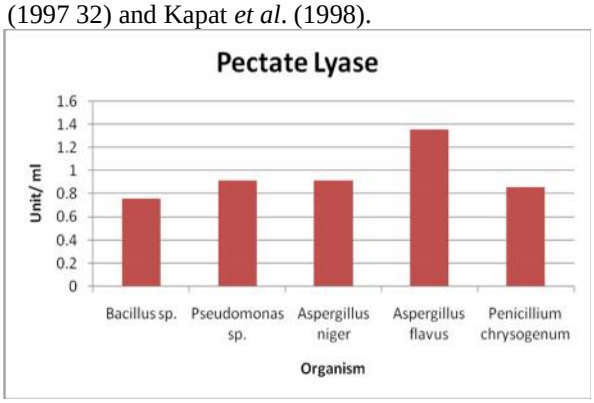

Figure - 2: Pectate lyase activity of the cultures from fruit peeling waste

Assay method for the detection and measurement of pectinolytic activity ranged from pure qualitative methods (Hildebrand, 2001) for demonstrating the presence of enzymes activity to quantitative methods (Collmer *et al*., 2008; Conway *et al*., 2008), which determine the activity in terms of actual linkages hydrolyzed. The most common method for determining pectin esterase activity is the titrimetric method of estimation of carboxyl groups formed in pectin by the enzyme (Cole and Wood, 2006; Tolboys and Busch, 2000). Pectin esterase can also be assayed by measuring the methanol liberated from pectin during the reaction. Zhao *et al*. (1996) have described a simple spectrometric method for the estimation of methanol released from pectin.

In the present study, the isolated cultures were screened for further studies based on the activity of pectinolytic enzymes such as pectin esterase and pectate lyase (PL). Among the bacterial isolates, *Pseudomonas* sp., and *Bacillus* sp. recorded maximum Pectinase activities (Figure - 1 and Figure - 2). The fungal cultures *viz*., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum* exhibited highest pectinolytic enzyme activities and hence these isolates were selected for further experiments.

The effect of different concentrations of sucrose on the production of pectinase by microbial cultures was tested. Bacterial and fungal cultures were inoculated in the medium prepared with 0.1, 0.2, 0.3 and 0.4 percent of sucrose. Among the bacterial isolates tested in the present study, *Bacillus* sp. grown at 0.4 percent sucrose medium along with one percent pectin showed maximum pectin esterase enzyme activity (0.29 meq. of NaOH min^{-1} ml^{-1}). Maximum pectin esterase activity was shown by the fungus *Aspergillus niger* at 0.4 percent concentration of sucrose (Figure – 3). Mehta *et al*. (2002) were also of the same opinion that the addition of sugars to the fermenting medium allows the organism to produce enzymes without catabolite repression.

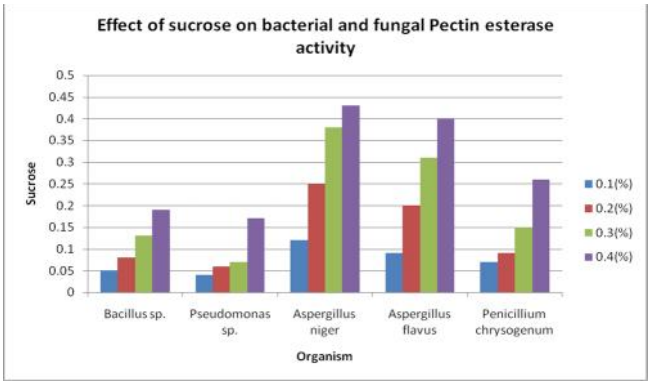

Figure - 3: Effect of sucrose on pectin esterase activity

The pectate lyase activity of the bacterial cultures grown in medium containing different concentration of sucrose was presented in Figure - 4. Among the bacterial and fungal isolates, *Aspergillus niger* recorded the maximum pectin esterase activity (1.46 unit/ml) followed by *Aspergillus flavus* and *Penicillium chrysogenum*. The bacterial isolates *Bacillus* sp (1.32 unit/ml). and *Pseudomonas* sp. (0.71 unit/ml) recorded least pectin esterase activity. The result was in confirmation with the report of Chatterjee *et al*. (2010) who reviewed that *Erwinia chrysanthemi* when grown in two percent glucose produced 2.42 Unit ml^{-1} of

enzyme. Elumali and Mahadevan (2005) were also of the same opinion that in *Pseudomonas marginalis*, the extracellular secretion of pectate lyase gets enhanced with the addition of glucose.

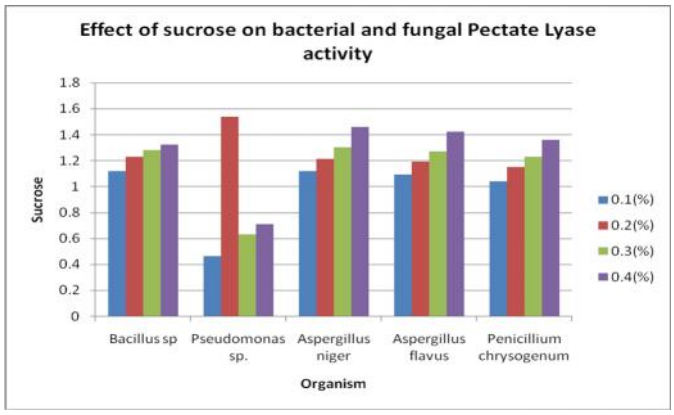

Figure - 4: Effect of sucrose on pectate lyase activity

Conclusion

Most of the microorganisms can capable to produce various extracellular and intracellular enzymes using various cheap sources. Pectinase is an extracellular enzyme, which is produced from various microorganisms and research on pectinase has progressed very rapidly over the last five decades and potential industrial applications of the enzyme especially in solid waste management have been identified. Major impediments to exploit the commercial potential of pectinases are the yield, stability and cost of enzyme production. Although, terrestrial strains of microbes have been extensively studied by many researchers. The bacteria *Pseudomonas* sp. and *Bacillus* sp. and the fungi *Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum* were used for the production of pectinase and fruit peel wastes were used as a substrate. The present research concluded that the bacterial isolate *Bacillus* sp. produced highest pectin esterase at 0.4 percent sucrose concentration whereas, the isolate *Bacillus* sp. showed maximum pectin esterase activity in glucose medium. All the fungal isolates showed maximum activity of pectin esterase at 0.4 percent sucrose concentration and maximum pectate lyase activity was recorded by *Aspergillus niger* in medium with 0.4 per cent sucrose.

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