Alkaline Polygalacturonases from Thermotolerant Pectinolytic Bacteria from Diverse Sources

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Abstract- Diverse pectin rich sources like food dumps, food waste waters, spoiled fruits, vegetables and alkaline soils were screened for alkaline bacterial pectinolytic isolates by enrichment culturing and ruthenium red plate assay. Six different bacterial isolates with higher zones of pectin hydrolysis from the source food waste waters were selected and tested for polygalacturonase production at room temperature and at neutral pH. Two isolates as *Bacillus sp 2* and *Bacillus sp* 3 with higher polygalacturonase activity were studied for their response to alkaline, thermophilic production. Polygalacturonase production was highest for *Bacillus sp* 3 at an alkaline pH of 10.5, a thermophilic temperature of 60°C, an agitation speed of 200 rpm and an inoculum size of 2%. It showed an enzyme production of 890 U/ml. Selected *Bacillus sp* 3 was both alkaline and thermophilic and had a short fermentation cycle with good polygalacturonase production. Alkaline polygalacturonase could have ample application in food wastes and food waste water treatment. It being highly alkaline it could have ample application in cotton scouring which can be eco friendly.

Index Terms- Alkaline polygalacturonase, *Bacillus sp*, Fermentation cycle, Pectinolytic, Thermophilic.

I. INTRODUCTION

Pectinases are depolymerizing enzymes that degrade pectins present in middle lamella and primary cell walls of plant tissues [5]. Pectinases produced by different microbes are divided into depolymerizing enzymes and saponifying enzymes. Depolymerizing enzymes are polymethylgalacturonases, pectin lyases, polygalacturonases and pectate lyases and saponifying enzymes are pectin esterases [15]. The production of pectinolytic enzymes has been widely reported in bacteria and filamentous fungi [10]. Acidic pectinases have wide spread applications in the food industry in clarification of fruit juices, wines [1], [14]. Alkaline pectinases are industrially significant enzymes with wide- ranging applications in cotton bioscouring, [6] degumming of bast fibers, [7] paper making and pre-treatment of food industry waste waters [11], [12]. Cotton bioscouring is traditionally performed with caustic alkaline solution at high temperature for uniform dyeing and finishing. Degradation and elimination of pectins from complex cotton fibers can be achieved by alkaline pectinases [8], [4] and this is an eco friendly process. Cotton being India's one of the important commercial crops and textile industry an important industry the application of alkaline pectinases for cotton scouring is significant for producing good quality fabric and thus generating revenue for the country.

II. MATERIALS AND METHODS

2.1 Screening and selection of alkaline pectinolytic isolates: Diverse pectin rich source samples like food dumps, food waste waters, spoiled fruits, vegetables and alkaline soils were screened for alkaline bacterial pectinolytic isolates by enrichment culturing. These were collected in sterile polythene bags, serially diluted and inoculated on Czapek agar plates enriched with pectin and at pH 9 & 10. The plates were incubated at 37°C for 24 hours. Plates with bacterial colonies were screened for pectinolytic isolates using ruthenium red plate assay [13]. The plates for assay were inoculated in duplicates to facilitate isolation of culture for study. Colonies in one of the plates were flooded with 0.5ml of 0.02% ruthenium red solution, incubated for one hour at room temperature and washed with sterile water to remove unbound ruthenium. Positive pectinolytic isolates were detected based on clear (colour less) zones of pectin hydrolysis around the colonies. Six bacterial colonies with higher zones of pectin hydrolysis were selected, were sub-cultured, identified morphologically and studied for polygalacturonase (PGU) production in liquid medium using commercial pectin as substrate (Citrus peel pectin, SD fine chemicals).

2.2 Enzyme production: Submerged fermentation was carried out in 250ml Erlenmeyer flasks containing 50ml pectin enriched Czapek broth. Flasks were incubated for 24 hours at 37°C. Broth samples were collected and assayed for the enzyme activity at 24 hours. Two isolates identified as *Bacillus sp* 2 and *Bacillus sp* 3 having more enzyme activity were selected and studied for enzyme production in pectin rich medium at both alkaline pH and thermophilic temperatures for a period of 24 hours. The pH range studied was 8-11 and temperature range studied was 40-70°C.

2.3 Fermentation conditions for Alkaline Polygalacturonase enzyme production: Submerged fermentation studies were done with selected isolates *Bacillus sp* 2 and *Bacillus sp* 3 for production of poly-galacturonase (PGU) using pectin enriched Czapek broth. Different fermentation conditions like pH (a range of 9-11.5), agitation speed (a range of 150-225 rpm), and fermentation cycle for 30 hours with a time gap of 6 hrs were studied.

2.4 Polygalacturonase assay: One ml of culture broth was centrifuged at 5000 rpm for 10 minutes. Supernatant was taken as enzyme source. The enzyme was assayed at 60°C by measuring the D-galacturonic acid released from polygalacturonic acid as substrate by DNS method [9]. One unit of enzyme activity is defined as the amount of enzyme required to produce 1 µ mole of galacturonic acid per minute at 60°C.

III. RESULTS

Different pectin rich source samples gave different pectinolytic bacteria that produced colourless hydrolytic zones around colonies in ruthenium red plate assay [13]. Pectinolytic bacterial isolates having 0.2-0.5 cm zones of hydrolysis were obtained (Figure 1). Six different bacterial isolates from the source food waste waters, namely *Xanthomonas sps*, *Pseudomonas sps*, *Bacillus sps1*, *Bacillus sps2*, *Bacillus sps3 and Erwinia sps* showed comparatively higher zone of hydrolysis and so were selected and tested for Polygalacturonase production (Figure 2). With interest in only PGU production at alkaline pH and high temperature two good bacterial isolates were further tested for this enzyme production at pH 9-11.5 (Figure 3), agitation speed of 150-225 rpm (Figure 5)and temperature 40°-70° C using commercial citrus pectin as substrate (Figure 4). Among the two isolates *Bacillus sp* 3 produced higher amount of alkaline polygalacturonase at thermophilic temperature. The isolate has a relatively shorter fermentation cycle (Figure 2) and so is beneficial as a commercial strain.

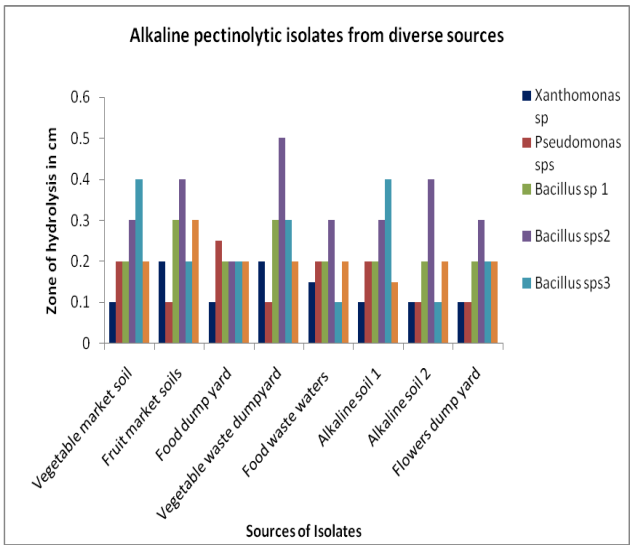

Figure 1: Alkaline pectinolytic isolates from diverse sources screened by ruthenium red plate assay.

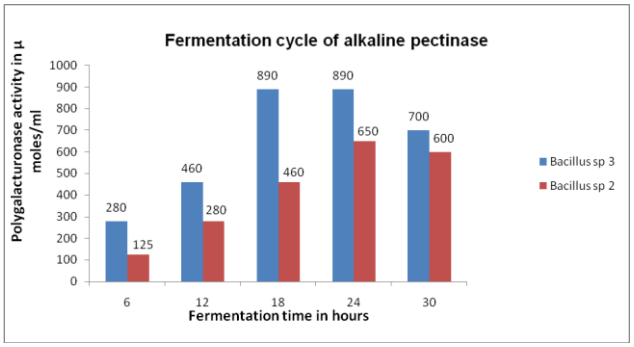

Figure 2: Comparison of Fermentation cycles of alkaline pectinolytic isolates.

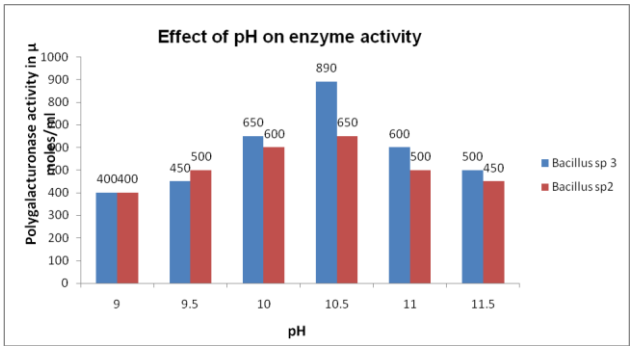

Figure 3: Effect of pH on polygalacturonase production at thermophilic temperature of 60^0 C.

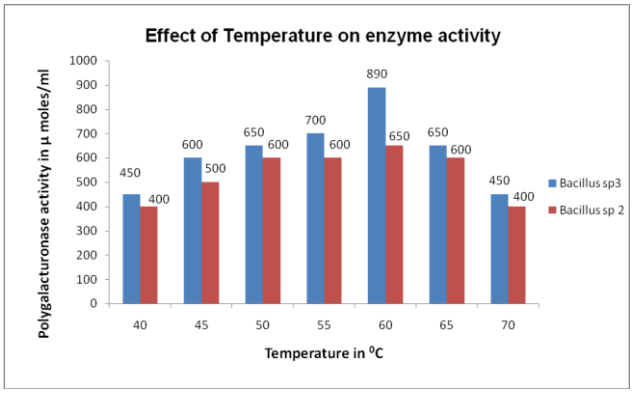

Figure 4: Effect of temperature on polygalacturonase production at a pH of 10.5.

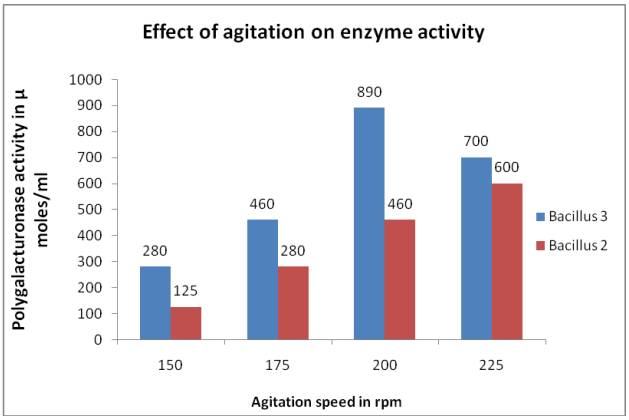

Figure 5: Effect of agitation on polygalacturonase production at a pH of 10.5 and temperature 60^0 C.

IV. DISCUSSION

Pectin being predominant component of fruits and vegetables promotes the growth of pectinolytic isolates hence good pectinolytic organisms were obtained from sample like food waste water. Ruthenium red is a chromogen that specifically binds to free carboxyl groups in pectin giving a red colour and a colourless halo in absence of pectin, hence ruthenium red plate assay was an efficient and significant screening strategy for pectinolytic isolates [13]. Bacteria are known to produce pectinases especially polygalacturonases [2], [3] at different temperatures and pH [16]. Bacteria are preferred as commercial

production strains as they have short fermentation cycle. Klug-Santner and his group reported that up to 80% pectin removal from the outer layer of cotton is done by a purified pectinase like endo-pectate lyases from *Bacillus pumilus BK2* [8]. Scouring of cotton fabric is typically done with a hot sodium hydroxide which could cause pollution when this after use is not treated but dumped. Use of alkaline pectinase from the selected isolate for cotton scouring can be both an efficient and an eco-friendly approach.

V. CONCLUSION

An efficient alkaline pectinolytic bacterial isolate identified as *Bacillus sp3*. was isolated by screening different source samples. The isolate showed good growth at pH of 10.5, agitation speed of 200 rpm and a temperature of 60°C. It produced a maximum of 890 U/ml of polygalacturonase. This enzyme can be used for cotton scouring. Cotton being India's one of the important commercial crops and textile industry an important industry the application of alkaline pectinases for cotton scouring is significant for generating revenue for the country.

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