Bacterial Isolates for Polygalacturonase Production from Diverse Sources

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Abstract - Seventy pectinolytic bacterial strains were isolated from diverse pectin rich sources and waste dump yard soils by enrichment culturing and ruthenium plate assay. Screening of nine different pectin rich sources and soils like fruit and vegetable dump yard soils, fruit and vegetable market soils, sugar cane bagasse soils yielded five primary bacterial isolates based on zones of pectin hydrolysis in ruthenium plates. These five primary isolates with higher zones of pectin hydrolysis were tested for polygalacturonase production. One efficient bacterial isolate identified as Bacillus sp 1 with highest polygalacturonase activity was selected for further study. The effect of temperature, pH and fermentation cycle was studied for polygalacturonase production by Bacillus sp in submerged fermentation conditions using crude pectin. The selected pectinolytic isolate Bacillus sp 1 could have application for enzymes production, fruit juice clarification and degradation of fruit and vegetable waste in process of urban waste disposal.

Keywords: Baciilus sp, Pectin, Polygalacturonase, Ruthenium plate assay.

I. INTRODUCTION

Pectin is a complex polysaccharide. It is a major component of middle lamella and primary cell walls of plant tissues which are degraded by pectinases. Pectinases belong to a class of depolymerizing enzymes that degrade pectin by hydrolyzing the pectic substances [1]. Pectinases have potential application food industry and hence are one of the fast upcoming enzymes of commercial sector with 25% share in global market. Pectinases are of major importance in clarification of concentrated fruit juices and so are extensively used in processing of fruits and vegetables [2] .They are also industrially very significant with their all embracing applications in clarification of fruit juices, wines [3, 4], extraction of oils, flavors and pigments from plants, coffee and tea fermentations, retting of jute fibers, degumming of plant bast fibers, waste water treatment and purification of plant viruses [5, 6]. Diverse sources for pectinolytic isolates in nature are rinds of fruits or peels, rotten vegetables and their dump yards [7]. Microbial pectinases are classified into depolymerizing enzymes and saponifying enzymes. Depolymerizing enzymes are further classified as poly-methylgalacturonases, pectin lyases, polygalacturonases and pectate lyases and saponyifying enzymes are pectinesterases [8]. A wide range of filamentous fungi and bacteria have been reported as good producers of pectinase. [9] .The present study was on pectinolytic activities of bacteria strains isolated from pectin rich soil and samples of vegetable and fruit dump yard soils.

II. DESIGN OF THE STUDY

The design of the study was collection of pectin rich soils from diverse environments for screening of pectinolytic bacterial isolates by enrichment culture technique and ruthenium plate assay. Potential primary isolates were tested for polygalacturonase activity and one efficient isolate was selected for further study for optimization fermentative conditions.

III. PREVIOUS WORK

There are reports of pectinases produced from different bacteria like *Agrobacterium tumefaciens*, *Bacteroides thetaiotamicron*, *Ralstonia solanacearum*, *Bacillus* sp [5]. At present almost all the pectinolytic enzymes used for industrial applications are produced by fungi and there are a few reports of pectinase production by bacterial strains. *Bacillus sphaericus*, a bacterium isolated from soil was found to produce good amount of polygalacturonase activity at neutral pH while alkaline pectinases were found to be produced by bacterial isolate *Bacillus sp* [10]. Pectinolytic bacterial strains were earlier isolated from soils and vegetable sources [11]. Hence good pectinolytic isolates were obtained from samples of vegetable and fruit dump yards or their peels were used for screening [12]. Bacteria are known to produce pectinases especially polygalacturonases [13,] at different temperatures and pH [14].

IV. METHODOLOGY

SCREENING AND SELECTION OF PECTINOLYTIC ORGANISM:

Diverse pectin rich sources and soils like vegetable market soil, vegetable waste dump yard, fruit market soil, fruit waste dump yards, sugar cane bagasse dumped soil, various fruit and vegetable peels were screened for pectinolytic isolates. These samples were collected aseptically, serial diluted and were inoculated on Czapek agar plates enriched with pectin and incubated at 37°C for 24 hours. Duplicates of the plates were kept to facilitate isolation of culture for study. Pectinolytic bacterial isolates were screened using ruthenium red [15].

The plates were flooded with 0.5ml of 0.02% ruthenium red solution, incubated for one hour at room temperature and then washed to remove unbound ruthenium. Isolates with Clear colorless zones around the colonies indicating pectin hydrolysis were observed. Five colonies with larger zones of pectin hydrolysis were sub cultured, identified morphologically and studied further for polygalacturonase production in liquid medium using commercial pectin as substrate (Citrus peel pectin, SD fine chemicals).

ENZYME PRODUCTION:

The enzyme production was done by submerged fermentation using 250ml Erlenmeyer flasks each containing 50ml pectin enriched Czapek broth. The flasks were incubated at 37°C for 24 hours. Broth samples were collected after 24 hrs and assayed for the enzyme activity. One efficient bacterial isolate identified as *Bacillus sp1* having more enzyme activity was selected and studied for enzyme production in pectin rich medium for a period of period of 24 hours.

FERMENTATION CONDITIONS FOR POLYGALACTURONASE ENZYME PRODUCTION:

Submerged fermentation studies were done with selected isolate *Bacillus sp 1* for production of poly-galacturonase (PGU) using pectin enriched Czapek broth. Different fermentation conditions like pH (a range of 4-7), temperature (25-40°C) and fermentation cycle for 24 hours with a time gap of 4 hrs were studied.

POLYGALACTURONASE ASSAY:

One ml of fermented broth was cold centrifuged at 4^0 C, 5000 rpm for 10 minutes. Supernatant was taken as enzyme source. The enzyme was assayed by measuring the D-galacturonic acid released from polygalacturonic acid as substrate by the method of Nelson and Somogyi [16]. One unit of enzyme activity is defined as the amount of enzyme required to produce 1 µ mole of galacturonic acid per minute at 50^0C [17, 18].

V. RESULTS

Seventy primary isolates were obtained by enrichment culture technique using 1% pectin. Pectinolytic primary isolates that depolymerized pectin produced colourless hydrolytic zones around colonies in ruthenium plate assay. Bacterial pectinolytic primary isolates having 0.1-0.5 cm zones of hydrolysis were considered as potential pectinase producers, as indicated in Figure 1.

With interest in only polygalacturonase production, the primary isolates were subjected to secondary screening for this enzyme. Among the primary isolates, one *Erwinia sp*, one *Xanthomonas sp*, one *Psedomonas sp* and two *Bacillus sp.* identified morphologically and were tested for polygalacturonase production by submerged fermentation.

The results are represented in Figure 2. Among the polygalacturonase producers, one bacterial isolate namely *Bacillus sp1* from vegetable soils showed high enzyme activity. It was selected for further study as its enzyme production was comparatively higher. Submerged fermentation studies were done with selected isolate *Bacillus sp 1* for production of poly-galacturonase (PGU) using pectin enriched Czapek broth.

Different fermentation conditions like pH (a range of 4-7)Figure 3, temperature (25-40°C)Figure 4and fermentation cycle for 24 hours with a time gap of 4 hrs were studied Figure 5. Highest enzyme production was observed after 24 hours at pH 6 and at temperature 37°C.

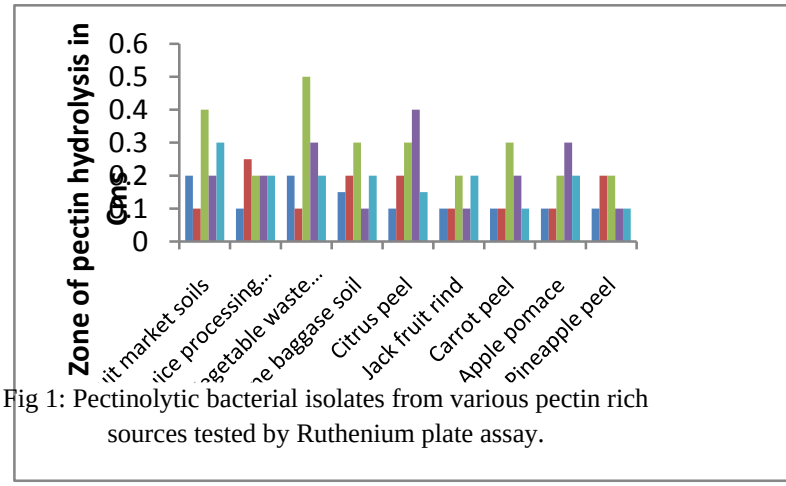

Fig 1: Pectinolytic bacterial isolates from various pectin rich sources tested by Ruthenium plate assay.

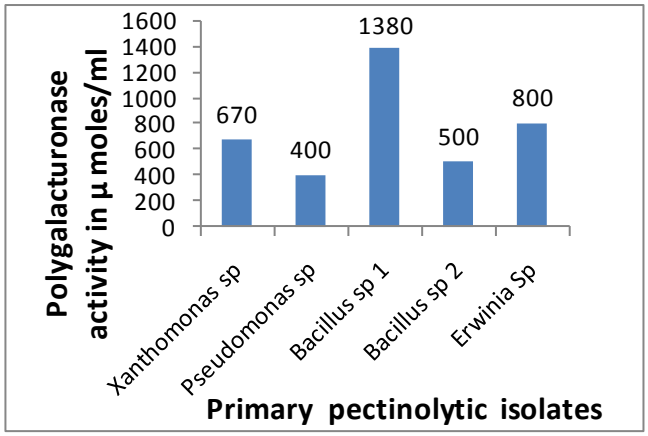

Fig 2: Polygalacturonase activity of selected pectinolytic bacterial isolates in Czapek broth.

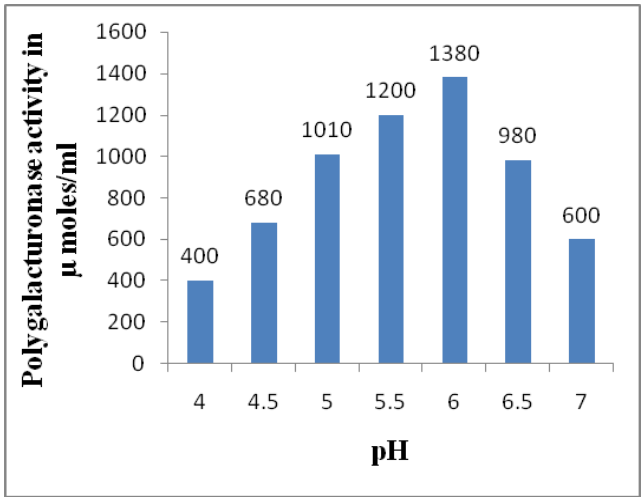

Fig 3: Polygalacturonase production by *Bacillus sp*1 at different pH

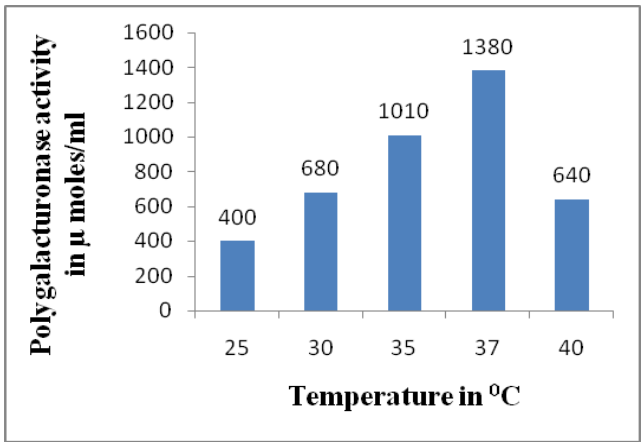

Fig 4: Effect of temperature on polygalacturonase production *by Bacillus sp* 1

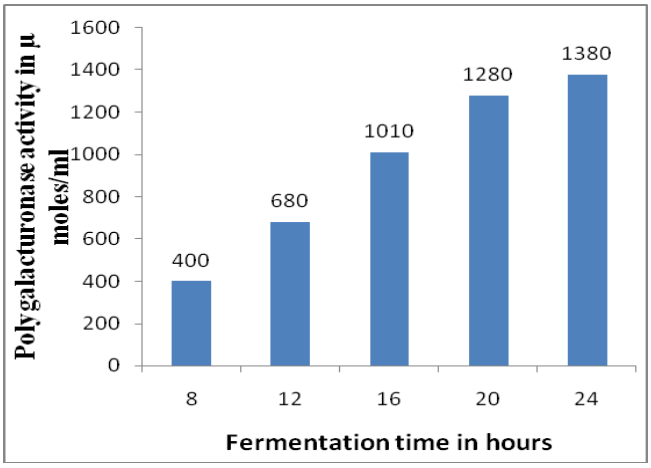

Fig 5: Production profile of polygalacturonase activity by *Bacillus sp*1

VI. CONCLUSION

An efficient pectinolytic bacterial isolate identified as *Bacillus sp1* was isolated from vegetable waste dump yard soil. by screening different source samples. The isolate showed good growth at pH of 6, and a temperature of 37°C. It produced a maximum of 1380 U/ml of polygalacturonase.

VII. FUTURE SCOPES

The selected pectinolytic *Bacillus sp.* isolate could have application for mesophilic enzymes production. The enzyme could have diverse applications in fruit juice clarification and degradation of fruit and vegetable waste in process of urban waste disposal.

VIII. ACKNOWLEDGEMENT

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