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Screening and Optimization of Pectinase Producing Bacterial Isolates from Fruit Wastes

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Abstract: ***Pectinase producing bacteria isolated from fruit wastes and it was screened for pectinase production. The enzyme responsible for hydrolyzing pectin to D- galacturonic acid was identified though novel assay techniques. In the present investigation, biochemical characterization such as Gram staining, Indole, MR-VP, citrate, catalase test and oxidase test for the isolates were perfomed. Further, the individual parameters in the media were used to screen the pectinolytic bacteria. The enzyme activity was confirmed by the zone media were optimized for pH (5-10), substrate concentration (0.5% to 3%), and temperature (20 to 60ºC). The significant factors and their optimized values are found to be pH - 6, Temperature - 60 ºC, Substrate Concentration – increases with respect to concentration. The enzyme activity is most effective in the acidic condition and is found to be pH 6. The present study proved that pectinase producing bacterial isolate was screened and optimized for enzyme production.***

Keywords: Pectinolytic bacteria; pectinase; biochemical assay and medium optimization

I. INTRODUCTION

Pectinases are natural biocatalysts that hydrolyse pectin; heterogeneous polysaccharides to D- galacturonic acid unit. Pectin is an important composition in cereals, vegetables, fruits.[1,2] They are highly complex molecular weight, heterogeneous and acidic structural polysaccharide. The pectinolytic enzymes derived from microorganisms becoming an attention for various researchers. Based on the substrate and their mode of action, the enzymes are grouped into depolymerases (hydrolase and lyases), esterase's, and proto pectinases on the base of their mode of action and substrate preferences [3,4 and5]. Pectinase enzyme is further categorized into two such as acidic and alkaline pectinase due to pH needs in order to have optimum enzymatic activity [6]. These enzymes have wide applications in various industrial processes. The marketing strategy of this enzyme production is also increasing worldwide. Pectinase is an enzyme that finds its applications in various industries such as fruit industry, coffee and tea fermentation, wine industry, extraction of vegetable oil, retting of plant based fibres, wastewater treatment, and other applications as well. Pectinase enzymes are produced by a wide range of microorganisms including bacteria [7,8 and 9], fungi [10,11 and 12] , yeasts [13 and 14] , and some Actinomycetes [15]. Among them the major producers are from fungal sources. *Aspergillus niger* is the most commonly used fungal species for industrial production of pectinolytic enzyme. With tremendous potential of pectinases, the screening of the microbe for the production will be a big task. The fruits and fruit wastes are the major sources for the commercial production nowadays. The present study is aimed at to (i) isolate pectinase producing bacterial species from fruit wastes dump, (ii) optimize the various parameters by altering the physio-chemical environment of the production medium and (iii) production of pectinase enzyme using the optimized parameters.

II. METHODS AND MATERIAL

A. Screening of Pectinase Producing Microorganism

The soil samples were collected from the fruit waste dump yard. 1g of waste dumps were added into the enrichment broth and kept in mechanical shaker for 3 days at room temperature. The enriched samples were serially diluted and spread over Yeast Extract Pectin agar (YEP) medium. The overnight culture plates were then screened for the production of pectinase. Primary screening was performed to identify pectinase producing isolate using pectinase screening media and secondary screening was used to measure the enzyme activity. The enzyme assay was done based on the DNS method (Miller 1959).

B. Biochemical Characterization Of Pectinase Isolates

The screened isolates were subjected to biochemical tests to identify pectinase producing property. The biochemical characterization such as Methyl Red, Voges Proskauer, citrate utilization, catalase and oxidase test.

C. Enzyme Assay

The reducing sugar after hydrolysis of pectin was determined. The pectinase enzyme assay was done based on the DNS method by Miller 1959.

D. Effect of Various Substrate Concentrations

To determine the effect of various substrate concentrations on the enzyme activity, various concentrations of substrate, Pectin 0.5% to 3% were added to YEP medium, which was inoculated with the isolate and incubated in rotary shaker for 48 h at 37 ˚C. After the incubation period, the culture was collected and subjected to the assay for determining the enzyme activity.

E. Effect of ph on Enzyme Activity

In order to investigate the influence of pH on the activity of pectinase, the isolate was grown in 100 mL of YEP medium. Each medium was subjected to various pH (5, 6, 7, 8, 9 and 10). After incubation of culture in rotary shaker for 48 h at 37 ˚C, the culture was collected and the activity was measured.

F. Effect Of Temperature On Enzyme Activity

Temperature is an important role for pectinase production. To identify the optimum temperature for pectinase production, the pectinase activity was tested at different temperature such as 20 ºC, 30 ºC, 40 ºC, 50 ºC and 60 ºC.

III. RESULTS AND DISCUSSION

The microbial strain was isolated from fruit dump were observed to have pectin hydrolysing activity by screening methods. The primary screening showed the zone of clearance and proved to be pectin hydrolysing organism. The zone around the colonies indicates that the substrate pectin which is also known as D- polygalacturonic acid was converted into monogalacturonic acid. (Fig No 1). Based on the assay procedures and characteristics of the pectinase, the pectinase of the screened and identifed isolates in this study resembles polygalacturonase. Similar results were reported and found to be *Bacillus subtilis* and it was a extracellular enzyme. [16,17]

The various biochemical characterizations found to be indole, citrate utilization and catalase positive, and methyl red, Voges Proskauer and oxidase were negative. The morphological appearance was gram positive and a rod shaped one. Similarly, [18 and 19] also followed similar procedures to isolate microorganisms from coffee husk and pulp

The various sets of temperature were employed for the pectinase production and the optimum temperature was found to be 60 ºC. Similarly, the effect of pH on production of pectinase is shown in the Fig No 8 and the optimum was found be at 6. The different substrate concentration was studied for the pectinase activity. The effect of substrate concentration on the production of pectinase is shown in figure No 9 that it keeps increasing with respect to concentration. [20, 21 and 22]. The specific activity was found to be at all the optimum ranges (substrate concentration, pH and temperature)

TABLE I
Biochemical Characterization

Test	Results
Indole	Positive
Methyl Red	Negative
Voges Proskauer	Negative
Citrate Utilization	Positive
Catalase	Positive
Oxidase Test	Negative

Fig No 1: Screening of pectinolytic isolates

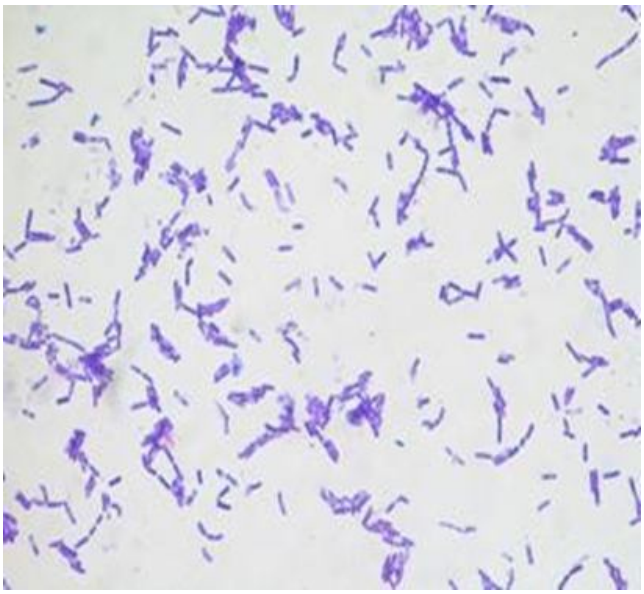

Fig No 2: Gram staining of fruit dump isolate

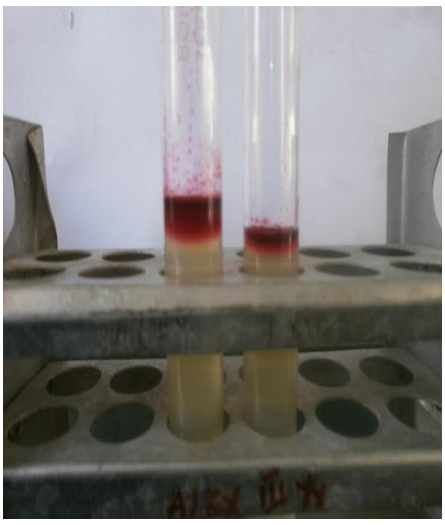

Fig No 3: Indole test of the screened isolate

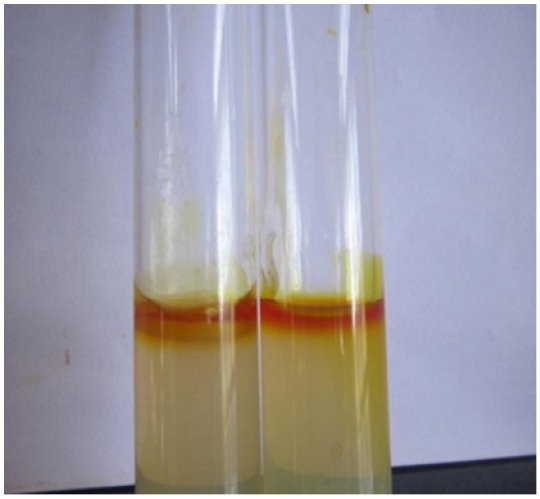

Fig No 4: Methyl red test of the screened isolate

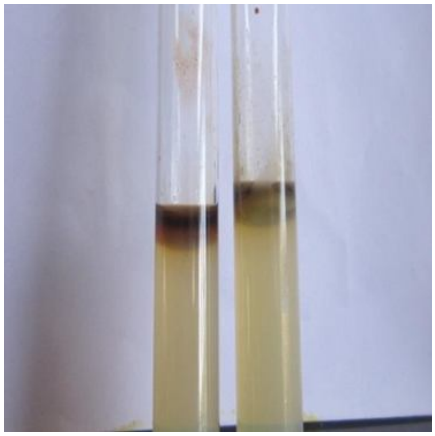

Fig No 5: Voges Proskeur test of the screened isolate

Fig No 6:Citrate utilization test of the screened isolate

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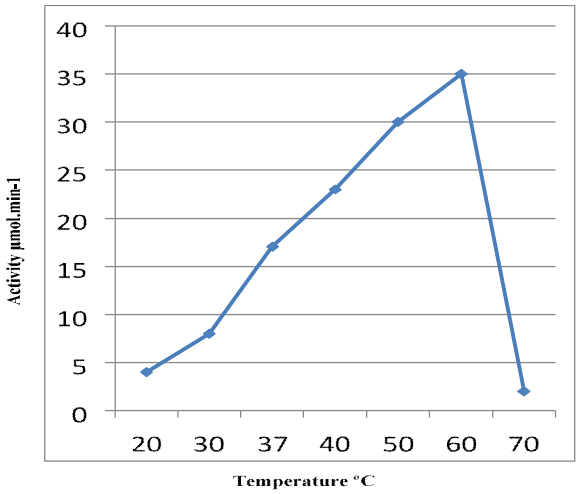

Fig No 7: Effect of temperature on pectinase activity

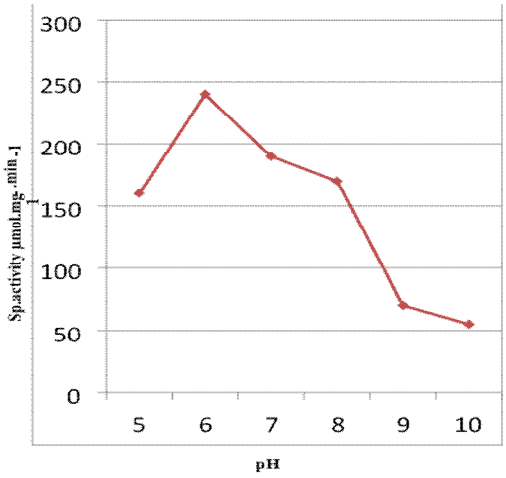

Fig No 8: Effect of pH on pectinase activity

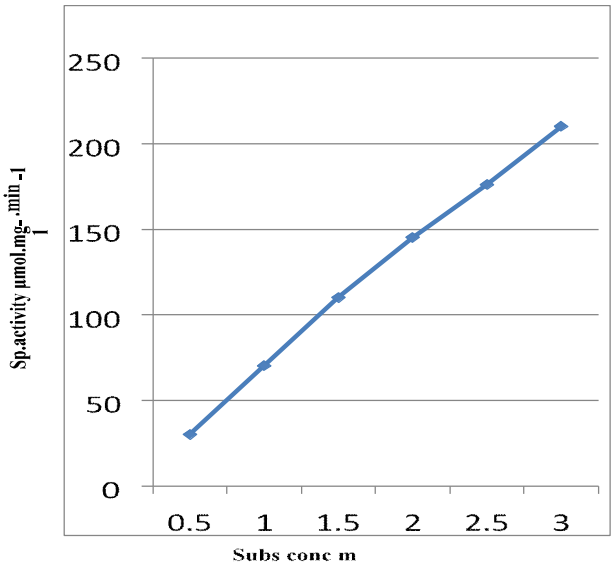

Fig No 9: Effect of substrate concentration on pectinase activity

IV. CONCLUSION

Pectinase are the enzymes that have the capability to degrade pectin which is a polysaccharide found in plant cell wall., Pectinase screening agar medium was used to screen the pectinolytic bacteria. The enzyme activity was confirmed by the zone of clearance by adding the iodine pellets. The enzyme suspected to be an extra cellular enzyme.

The significant factors and their optimized values are found to be pH - 6, temperature - 60 ºC , substrate concentration – increases with respect to concentration. The enzyme activity is most effective in the acidic condition and is found to be pH 6. The study concludes that pectinase producing bacteria enhance the hydrolysis of pectin by pectinase production and could have more applications in various industries such as fruit processing, coffee and tea fermentation and also wine industry.

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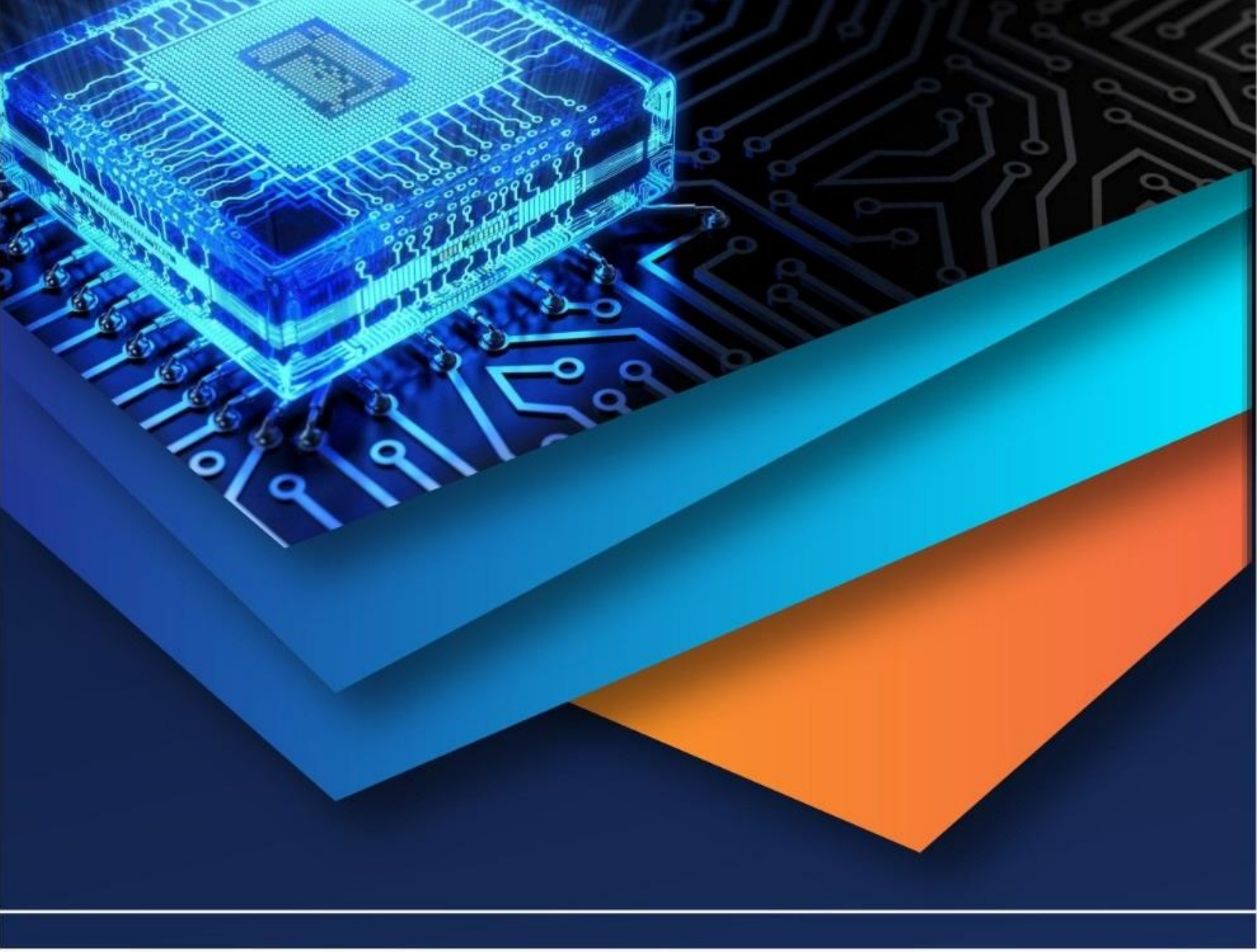

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