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# Isolation and Screening of Native Microbial Isolates for Pectinase Activity

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**Abstract:** ***The aim of this study was to isolate and to screen native bacterial isolates for pectinase activity. In this experiment twenty isolates from locally available decaying fruits and vegetables were screened for pectinase activity on solid medium based on diameter of clear zone produced. Out of these two isolates $P_1$ and $P_2$ which exhibited maximum pectinase activity were identified by 16S rDNA sequencing as Bacillus subtilis and Streptomyces rochi. These isolates were cultured at optimum pH and temperature and enzyme assay were carried out by using DNS method. Banana juice was treated with enzymes extracted from these two isolates and its microbial cultures were used. The extent of clarification was determined by measuring the absorbance at 660nm using UV Vis spectrophotometer.***

**Keywords:** Pectinase, 16S rDNA sequencing, DNS, Clarification

## 1. Introduction

Pectin is a structural hetero polysaccharide contained in primary cell walls of terrestrial plants [1]. It is a complex polysaccharide consisting mainly of esterified D-galacturonic acid resided in α- (1-4) -chain present in primary cell wall and middle lamella of fruits and vegetables [2]. During ripening, pectin is broken down by the enzyme pectinase and in this process the fruit becomes softer as the middle lamella breaks down and cells become separated from each other [3]. The pectic substances account for about 0.5-4% of the weight of fresh material [4]. The raw pressed juice is rich in insoluble particles mainly made up of pectic substances. The main uses of the pectinase enzymes are they are widely used in the food industry and in wine production.

Banana (*Musa* spp.) is the fourth most important food crop in the world after rice. Banana is a climacteric fruit. Due to highly perishable nature of fruit, shelf life of fully matured banana under tropical conditions is limited to a short period. And hence it is being wasted to a large extent. Hence, in order to minimise losses due to wastage, clarification is attempted [5]. Bananas have a high sugar and recognizable desirable flavour that could be utilized to produce banana juice with nutritional value and acceptable flavour. Banana juice is turbid, gray in colour, very viscous, tends to settle during storage and therefore clarification is must. Pectins make the clarification process harder because of their fibre like molecular structure [6].

In this study different locally available decaying fruits and vegetables like orange, apple, guava, tomato and banana were collected from household garbage and fruit shop for the isolation and screening of the microbial isolates for pectinase activity.

## 2. Materials and Methods

### 2.1 Sample Collection

Decayed fruits and vegetables were collected from Sakthan market, Thrissur.

### 2.2 Isolation of microorganisms

Collected samples were serially diluted with water and 1µl from $10^{-6}$ dilution were spread on nutrient agar and potato dextrose agar. These plates were kept for incubation overnight. The obtained microbial cultures were sub cultured for purification.

### 2.3 Screening for pectinase producers

The isolates were screened for pectinase activity. These was done by inoculating the organisms on the pectinase screening agar medium (PSAM) plates and incubated at 37ºC for 7 days. Then the plates were flooded with iodine solution and incubated for 15min at room temperature. A clear zone around the growth of microorganisms indicated pectinase activity [7]. The efficiency to solubilize pectin was assessed in terms of solubilization index calculated using the formula:

**Solubiization index = Colony + Halozone diameter/ Colony diameter**

Composition of the media used for isolation (g/l, wt/vol.): 1g pectin, 0.3g ammonium chloride, 0.2g $KH_2PO_4$, 0.3g $K_2HPO_4$, 0.01g $MgSO_4$, 2.5gm agar in 100ml.

### 2.4 Identification of microbial isolates

Strains were identified by using 16S r DNA sequencing for bacteria. The phylogeny and the family of the strains was accessed using BLAST search.

### 2.5 Enzyme Extraction

22ml of 0.1M phosphate buffer (pH 6.5) was added to cultures, the mixtures were shaken for 30 min at 19˚C and 140 rpm on a rotary shaker. The mixture was filtered through cheese cloth and centrifuged at 8000 rpm at 4˚C for 15min. The supernatant was filtered through Whatmann's number 1 filter paper and the filtrate was used as crude enzyme preparation [8]

### 2.6 Enzyme Assay

Enzyme assay was based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by dinitrosalicylic acid reagent (DNS) method. For this, to 0.2ml of 1% pectin 2.0ml of sodium citrate buffer of pH 5.0 and 1.0 ml of enzyme extract were added. The reaction mixture was incubated at 35˚c for 25 min. After 25 min, 1.0ml of this reaction mixture was withdrawn and added to test tubes containing 0.5ml of 1M sodium carbonate solution. To each test tube, 3.0ml of DNS reagent was added and the test tubes were shaken to mix the contents. The test tubes were heated to boiling on water bath for 10-15 min. Then these were cooled and 20ml of distilled water was added to contents of each tube and the absorbance was measured at 570 nm using spectrophotometer. The enzyme and the substrate were run parallel. The standard curve was prepared for reducing sugars with glucose.

### 2.7 Clarification

Clarification is the process by which insoluble matter suspended in juice is removed. The banana fruits were washed, peeled manually and cut into small pieces. Juice of 10ml from these were taken after heating at $85^0$C for 3 min to inactivate the natural fruit enzymes or microbes present and then cooled to $40^0$C before adding pectinase enzymes and microbial cultures. Varying concentrations of enzyme (0, 1.5, 2.5 and 5mg respectively) and microbial cultures (0, .5ml, 1ml) were added. The samples were incubated for 4 hours and then treated at $85^0$C for 3 min to inactivate the enzymes and microbes. Centrifuged at 3000rpm for 20 min and the supernatant were filtered out using Whatmann's filter paper. Clarity of the juice was determined by measuring the absorbance at 660 nm using a UV– Vis spectrophotometer, distilled water was used as blank. The clarity was expressed in terms of percentage by using Absorbance value (Abs).

$$\%\ \boldsymbol{Clarity} = (\boldsymbol{Control} - \boldsymbol{Sample} \div\ \boldsymbol{control}) \times \mathbf{100}$$

## 3. Results and Discussion

Pectinases are produced by a large number of organisms such as bacteria, fungi, actinomycetes and yeasts. Pectinases have been used in processes and industries where the elimination of pectin is essential; fruit juice processing, coffee and tea processing, macerating of plants and vegetable tissue, degumming of plant fibre, treatment waste water, extracting vegetable oil, bleaching of paper, adding poultry feed and in the textile, alcoholic beverages and food industries.

We have isolated a few microorganisms from decayed fruits and vegetables from vegetable market in Thrissur, Kerala. The isolates were streaked on PSAM plates. These plates were subjected to incubation for 37˚C for 7 days. The plates which showing clear zone around the streaked area of test organism were selected as pectinase producing strains

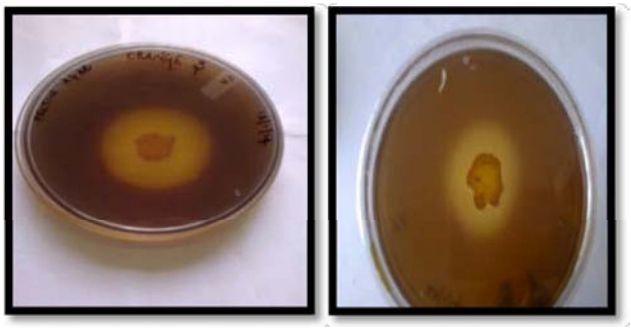

**Figure 1 and 2:** Clear zone formation by microbes on PSAM agar

Identity and phylogeny of the isolate was analysed using 16S r DNA analysis. The strains showed maximum homology with *Bacillus subtilis* and *Streptomyces rochei*. These microorganisms are known to produce variety of extra cellular enzymes and they have a wide range of industrial applications [9] The bacterial colonies with maximum zone diameter were selected as the best isolates for further studies (Tab.1).

**Table 1:** Showing Solubilisation Index of Various Isolates

| *Sample* | *Diameter* |
|---|---|
| P1 | 4.83cm |
| P2 | 3.16cm |
| P3 | 3.00cm |
| P4 | 2.46cm |
| P5 | 2.28cm |

DNSA enzyme assay was carried out using the extracted enzymes. In 0.5ml of enzyme sample extracted from *Streptomyces rochei* and *Bacillus subtilis* showed 248.79µg and 235.87µg respectively (fig 3). And hence *Streptomyces rochei* was found to produce more enzyme concentration compared to *Bacillus subtilis*.

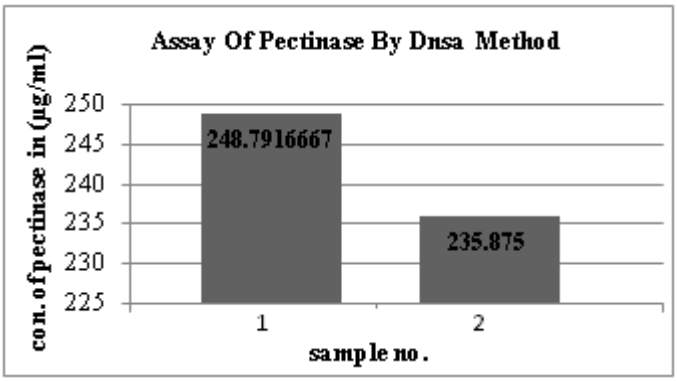


**Figure 3:** Graph showing concentration of 0.5 ml enzyme, extracted from microbial cultures

Banana juice clarification was attempted using microbial culture and crude enzyme. In microbial clarification we used culture of *Streptomyces rochei* (32 x $10^4$ CFU/ml) and *Bacillus subtilis* (69 x $10^5$ CFU/ml). 93% activity for *Streptomyces rochei* and 87% activity for *Bacillus subtilis* using 1ml microbial culture (fig 4). And in the case of the enzyme clarification, for the *Streptomyces rochei* showed 83% activity and 74% activity by *Bacillus subtilis* (fig 5). Thus resulting the microbial culture has more activity than enzyme towards the banana clarification. When comparing within the cultures used for clarification the *Streptomyces*

*rochei* showed much higher potential for clarification than that of the *Bacillus subtilis*. The observation may be due to degradation of pectic substances which lead to increase in the yield of juice [10]

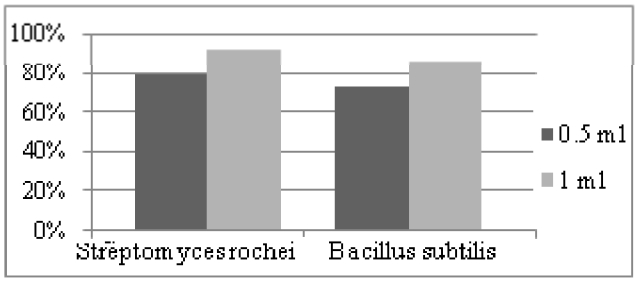


**Figure 4:** Extent of clarification of banana juice by pectinase producing microbes

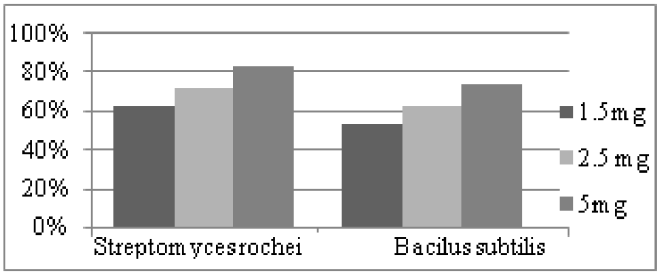


**Figure 5:** Extent of clarification of banana juice using crude pectinase from microbes

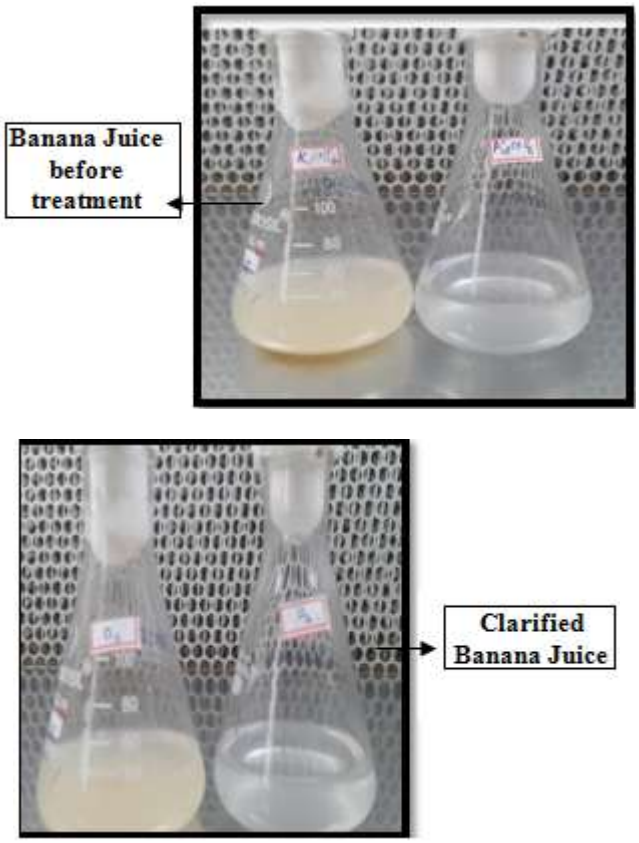


**Figure 6 and 7:** Clarified banana juice obtained from the treated samples

## 4. Conclusion

The search for promising strains of pectinase producer is a continuous process. Two isolates which showed higher pectianse activity were selected and identified as *Streptomyces rochei* and *Bacillus subtilis*. On the basis of the data obtained in the present work it can be concluded that *Streptomyces rochei* and its enzymes have higher ability to clarify the banana juice than that of *Bacillus subtilis*.

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## References

[1] Anisha S.K, Ashwini S, Girish K. (2013).'Isolation and screening of Aspergillus spp. for pectinolytic Activity' Electronic Journal of Biology .9(2): 37-41
[2] Ahamd Asgharzadeh, Mohammad H. Maleki, Mohammad A. Tajiek Ghanbary, Gholamali Ranjbar and Abolfazl Lotfi (2011). 'Screening of Some Zygomycetes strains for pectinase activity'. Journal of Microbiology and Biotechnology Research. 1(2):1-7
[3] Arunachalam, c ., Asha, s., (2010). 'Pectinolytic enzyme –a review of new studies'. Adv Biotech J Online.
[4] Neelam G and Vibha B .(2010).'Exploitation of microorganism for isolation and screening of pectinase from environment'. University of Malaya.
[5] Tapre A. R and Jain R. K. (2012). 'Optimization of process variables for enzymatic clarification of banana pulp'. International Journal of Emerging trends in Engineering and Development 2(7).
[6] S. Yusof, B.S Baharin,W. C Lee, N.S.A Hamid.(2006). 'Optimizing conditions for enzymatic clarification of banana juice using response surface methodology'. Journal of Food Engineering 73(55-63).
[7] E.Venkata Naga raju, G. Divakar(2013). 'Screening and Isolation Of Pectinase Producing Bacteria from Various Regions in Banglore'. International Journal of Research in Pharmaceutical and Biomedical Sciences. Vol. 4(1).
[8] P. Lakshminarasimha Reddy and A Sreermulu (2012). 'Isolation , identification and screening oof pectinolytic fungi from different soil samples of chittoor district' International Journal of Life Science Biotechnology and Pharma Research 1(3)
[9] N. Annamalai , S.Elayaraja, S.Vijayalakshmi and T.Balasubramanian, Afr.j.Biochem.Res., 2011,5(6),176-181.
[10] Gerhartz, W. (1990) Enzyme in Industry. Food Sc. And Tech. 64:1210-1211