Pectinase Production From *Aspergillus niger* And Activity Assay With Pectin Isolated From Orange Peel

Yashaswini R Bhat¹, Srujana S Ramaswamy¹, Brinda V¹, Aishwarya Bhat¹
Students of Department of Biotechnology
R.V College of Engineering
Mysuru Road, R. V. Vidyamitran Post, Bengaluru, Karnataka 560059
India

**Abstract:** The paper discusses a method of extracting the enzyme pectinase which is involved in the degradation of plant cell wall. The paper describes the extraction of pectinase from *Aspergillus niger*, its enzymatic assay and activity. The paper also discusses a method of extracting a flavouring component, Limonene from the waste orange peels.

**Keywords:** Pectinase; *Aspergillus niger*; Limonene; orange peels

I. Introduction

Pectinase is an enzyme that is known to break down pectin. Pectin is a gel-like matrix present in the walls of the plant cell. The basic function of pectinase is to firstly break down the plant material and allows the extraction of flavours as a by-product. Pectinase enzymes are majorly used in the process for extracting juice as the pectinase breaks down the pectin in the fruit. Pectin is heteropolysaccharide contained in the cell walls of terrestrial plants. The structural type of pectin is rhamnogalacturonan II, which is less frequent, complex polysaccharide. Isolated pectin has a molecular weight of typically 60000 to 130000 g/mol. There are two types of pectin a). High ester pectin b). Low ester pectin. [1]

In high ester pectin at soluble solids content above 60% and a pH value between 2.6-3.6, hydrogen bonds and hydrophobic interactions bind the individual pectin chains together. In low ester pectin, ionic bridges are formed between calcium ions and ionized carboxyl groups of the galacturonic acid. Normally in low ester pectin form a gel with a range of pH from 2.6 to 7.0 and with a soluble solid content between 10 and 70%. The typical levels of pectin present in plants range from; apples -1-1.5%, apricots- 1%, cherries - .4%, citrus peels – 30% and thus conclusively the pectin production from citrus peels is higher. [2]

In retrospect of this project, the isolation of *Aspergillus niger* from a soil source and correspondingly pectinase was extracted. In order to verify the action of pectinase, pectin was extracted from orange peels and assayed against pectinase to verify the pectin degrading properties of pectinase. The pectinase was then analyzed with a commercially bought pectinase to quantify the purity of the pectinase produced. As pectinase also helps in the production of flavours, the by-product of the production of pectinase is the production of limonene which is a colourless liquid hydrocarbon classified as a terpene. [2]

Limonene takes its name from the lemon (or other citrus fruits) giving the flavour odour. It is a colourless hydrocarbon and is classified as a cyclic terpene. It possesses a strong smell of orange. Limonene is obtained commercially from citrus fruits through two primary methods: Centrifugal separation and Distillation. Limonene is commonly used in cosmetic products. In natural and alternative medicine, limonene can be used to relieve gastroesophageal reflux disease and heartburn. Limonene is also finding increased use in filament 3D printing and also in bio fuel.

II. Materials and Methods

A. Citrus Pectin Production

Citrus Peel contains about 20-30% pectin. Hence Citrus peel was used for pectin extraction. *Citrus sinensis* (orange) was the citrus fruit that we used for extraction. [3]

One kilogram (5 oranges) of oranges were bought from the market and the pith (the white part) was separated from the orange peels. The peels were shredded into tiny pieces and soaked in lemon juice (of 2 lemons) for an hour. This was then boiled with a 300mL of water to get a jelly-like consistency and sieved through 4 layers of clean gauze cloth.
A. Isolation of Aspergillus niger
Two soil samples from two parts of Bengaluru were collected and mixed into equal concentrations. 1g of the mixed soil sample was weighed and added to 10 mL of distilled water and then 1 mL of this was added to 9 mL of distilled water and so on. This was continued up to $10^5$ dilution. Mineral salt agar medium was used for the isolation and growth of the Aspergillus niger. The serially diluted soil samples were plated onto Mineral Salt Agar petri plates. This is subcultured and preserved in agar slants. The Aspergillus niger growth was morphologically confirmed through microscopic identification.

B. Production of Pectinase from the isolated Aspergillus niger culture
Aspergillus niger was cultured in Mineral salt broth medium for 6-7 days under controlled conditions. 22 ml of 0.1M Phosphate buffer (pH 6.5) was added to the culture and then incubated at 20°C for one hour in rotary shaker at 200 rpm. This was then filtered through filter paper and the liquid was centrifuged at 8,000 rpm at 4°C for 15 mins. This was the crude enzyme.

C. Limonene production
The leftover orange peels were then distilled by simple distillation with water. 200 grams of orange peels were mixed with 500 mL water and distilled at 100°C. The distillate collected was 200 mL. This distillate was then mixed with hexane and then separation funnel was used to separate the two layers. The limonene had now gone into the hexane layer. The hexane was later evaporated at 68°C to get pure limonene. 200 grams of orange peel yielded 5 ml of limonene. Yield of limonene = 2.5%
A. Enzyme identification and assay

The pectinase activity was also determined by well method. Wells were made in Mineral Salt Agar Medium and 30µL of crude enzyme was added to the wells and incubated in the incubator for 24 hours. After 24 hours of incubation growth zones were observed. These growth zones are formed when the pectinase enzyme start acting on the pectin present in the Mineral Salt Agar Medium. The zones of pectinase activity were found by flooding the plates with Iodine solution containing 0.25% iodine, 0.5% potassium iodide and 31 ml of 20% ethanol. Zone of pectinase activity was measured as 2.5cms diameter.

Table 1: Enzymatic assay procedure and constituents

<table>
<thead>
<tr>
<th>Components</th>
<th>Test</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus pectin (1:3 dilution with water)</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>Acetate buffer (32.15mL of 0.1M Sodium acetate + 17.85mL Acetic acid) pH 5</td>
<td>10mL</td>
<td>10mL</td>
</tr>
<tr>
<td>Crude enzyme</td>
<td>6 mL</td>
<td>-</td>
</tr>
<tr>
<td>Incubation at 37°C for 20 mins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNS reagent</td>
<td>15 mL</td>
<td>15 mL</td>
</tr>
<tr>
<td>10 mins water bath at 40°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measure at 540 nm</td>
<td>0.12</td>
<td>0</td>
</tr>
</tbody>
</table>

The enzymatic assay was done as in Table 1. D-Galacturonic acid was used as a standard to plot the standard curve. The concentration of the D-galacturonic acid in the test sample was determined by comparing with the standard plot. This was used to calculate the pectinase activity. The absorbance of the test sample was found to be 0.12 with the control as blank.
IV. Discussion

Pectinase is one of the major commercial enzymes, which degrades pectin that occurs in the middle lamella as structural polysaccharides in primary cell walls of plants into a simpler galacturonic acid. Pectinases are primarily used in the food and cloth industry. Pectin is mainly used in the food industry for juice clarification and in the cloth industry for retting of fibers. Pectinase also increases yield of juices, coffee oil during extraction. It helps in better digestion of food in animals when pectinase is mixed into the food.[5] Limonene has numerous uses. Limonene is a cyclic terpene, found to be useful as an anti-obesity drug and is found to also have anti-cancerous properties. It can also be used in the food industry as a flavouring agent and as a fragrance in cosmetics. Also, limonene is used as a cleaning solvent. As it is combustible, it can be used as a biofuel.

V. Conclusion

The fungus Aspergillus niger was successfully isolated in mineral salt agar medium from serially diluted soil samples and citrus pectin was obtained from orange peel. Pectinase was successfully produced from the isolated culture. The assay was done by method because of the advantages and convenience it provides over the other methods. The diameter of growth zones confirms the pectinase activity. Pectinase activity was found by the colorimetric assay with DNS reagent. Limonene was extracted from the orange peel by distillation method. Hexane was added to the distillate to purify limonene. This hexane was evaporated to get pure limonene. Citrus pectin and limonene were extracted from orange peels which are the waste of food industry. This was a way of extracting commercially important enzymes and chemicals from waste and seems like an economical method. Effective management of waste and by products is possible because of this method of extraction. This can be employed in industries on a larger scale for better management of waste and better profit.

VI. References


