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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Production of Pectinase by *Trichoderma* Spp. Under Solid State Fermentation Conditions by Using Pineapple Peel

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DOI: 10.55248/gengpi.4.623.47301

ABSTRACT

The purpose of this investigation is to isolate and exploit diversified pineapple peels from well drain habitat and their commercial utilization in industrial area. Pectinases are enzymes with a wide range of industrial and commercial uses. The pectin found in plant material is broken down into simple chemical compounds by the enzyme polygalacturonase, which is produced by bacteria. The enzymatic breakdown of α-1, 4-glycosidic linkages in polygalacturonic acid is catalysed by Polygalactouronic acid, releasing D-galacturonate which is a simple chemical substance. The production of Polygalacturonase by *Trichoderma* was enhanced by optimization of different nutritional parameters. Highest polygalacturonase activity was observed at 120 hour at 35°C. The pH of 6.0 was ideal for the synthesis of increased polygalacturonic acid. Cellulose and malt extract were the carbon and nitrogen sources that improved enzyme activity. Highest enzyme specificity was recorded when the substrate used was citrus pectin pineapple peels. Polygalacturonase from *Trichoderma* is suitable for industrial scale production as it has high productivity of pectinase enzyme.

Keyword- Pectinase, well drained habitat, pineapple, *Trichoderma sp.*

1. Introduction

Pectinase is a class of enzymes. Pectin, a polysaccharide present in plant cell walls, is broken down by a group of enzymes called pectinase through hydrolysis, trans elimination, and desterification processes. They are also referred to as pectic enzymes and include polygalacturonase, one of the most extensively researched and utilised commercial pectinases, as well as pectolyase and pectozyme. Pectin is the jelly-like matrix that helps bind plant cells together and contains Pectinases along with other components of cell walls like cellulose fibrils. In order to accelerate the extraction of fruit juice from fruit, such as apples and sapota, pectinase enzymes are frequently utilised in processes involving the. Pectinase enzymes are naturally produced by various plants, fungi, yeasts, insects, bacteria and microbes, but cannot be synthesized by animal or human cells. In plants, pectinase enzymes hydrolyze pectin that is found in the cell wall, allowing for new growth and changes to be made. Similar to their role in plants, pectinases break down pectin during the developmental stage of fungi. Pectinases depolymerize pectin through hydrolysis, trans-elimination and desterification reaction processes, breaking down the ester bond that holds together the carboxyl and methyl groups in pectin. Endo-polygalacturonase progresses through a reaction along the following pathway: 1,4-alpha-D-galacturonosyl + H2O = (1,4-alpha-D-galacturonosyl)n + (1,4-alpha-D-galacturonosyl)m. They are used for clarification in fruit juices and also speed up fruit juice extraction through enzymatic liquefaction of fruit pulp. In addition, pectinase enzymes aid in formation of pulpy products in the fruit juice industry. Pectinase enzymes are used for extracting juice from purée. This is done when the enzyme pectinase breaks down the substrate pectin and the juice is extracted. The enzyme pectinase lowers the activation energy needed for the juice to be produced and catalyzes the reaction.

Pectinases can be extracted from fungi such as Trichoderma. The fungus produces these enzymes to break down the middle lamella in plants so that it can extract nutrients from the plant tissues and insert fungal hyphae. If pectinase is boiled it is denatured (unfolded) making it harder to connect with the pectin at the active site, and produce as much juice

Based on how they work, pectinase enzymes are broadly divided into three categories. hydrolases, lyases, and pectin esterase. In order to create pectic acid, pectin esterase catalyses the de-esterification of the methoxyl group of pectin. In pectic acid and pectin, respectively, hydrolases (Polygalacturonases and Polymethylgalacturonases) catalased the hydrolytic breakage of the 1,4-glycosidic bond through a trans-elimination reaction, resulting in the formation of unsaturated galacturonates and methyl galacturonates. Pectinases are categorised according to their method of action and end products. Both submerged and solid state fermentation (SSF) are methods for making pectinase. The extraction of juices from fruits like apples, grapes, wine, strawberries, etc. uses this specific enzyme. The turbidity and viscosity of fruits are significantly influenced by pectin. Up to 50% less time is spent filtering. .

2. Materials and methods

Collection of pineapple peels

The pineapple peels were collected in sterilized conditions to avoid contamination from juice corner in Meerut nearby Meerut Institute of Engineering and Technology, Meerut, Utter Pradesh, India.

Isolation Of Fungal Strain

Before beginning the process of producing an enzyme of interest, potassium fungal strain isolation is crucial. Trichoderma was employed in this experiment to produce pectinase under solid state fermentation conditions. Using a sterilising technique, the fungus Trichoderma was isolated from the pineapple peels. The pineapple peels were autoclave-sterilized for two to three hours. Inhibition can also be achieved with Potato Dextrose Agar (PDA) medium using the plating method. The petri plates were incubated at 21°C for 3–4 days..

Effect Of Carbon Source:

Potato dextrose/ starch were used in the basal medium at 1% level as carbon source to support the microbial growth. Flask was incubated at 30°C for 5 days. The content was filtered and enzyme activity was assayed.

Effect Of Nitrogen Source:

In enzyme production the effect of different nitrogen source was observed by adding ammonium chloride, ammonium nitrate, ammonium sulphate, and sodium nitrate separately to the fermentation medium and incubation them for 90 hours at room temperature.

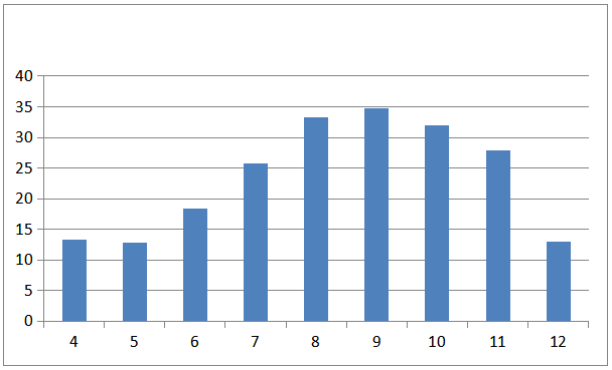
Procedure For Crude Enzyme Extraction

To obtain the crude extract from the production media we added 50ml distilled water in the flask. Then the filter suspension in a separate flask using filter paper. After that , repeat the filtration step again 2 times.

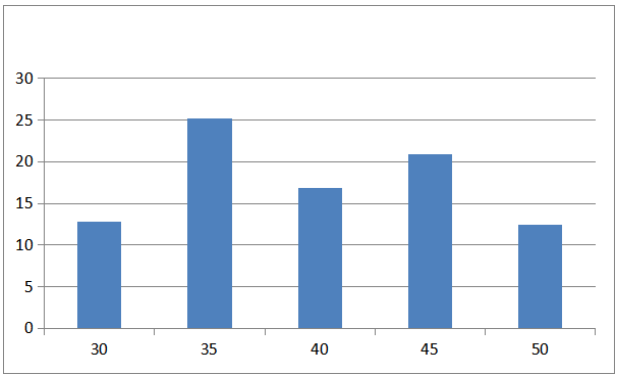
3. Results and Discussion

The enzyme were isolated well drained habitat and screen of pectinase enzyme activity. The production of pectinase is regulated by physico-chemical and nutritional factor. For microbial production of pectinase, Solid State Fermentation (SSF) was preferred. SSF process employs natural raw material as carbon or energy source.

Graph 1: Effect of different incubation period (in days) on Pectinase production by *Trichoderma* under solid state fermentation conditions.



Graph 2: Effect of temperature (°C) Pectinase production by *Trichoderma* under SSF conditions.



Graph 3: Effect of carbon source on production of pectinase by Trichoderma under SSF conditions.

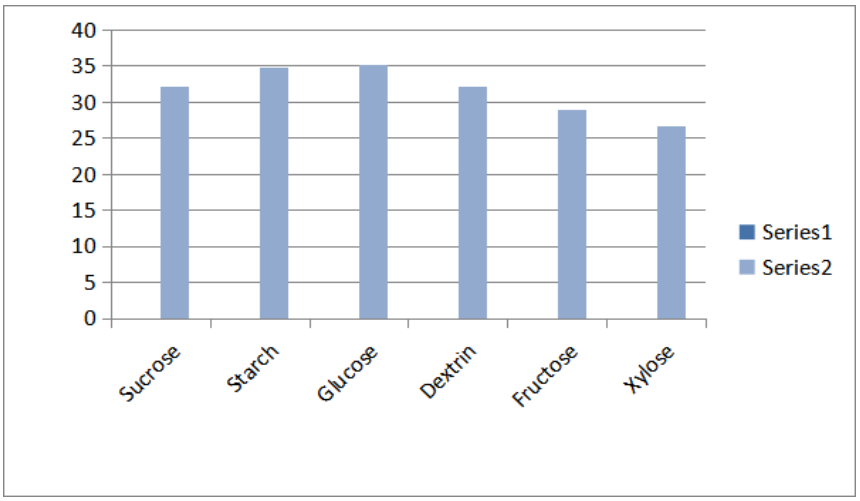


Table 1-Effect of pH on the production of Pectinase by *Trichoderma* under SSF conditions.

pH	Pectinase activity(1U/ml)
4	12.72
4.5	13.25
5	16.51
5.5	18.23
6	24.86
6.5	27.02
7	15.82
7.5	12.18
8	11.04

Table 2 - Effect of different nitrogen source on the production Pectinase by *Trichoderma* under SSF conditions.

Nitrogen Source	Pectinase activity(1U/ml)
Casein	24.10
Peptone	36.06
Urea	31.26
Gelatin	22.78
Yeast Extract	23.25

4. Acknowledgement

I wish to acknowledge the Department of Biotechnology and Microbiology M.I.E.T for providing me the facilities to carry out the research work. I am thankful to Aditi Tiwari for helping me in the drafting of this work.

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