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Pectinase production and purification from *Bacillus subtilis* isolated from soil

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ABSTRACT

Pectinases are industrially important enzymes. The aim of this work was the isolation of pectinases producing Bacillus species from soil sample and their identification by phenotypic tests as well as production and purification pectinases enzyme. Isolated bacteria Subjected to various screening activity and gives pectinases screening test positive i.e. bacteria produced pectinases enzyme. The enzyme produced and purified By Ammonium sulphate precipitation followed by Dialysis and ion-exchange chromatography.

Keywords: Screening tests, Ammonium sulphate, ion-exchange chromatography, *Bacillus subtilis.*

INTRODUCTION

Pectinases are the enzymes, such as pectolyase, pectozyme and polygalacturonase, commonly known as pectic enzymes. These break down pectin, a polysaccharide substrate that is found in the cell walls of plants. One of the most studied and widely used commercial pectinases is polygalacturonase [1]. It is useful because pectin is the jelly-like matrixes which helps cement plant cells together and in which other cell wall components, such as cellulose fibrils, are embedded.

Occurrence

Pectinolytic enzymes can be derived from different sources such as plants, animals and microorganisms like bacteria and fungi such as *Aspergillus niger* [1].The fungus produces these enzymes to break down the middle lamella in plants so that it n extract nutrients from the plant tissues and insert fungal *hyphae.* It was found that some species of *Bacillus* like *Bacillus sabtilis* can be a good source of pectinase production [2].

Industry Demands and Uses

Pectinases enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples. Pectinases have also been used in wine production since the 1960s. Enzymes were used industrially because of their high catalytic power, specific mode of action, stereo specificity, eco-friendly use, reduced energy requirement. The commercial application of pectic enzymes was noted in 1930 for the preparation of wines and fruit juices. But the chemical nature of the plant tissues was apparent only in 1960's. In 1995 was one billion dollars, of which 75 million dollars. By 2005 the whole world market for industrial enzymes is expected to be 1.7-2 billion dollars. They are of prime importance for plants as they help in cell wall extension and softening of some plant tissues during maturation and storage. They also aid in maintaining ecological balance by causing decomposition and recycling of waste plant materials.

MATERIALS AND METHODS

Isolation of micro organisms
Bacterial species were isolated on NAM media from the soil enumerated for 20 days with fruit peels as a sole of carbon source by the serial dilution in the range of 10^{-1} to10^{-6}. Few drops were spread from each sample on media for growth.

Biochemical tests
Some biochemical and staining methods ware used for the identification of the bacteria these included gram staining method, endospore staining, catalase test, Simmon citrate agar test, MR-VP tests etc

Screening
Various screening tests were performed by inoculating bacteria on mannitol agar media, Amylase screening test also performed and results obtained positive for pectinase screening test.

Fermentation and media
A basic liquid medium was used for the production of pectinase having compostion (g/ml), Pectin (1.0), Ammonium dihydrogen sulphate (0.14), Potassium dihydrogen phosphate (0.2), Potassium hydrogen phosphate (0.6)/Magnesium sulphate (0.02) at pH 7.2 . The production media was incubated for 48 h for submerged fermentation.

Enzyme extraction and purification
Culture medium was centrifuged and supernatant was used as crude enzyme source. The crude enzyme was precipitated by 60 % ammonium sulfate saturation, incubated for night at 4^0 C and centrifuged at 5000 rpm for 20 min and palates were dissolved in T.E. buffer at pH 7. Dialysis was also performed against T. E. buffer overnight at 4^0 C.

Ion exchange chromatography
Dialyzed buffer was directly applied to the agarose column (1×30 cm.) equilibrated with TE buffer (pH 7.0). Elution was carried out by liner gradient of NaCl (0.1M - 0.6M). About 5 ml of fraction collected and activity was observed.

Determination of protein
Total protein was determined by the method of Lowery *et al.* (1951) by using BSA as standard.

Enzyme assay
Pectinase activity was measured by estimation of glucose by DNS method using pectin as substrate. Standard graph prepared by concentration of standard glucose solution. One unit of Pectinase activity was defined as the amount of enzyme which liberated 1µm glucose per min.

RESULTS AND DISCUSSION

Pure colonies of *Bacillus species* was isolated from 20 days Soil and mixed fruit waste and subjected to various phenotypic identification tests. The Results of the phenotypic Tests are given in the Table No.-1. Further. Again pure colonies are used for the screening test like amylase test and bacteria gives positive result for the production of pectinase test, Earlier these studies performed by various researchers [2].The enzyme petinase was produced by using isolated bacterial species was carried out with a basic pectinase production media (pH 7.2) by submerged fermentation after incubation of 48 h. The crude enzyme was recovered. The specific activity of the crude enzyme was found to be 3.1 Umg^{-1}.

The crude enzyme was purified by ammonium sulphate precipitation and followed by Dialysis and aliquates are applied to agarose ion- exchange column (1*30). After this step Specific activity was 4.5 Umg^{-1} and enzyme was 1.5 fold purified. Maximum yield observed about 80% for the process most recently this was [3] found specific activity 0.185 for *Bacillus subtilis*.

Table 1- Various tests for identification and screening of unknown bacterial species

S.NO.	Test	Results
1	Gram Staining	+++
2	Endospore Staining	-+-
3	Catalase Test	+++
4	Simmon citrate agar test	-++
5	Mannitol salt agar media	+-+
6	Amylase screening test	++-
7	MR and VP	---
8	Mannitol fermenting broth	-++
9	Pectinase screening test	++-

**All the experiments was performed in triplicate manner*

Table 2- Purification table for Pectinase Enzyme

sample	Total Protein (mg)	Activity (Unit)	Specific Activity (U/mg)	Fold	Yield
Crude	0.56	0.18	3.1	1	100
Ion Exchange	0.45	0.10	4.5	1.5	80

CONCLUSION

Bacillus subtilis are isolated from soil and mixed food wastes are able to produce the pectinase enzyme. The yield of purified enzyme was about 80% and maximum specific activity was obtained 4.5 Umg^{-1}. Pectinase is an important enzyme for industrial purposes so it is important to find new method for production and purification which should be cost effective and beneficial.

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