

Isolation, Production and Partial Purification of Pectinase Isolated from *Bacillus* Sp.

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ABSTRACT

Introduction: Pectin is a polysaccharide present in the cell wall of plants and occupies one-third of the dry weight of plants (Oumer, 2017). Pectin consists of long galacturonic acid chains with residues of carboxyl groups with varying degrees of methyl esters (Haile & Ayele, 2022). Pectinolytic enzymes (Pectinases) are group of pectin hydrolysing enzyme classified according to their mechanism of action including protopectinases, esterases, and depolymerases. Pectinase is involved in the metabolism of cell walls as well as in the growth of cells, ripening of fruits, softening of food (Guan *et al.*, 2020). In Industries acidic pectinases are used in the extraction and clarification of fruit juices, jams and jelly production, alkalophilic pectinases are used in the degumming of plant-based fibres, treatment of effluents discharged from food processing units as well as used in paper making, treatment of pectic wastewater and in coffee and tea fermentation (KC *et al.*, 2020).

Objective: Given the wide range of industrial applications for pectinases, this study aimed to isolate pectinase-producing bacteria (*Bacillus*) from soil. The pectinase was then produced and partially purified using the salt fractionation method.

Methodology: Pectinase producing *Bacillus* sp. was isolated from soil. Soil samples were collected from rhizosphere zone where fruit peels were buried for ten days. Bacterial cultures were isolated and serially diluted from the soil. The dilutions were plated on pectin-agar medium. Colonies were selected based on the size of the clear hydrolytic zones around them indicating pectin hydrolysis. The best pectin producer strain was purified, revived on nutrient agar, and stored at 4°C for further use.

For the production of pectinase, fermentation media with 1g% pectin, 0.2g% KH_2PO_4 , 0.2 g% K_2HPO_4 , 0.2g% KNO_3 and 0.3g% yeast extract was prepared and autoclaved. Overnight culture of *Bacillus* sp. was inoculated in the medium and incubated for 24h at 37 °C. After 24h, the cell free filtrate (CFF) was collected using cold centrifugation. Enzyme assay was determined by Dinitrosalicylic acid (DNS) method (Rozendo *et al.*, 2024), using 1 g% citrus pectin as substrate. Partial purification of pectinase was performed by salt fractionation technique. Varying concentration of ammonium sulphate was added in cell free filtrate followed by dialysis. Specific activity and total protein was estimated by Lowry's method (Satpathy *et al.*, 2020).

Result: Pectin degrading enzyme, pectinase was produced from *Bacillus* sp. isolated from soil. The Pectinase was produced with 2683 U/ml. The enzyme was then partially purified using different concentrations of ammonium sulfate. It was observed that 40 g% ammonium sulfate resulted in maximum specific activity of 815 U/mg with 3.08 fold purification.

Conclusion: The partially purified pectinase can be effectively utilized in various industrial sectors, including food processing, textile, and agricultural industries, thereby enhancing productivity and efficiency of the processes.

Keywords: Fermentation, *Bacillus*, Partial Purification, Pectinase.

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