Production of pectinase by *Bacillus sp.*, isolated from soil


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ABSTRACT

The significance of pectinase enzyme is emerging as a rapid fire, since these enzymes act as a tool in the variety of industries like fruit processing industry, vegetables processing industry, paper industry etc., so owing to their vast and variety applications, newer microorganisms are to be screened for the production of pectinase enzyme with desirable properties. The *Bacillus species* was isolated from soil, their phenotypic and genotypic identification has been carried out. The pectinase producing *Bacillus species* were screened under plate assay technique. The screened isolates were used as a source of pectinase production. Using various substrates orange peel extract, potato peel extract, tea waste, the pectinase enzyme was produced, the orange peel extract shows maximum enzyme activity and at various parameters like pH and temperature the activity of pectinase enzyme was determined by using titrimetric method. It has been identified that the pectinase shows its maximum activity at neutral pH and temperature of 35°C. Finally, the substrate, pH, and temperature influence the activity of pectinase enzyme was studied.

Keywords: Pectinase, *Bacillus species*, Solid waste, Temperature, pH

INTRODUCTION

Pectinases are a heterogeneous group of enzymes that catalyse the breakdown of pectin containing substrates by hydrolysis and transelimination as well as by deesterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin. In commercial terms pectinases refer to a mixture of primarily three different enzymatic activities: polygalacturonase, pectinesterase and pectinlyase (Semenova et al., 2006). Pectinolytic enzymes are naturally produced by many organisms like insects, nematodes, plant and microorganisms. Microbial pectinases are important in the phytopathologic process in plant-microbe symbiosis (Jayani et al., 2005) and several bacterial and fungal strains have been shown to produce different types of pectinolytic enzymes (Gumadi and Panda2003).

Pectinase are constitutive or inducible enzymes that can be produced either by submerged (Alkorta et al., 1998) or solid state fermentation (Kashyap et al., 2001). Various factors related to environment affect the production of pectinase. Some of them are concentration of nutrients, pH, temperature, moisture content, influence of extraction parameters on recovery of pectinases (Sakai et al., 1993). Pectinase enzyme has major applications in fruit processing industries (Pandey et al., 1999), like degradation of plant materials, such as speeding up the extraction of fruit juice from fruit, including apples, sapota and citrus fruits (Takuo Sakai et al., 1998), addition of pectinase lowers the viscosity and causes cloud articles to aggregate to larger units, which sediment and is easily removed by centrifugation (Apsara et al., 2002). The aim of our study to isolate, characterisation of microorganisms from soil, separation of pectin, production of pectin enzyme using various parameters and determination of enzyme activity.

EXPERIMENTAL METHODS

Isolation of organisms: 1 gm of soil diluted with 100 ml of distilled water. Serially diluted sample was spreaded on nutrient agar and incubated at 30°C. The colonies formed on the nutrient agar plates were observed at regular intervals and the subculture was done. Finally plate assay was performed to detect the presence of bacteria that degrades pectin.

**Confirmation test for pectin degradation:** Nutrient agar combined with pectin acts as the culture medium for plate assay. A hole was punched on the agar plate using gel puncture and the strain was inoculated into the punctured hole. The plates were incubated for 48 hours at 30°C and the pectin was degraded by the inoculated strain has been observed.

**Biochemical test:** Idenfication of organisms by biochemical characterization and morphological identification by staining. Based on this, the isolate was found to be gram positive, motile rod shaped *Bacillus*.

**Separation of pectin from orange peels:** Orange peel powder was collected and 0.05N HCL was added and boiled for 1 hour at 100°C. The mixture was then cooled at room temperature. The cooled mixture was filtered twice and double the amount of absolute alcohol was added. Thus precipitation occurs and the extract was separated.

**Conformation of pectin:** The sample isolated from the above procedure, was dried and grinded into powder. The pectin power was given for FR-IR analysis. (Version FT-IR: 8400S, KBr pelleting technique).

**Pectinase production:** The pectinase enzyme is produced using the isolated strains of *Bacillus Sp.*, in the sterilized pectinase medium. The isolated strain was inoculated in three different substrates namely tea waste, orange peel waste, potato peel waste. The culture was kept for 72 hours incubation. After incubation the three different cultures were centrifuged for 10 minutes at 1200 rpm. The supernatant from the centrifuged samples were used as a crude enzyme for the determination of enzyme activity.

**ENZYMATIC ASSAY**

**Determination of enzyme concentration by lowry’s method:** The concentration of the extracted enzyme was determined by the Lowry’s method. The Bovine Serum Albumin (1mg/ml) serves as a standard for the determination of enzyme concentration. 0.1 ml of the crude enzyme was taken and make upto 1 ml with distilled water. About 4.5 ml of the lowry’s reagent was added to each test tube and the mixture was incubated at room temperature for 10 minutes. Then about 0.5 ml of Folin-Phenol reagent was added and it was then incubated for 30 minutes and the blue colour was formed and measured at 660nm in spectrophotometer.

**Determination of enzyme activity of Pectinase enzyme using titrimetric method:** The activity of the pectinase enzyme obtained was determined by the sigma Aldrich laboratory protocol. Pipetted out 4.9 ml of reagent A (polygalacturonic acid) into Erlenmeyer flask for sample and 5ml of reagent A for blank. Eqilibrate to 25°C and the added 0.1ml of reagent G (pectinase) only to sample.
Mixed them by swirling and incubate at 25°C for exactly 5 minutes. After that added 5ml of reagent B (I2/KI) and add 1ml of reagent C (Na2CO3) in both sample and blank. Mixed them by swirling and store in dark place for 20 minutes and then added blank with reagent E (Na2S2O3) until it becomes light yellow. Then added one drop of reagent F (starch indicator) and continue titration with reagent E until solutions are colourless. This titration was carried out for various substrates, pH, temperature and the enzymatic activity was observed.

**In-vitro characterisation of pectinase activity:**

**Effect of substrates on enzyme activity:** 250 ml of Erlenmeyer flask containing 150 ml of various substrates orange peel extract, potato peel extract, tea waste and 50 ml of mineral solution. The bacterial culture was inoculated into the substrates and kept for 3 days incubation. After that the sample was centrifuged at 12000 rpm for 10 minutes. The supernatant was used as a crude enzyme for determination of enzyme activity.

**Effect of pH on enzyme activity:** 100ml of Erlenmeyer flask containing 75ml of pectin and 25ml of mineral solution. The pH was adjusted from 5 to 9. The bacterial culture was inoculated and incubated at 37°C for 3 days. After that the sample was centrifuged at 12000 rpm for 10 minutes. The supernatant was used as a crude enzyme for determination of enzyme activity.

**Effect of temperature on enzyme activity:** 100 ml of Erlenmeyer flask containing 75 ml of pectin and 25ml of mineral solution. The bacterial culture was inoculated in to the substrate and kept for incubation at various temperatures (30, 35, 40, 45 and 50) for 3 days. After that the sample was centrifuged at 12000 rpm for 10 minutes. The supernatant was used as a crude enzyme for determination of enzyme activity.

**RESULTS AND DISCUSSION**

**Isolation of pectinase producing microorganism from soil:** The collected soil sample was used to identify pectinase producing microorganisms. The microorganisms were grown in agar plates; seven isolates (A, B, C, D, E, F and G) were subcultured and tested for pectinolytic activity (Fig 1).

**Conformation of pectinase producing isolates:** The isolated strains (A, B, C, D, E, F and G) were confirmed for the presence of pectinase activity using plate assay method (nutrient agar plate with pectin). The pectinase producing microorganism (strain G) degrades the medium containing pectin was identified. The plate shows clear zone formation (Fig 2).

**Growth curve of Bacillus species:** Growth curve of Bacillus species was obtained by plotting the graph between time intervals (hours) and absorbance at 660nm. The growth curve reached stationary phase after 19hours.

**Separation of pectin:** Pectin has been separated from orange peel using ethanol by precipitation method (Fig 3), Pectin characteristic has been studied under FT-IR spectrometry.

**Confirmation of pectin:** The pectin separation was confirmed by studying the peaks in FT-IR analysis (Fig 4). The spectrum showed peak at 3315.41 cm⁻¹ due to the stretching of OH groups. The peaks at 2964.39 cm⁻¹ indicated –C-H stretching vibration. The peak at 1670.24 cm⁻¹ indicated C=O stretching vibrations due to the presence of -CO-OCH₃ group. The peak at 1336.58 cm⁻¹ suggested –CHOH in aliphatic alcohol. The peak at 1193.85 cm⁻¹ suggested –CH-OCH stretching the above mentioned bonds can be seen in structure of pectin which confirms the pectin formation.

**Production of pectinase enzyme:** The production of pectinase enzyme by Bacillus species was investigated in a Erlenmeyer flasks in shaker. There are five different substrates orange peel, potato peel, tea waste has been chosen and kept for 72 hour incubation at various pH and temperatures. After the samples were collected and the crude extracts were assayed for pectinase production. It was observed that the enzyme production was higher in the medium containing orange peel extract. The concentration of enzyme and their activity was obtained by using the Lowry’s method and titrimetric methods.
CONCLUSION

The microorganisms were isolated from the soil and serially diluted, the cultures were grown in nutrient agar plates. Seven strains (A,B,C,D,E,F and G) were tested for pectinolytic activity using plate assay method. Of them strain G was identified as pectinase producing microorganism. Biochemical characterization was carried out and the strain G was identified as Bacillus species. Pectin was separated using ethanol precipitation method and confirmation of pectin was carried out by FT-IR spectroscopy, various peaks confirm the sample as pectin. Production of pectinase was carried out by using different substrates orange peel, potato peel, tea waste, and the concentration of enzyme and enzyme activity was done by Lowry’s method and titrimetric method, of them orange peel extract shows maximum production and enzyme activity is 166.5 units/ml. The enzyme activity at different pH and temperatures also done. The maximum activity of the enzyme was found at neutral pH is 187.0 units/ml and room temperature is 196.0 units/ml. Here, we conclude that the orange peel extract as a substrate shows maximum activity and production of pectinase enzyme when compared to the other substrates that were given as raw source.

REFERENCES