PRODUCTION AND OPTIMIZATION OF PECTINASE BY BACILLUS SP. ISOLATED FROM VEGETABLE WASTE SOIL

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ABSTRACT

Microbial enzymes have shown tremendous potential for different applications. Over the years due to their remarkable features enzymes have occupied the centre stage of all the biochemical and industrial processes. Pectinases are a group of enzymes responsible for the hydrolysis of pectic materials found in plants and are important industrial enzymes. In the present study, pectinase is produced from Bacillus sp. that was isolated from vegetable waste dump soil samples. A total of five isolates showed pectinase production and designated as PPB1 to PPB5. The screened isolates were used as a source of pectinase production using cassava waste as a substrate. Isolate PPB5 showed maximum enzyme activity of 0.641 IU/ml. Pectinase activity was optimized for various parameters like incubation time, temperature, pH, different carbon and nitrogen sources. Enzyme activity was observed maximum at 96 hr of incubation, 35°C temperature and at pH 6. The best carbon was found to be glucose. Among organic and inorganic nitrogen sources yeast extract and ammonium nitrate was founded to be better than other nitrogen sources. Among the five isolates, the isolate PPB5 showed maximum activity at all optimum conditions. This isolate is best producer and can be used in future for further pectinase production.

INTRODUCTION
Enzymes are biological molecules that catalyze chemical reactions [1]. Like all catalysts, enzymes work by lowering the activation energy (Ea) for a reaction, thus dramatically increasing the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. Most enzymes reaction rates are millions of time faster than those of comparable un-catalyzed reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life. Enzymes are protein catalysts synthesized by living system and are important in synthetic as well as degradative processes. The history of pectinases began with an understanding the structure of pectin substances and mechanism by which pectolytic enzymes degrade pectic substances. Later, the microbial production of pectinases became prominent for many decades. Many microorganism viz., bacteria, yeast, fungi could produced pectinases. Pectinases can be produced from different carbon sources. In the course of time, numerous reports have appeared on the optimization of fermentation and microbiological parameters and different fermentation strategies for the production of pectinases. Among the various pectinase, bacterial extracellular pectinase are the most significant, compared with animal, plants, viruses and fungal extracellular pectinase. Extracellular pectinase produced by Bacillus and Coccii species are of main interest from a biotechnological perspective, and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as foods, pharmaceutical and paper industries. Pectinase accounts for 10% of global industrial enzymes produced and their market is increasing day by day [2]. Bacteria produces enzyme to breakdown middle lamella in plants so that it can extract nutrients from the plant tissue and insist fungal hyphae. Addition of chelating agent or pre-treatment of plant material enhances the effect of enzymes. Hence pectinolytic enzymes or pectinase are a heterogeneous group of related enzymes that hydrolyse the pectic substances. Keeping in mind the importance of pectinase, the present investigation was undertaken and attempts were made to isolate and characterize Bacillus sp. isolated from vegetable waste soil and optimize pectinase production by using cassava waste as substrate.

MATERIALS AND METHODS
Isolation of organisms
Bacillus sp. was used for the production of pectinase enzyme isolated from vegetable waste soil samples which was identified by morphological and biochemical characterization. Based on this, the isolates were found to be Gram positive, motile, rod shaped Bacillus.

Screening of pectin producing bacteria
Screening was performed to detect the presence of bacteria that degrades pectin. The YEP medium was used for isolation of cultures supplemented with 2% agar. Pure culture was inoculated by puncture in the medium and incubated for 48hrs at 30°C. After incubation, iodine-potassium iodide solution was added to detect the clearance zone [3].

Pectinase assay
Substrate
Cassava waste (100g dry residue) was collected from local fruit market. The cassava waste was oven dried (80°C for 24 h), and the powder was stored at 4°C till use [4].

Pectinase production medium
A basic liquid medium showing the following composition (g/l): Cassava waste 0.3, Sucrose 10, KNO₃ 0.6, KH₂PO₄ 0.1, MgSO₄ 0.25, CaCl₂ 0.1, NaNO₃ 2, K₂HPO₄ 0.5, KCl 0.5 and yeast extract1 used for the production of pectinase [5]. Erlenmeyer flasks containing 100 ml of basal medium amended with 3% cassava waste were inoculated with one ml of overnight grown bacterial culture and incubated for 3 days at 37°C under agitation (125 rpm). The culture medium was centrifuged and the supernatant was used as crude enzyme source.

Quantitative assay for pectinolytic activity
The crude enzymes were taken for pectinase activity from all the isolates 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 makes up to 1 ml with distilled water. About 4.5 ml of the Lowry’s reagent was added to each test tube and it 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.

Effect of incubation time, temperature, pH
The bacterial isolates were subjected to different culture conditions to derive the optimum conditions for pectinase production. Pectinase production was estimated at regular time intervals (12, 24, 48, 72, 96 and 120 hr), at selected temperatures (25, 30, 35, 40, 45, 50 and 55°C) and pH (4, 5, 6, 7, 8 and 9). All the experiments were carried out in 500ml Erlenmeyer flask containing 100 ml of basal medium [6].

Effect of carbon and nitrogen Sources
Carbon sources viz., starch, fructose, lactose, glucose, maltose and nitrogen sources viz., tryptone, yeast extract, peptone among organic and urea, ammonium nitrate and ammonium chloride among inorganic were supplemented as individual components to the basal media to check their effect on pectinase production.
RESULTS AND DISCUSSION

Pectinase are among the most important enzymes and are great significance in present day biotechnology. Although they can be derived from several sources such plants, animals and microbes, the enzymes from microbial sources generally meets industrial demands. The present study was planned to study the pectinase producing microorganisms and to optimize the factors which effect pectinase production.

Isolation of pectinase producing bacteria from soil

The collected vegetable waste soil samples were used to identify pectinase producing microorganisms. The colonies showing clear zones upon flooding with 1% acetylmethyl ammonium bromide were confirmed as pectinase producers. Five isolates (PPB1, PPB2, PPB3, PPB4 and PPB5) showed clear zones and found as pectinase producers were subcultured and tested for pectinolytic activity.

Pectinase assay

The pectinase enzyme was produced using the isolated strains of Bacillus sp., in the sterilized pectinase medium. On calculating the obtained values of absorbance for each isolated enzymes, it was concluded that Bacillus sp. PPB5 showed maximum enzyme activity of 0.641 IU/ml. Enzymes extracted from Bacillus sp. PPB1, PPB2, PPB3 and PPB4, showed activities of 0.436, 0.321, 0.534 and 0.421 IU/ml respectively.

Factors affecting enzyme activity

Effect of incubation time

The pectinase production by different isolates of Bacillus sp. was found maximum at 96hr of incubation period (Fig.1). Further increase in incubation time marks the decrease of pectinolytic activity. Isolate PPB5 showed maximum enzyme activity after 96hr of inoculation. In submerged fermentation the pectinase production reached maximum at 96 hr of incubation. Further increase in incubation period did not show any significant increase in pectinase production rather it was decreased [6]. Enzyme production increase with increase in time duration up to 96 hr then it decreases [7]. These investigations are in line with the present study. But in some studies incubation period of 48 hr for optimal production for some fungal strains was reported [8]. While Fuijo and Eledago, reported a 72 hr incubation time for polygalacturonase production by Rhizopus oryza [9].

Fig.1. Enzyme activity of pectinase at different Incubation time

Effect of Temperature

Activity of enzymes extracted from the isolates was determined to check out the optimum range of temperature for pectinase enzyme. Pectinase activity was found maximum at 35°C (Fig. 2). Isolate PPB5 showed maximum enzyme activity. When temperature is altered below or above the optimum, the activity is decreased. The maximum production of pectinase enzyme was obtained at 37°C by the Bacillus subtilis [10]. Kumar et al. found pectinase production by Bacillus sp. MFW7 maximum at 35°C. Further increase in the temperature results in the decrease of pectinolytic activity [6]. Temperature of the fermentation medium was found to be optimum at 35°C. The maximum production of pectinase enzyme was obtained at this temperature by the Bacillus subtilis [10]. Maximal growth of Bacillus sp. DT7 as well as maximum pectinase production was observed at 37°C incubation [11]. The increasing of kinetic energy can lead to increasing of collisions between enzyme and substrates to form a complex of enzyme substrates (ES) and finally can increase the product. Production of enzyme was high at 28°C [12, 13].

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Effect of pH

Enzyme activity of different isolates was checked at different pH (4-9). Maximum activity of enzyme was observed at pH 6 for all the isolates. Thus this is considered as optimum pH for enzyme production (Fig. 3). Isolate PPB5 showed maximum activity at this pH. Pectinase production by Bacillus sp. by using orange peel as substrate at the concentration of 1% was found to be maximum at pH 6.5 [10]. Bacillus firmus isolated from soil and isolate FW2 which belong to Bacillus sp. produced pectinase maximum at pH 6 [5,7]. Isolated strains of Bacillus firmus provided optimum conditions for pectinase production at pH 7-8 and high pectinase production was observed at pH 6 by fungi Aspergillus terreus by using banana peel as substrate [12,14]. Sugarcane bagasse gives maximum pectinase yield during the fermentation period at pH 5 and Bacillus sp. produced significant amount of pectinase at pH 6.5 [15,16].

Effect of carbon sources

Supplementation of carbon sources in the form of carbohydrates resulted increase in pectinase production by Bacillus sp. (Fig. 4). Highest production was recorded when glucose was used as carbon source followed by lactose. Prakash et al. observed highest production of pectinase with lactose and glucose [17]. The synthesis of pectinase was greatly hidden when the bacterium was grown either on starch and production was found to be good when the bacterium was grown on glucose [6]. Jayani et al. reported citrus pectin and xylose as best carbon source for the pectinase production by Bacillus sphaericus [18].
Fig. 4. Enzyme activity of pectinase at different carbon sources

**Effect of nitrogen sources**
The influence of organic nitrogen sources such as tryptone, yeast extract, peptone and inorganic nitrogen sources such as urea, ammonium nitrate and ammonium chloride on amylase production was determined. Yeast extract and ammonium nitrate was found to be better among organic and inorganic nitrogen sources respectively (Fig. 5). The organic sources like peptone and inorganic sources like NH₄Cl were found to stimulate the pectinase production [6]. Prakash et al. also observed pectinase production in different organic sources and reported that peptone and yeast extract were better than other nitrogen sources [17]. Tryptone also served as better nitrogen source for pectinase production [10].

Fig. 5. Enzyme activity of pectinase at different organic and inorganic nitrogen sources

**CONCLUSIONS**
As there is need of bulk production of enzymes at a cost effective rate. In order to meet this goal, such strategies should be explored by which cost-efficient and ecofriendly method for bulk production can be achieved. Present study illustrated the usage of cassava wastes as a substrate for pectinase production and isolate PPB5 showed maximum enzyme activity, so this can be used for pectinase production on large scale.

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**REFERENCES**


