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Production of Pectinase by *Bacillus* Species Cultured on *Parkia biglobosa* Fruit Pulp

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In recent years, the cost of carbon source for microbial enzymes production has necessitated a drive towards cheaper and sustainable sources. In this study, *Parkia biglobosa* fruit pulp was utilized as substrate for pectinase production by *Bacillus* sp. The pectinolytic activity of the isolates were screened base on haloes of clearance on pectin agar, The two *Bacillus* species screened, *Bacillus lentus* and *Bacillus laterosporus* showed pectinolytic activity with *Bacillus lentus* having the highest activity of 80 mm and was therefore selected for the experiment. The effect of different ranges of temperature (35 °C–55 °C), pH (4–12), incubation period (24, 48, 72 and 96 hrs), inoculum size and substrate concentration on pectinase production was investigated while the pectinase activity of the selected *Bacillus* species was monitored. The optimum temperature for pectinase production was 45 °C with an activity of 97.61 mMol/L. Pectinase production by *Bacillus lentus* was optimum at pH 4 and a temperature of 45 °C. The optimization of the fermentation conditions enhanced the enzyme production to 827.42 mMol/L of the 3% substrate concentration. The enzyme production was enhanced after 3 days of incubation using *Parkia biglobosa* pulp as carbon source. The results of this study suggests that *Parkia biglobosa* fruit pulp can be harnessed at low concentration for large scale *Bacillus* pectinase production, and the pectinase produced by this *Bacillus lentus* could have novel characteristics suitable for industrial exploitation.

KEYWORDS: Pectinase, *Parkia biglobosa*, *Bacillus lentus*, Activity, Optimum.

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1. INTRODUCTION

Citrus fruits constitute an important group of fruit crops produced all over the world. The family of citrus fruits consists of Oranges, Kinnow, Khatta, Lime, Lemon (Galgal), Grapefruit, Malta, Mausami, Sweet orange etc. These all are known to contain appreciable amounts of pectin. Besides these, other fruits like Mango (*Mangifera indica*), Avocado Pear (*Avocado avocado*), Guava (*Psidium guajava*), Banana (*Musa sapientum*), Papaya (*Carica papaya*), Cashew Apple (*Anacardium occidentale*), Garden-egg (*Solanumnigrum linn.*), Star Apple (*Crysophylum albidium*), and Tomato (*Lycopersicum esculentum*) also contain substantial amounts of pectin having a high gelling grade.[1]

Pectinases hydrolyze pectins, the soluble complex polysaccharides that occur widely in plant cell walls. Pectinase accounts for 10% of global industrial enzymes produced and their market is increasing day by day. Pectinases are widely used in the food industry for the production and clarification of fruit juices, to improve the cloud stability of fruit and vegetable juices and nectars, for depectinization in order to produce high density fruit juice concentrates, and for haze removal from wines Pectic enzyme preparations are also used for the production of low methoxypectin for diabetic foods, in the degumming of natural fibers in the textile industry, and in making commercial softwoods, such as Sitka and Norway spruce, more permeable to preservatives.[1]

Pectinases can be produced by both submerged and solid state fermentation (SSF).[2] As a bulk enzyme that is not recovered after use, pectinase must be produced cheaply. Several pectin rich substrates have been previously used to produce microbial pectinases by fermentation.[3] Microbially derived Pectinase enzymes find more use due to their advantage over plant and animal derived pectinases. The reasons being cheap production, easier gene manipulations, and faster product recovery; also, microbial enzymes are usually free of harmful substances.[4] Pectinolytic enzymes are produced by many organisms like bacteria, fungi, yeasts, insects, nematodes, protozoan and plants. Many studies have been

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conducted on the production of pectinases from various microorganisms. The difficulties to obtain the appropriate substrate might be the biggest problem to develop such studies. A suitable substrate should be cheap and should be able provide all necessary nutrients to the microorganism.[5]

In Nigeria, several tree species serve as sources of wood, food, fodder and medicine to indigenous people. The trees also provide ecological services including soil fertility and microclimate amelioration. In addition to the direct domestic use of the tree products, they also serve as a source of cash for many poor people. *Parkia biglobosa* popularly known as the African locust bean tree belongs to the family *Fabaceae* it is a perennial deciduous tree with a height ranging from 7 to 20 m, although it can reach 30 m under exceptional conditions.

The trees of the *Parkia* species are carefully preserved by the inhabitants of the area where they grow, because they are valuable sources of food, especially the seeds, which serve as source of useful ingredient for consumption. It has been reported that the husks and pods are good food for livestock.[6]

The present study was carried out to evaluate the pectinase activity of pectinolytic bacteria using *parkia biglobosa* fruit pulp as a feed substrate in submerged state fermentation.

2. MATERIALS AND METHODS OF SAMPLE COLLECTION

Waste mango fruits were collected in clean polythene bags and were taken to the Microbiology Research Laboratory of Usmanu Danfodiyo University, Sokoto. 150 g of *Parkia biglobosa* fruit pulp was purchased from the Central Market in Sokoto, Nigeria, in clean polythene bags.

3. MICROBIOLOGICAL ANALYSIS, ISOLATION AND CHARCTERIZATION

One gram (1 g) of waste mango fruit was weighed and dissolved in 9 ml of sterile distilled water and mixed. An aliquot of 1 ml was taken and serially diluted. An aliquot of 0.1 ml from test tube was plated unto sterile molten nutrient agar plate and incubated at 37 °C for 24 hours. The colonies that developed after 24 hours of incubation were counted using the colony counter and subcultured until a pure culture was obtained. The pure cultures were then cultured on nutrient agar slants, incubated for 24 hours and refrigerated. The isolates were maintained on the slant until required.

3.1. Screening of Isolates for Efficient Pectinase Producers

The YEP (yeast extract pectin) medium was used for isolation of cultures supplemented with 2% agar. Pure culture was inoculated by puncture in the medium and incubated for 48 hrs at 30 °C. After incubation, iodine potassium iodide solution was added to detect the clearance zone.

3.2. Inoculum Preparation

McFarland standard (0.1) was prepared and the turbidity of the inoculums was adjusted to approximately 300 cells/ml.

3.3. Production of Pectinase Enzyme

This was carried out according to the DNS method of Miller.[7] The liquid medium containing 40 g of substrate (*Parkia biglobosa* pulp) was added to a mineral salt medium containing 10 g KH_2PO4, 50 g NaCl, 1 g $MgSO_4 \cdot 7H_2O$, 1 g $CaCl_3$. The culture was grown at 37 °C for 24 hours. Samples were taken from the culture broth at different times during growth in order to determine the pectinase production by measurement of absorbance at 540 nm.

4. PECTINASE ASSAY

Pectinase activity was assayed by the DNSA method of Alabi et al.[8] Briefly, 0.5 ml of cell free supernatant was incubated with 0.5 ml of pectin in 0.1 M acetate buffer with pH 6.0 and the reaction mixture was incubated at 40 °C for 10 minutes in static condition. After adding 1 ml of DNS reagent, the mixture was boiled for 5 min at 90 °C. The reaction was stopped by adding 1ml of Rochelle's salt. Then the mixture was diluted by adding 2 ml of de-ionized water. The absorbance was measured spectrophotometrically at 595 nm. A standard graph was generated using standard glucose solution.

4.1. Optimization of Pectinase Production

The bacterial isolate was subjected to different culture conditions to derive the optimum conditions for pectinase production.

4.2. Effect of Temperature

In order to determine the effect of temperature on pectinase activity, the selected bacteria isolate was grown in *Parkia biglobosa* pulp broth adjusted to pH 10 and incubated at 35 °C, 45 °C and 55 °C for 72 hours.

4.3. Effect of pH

The effect of pH on pectinase activity was obtained by adjusting the *Parkia biglobosa* pulp broth to pH 4, 6, 8, 10 and 12 before bacterial inoculation. After 72 hours of incubation at 25 °C, the culture broth was centrifuged and the supernatant was used as crude pectinase.

4.4. Effect of Fermentation Period

To evaluate the effect of fermentation period on pectinase activity, the selected *Bacillus* sp was grown at 25 °C in *Parkia biglobosa* pulp broth adjusted to pH 10 and incubated for 24, 48, 72 and 96 hours, the culture broth was

then centrifuged to obtain supernatant which was used as crude pectinase.

4.5. Effect of Substrate Concentration

To evaluate the effect of substrate concentration on pectinase activity, the selected bacterial isolate was grown at 25 °C in 1, 2, 3 and 4% *Parkia biglobosa* pulp broth adjusted to pH 10 and incubated for 72 hours. The culture broth was centrifuged to obtain supernatant.

4.6. Effect of Inoculum Size

To evaluate the effect of inoculum size on pectinase activity, different inoculum sizes (1, 2, 3 and 4% of the selected *Bacillus lentus* was grown at 25 °C in *Parkia biglobosa* pulp broth adjusted to pH 10 and incubated for 72 hours. Culture broths were centrifuged to obtain supernatant.

5. RESULTS AND DISCUSSION

The microorganisms isolated from spoilt mango include *Bacillus lentus, Bacillus laterosporus* and *Micrococcus varian. Bacillus lentus* had the highest pectinolytic activity of 80 mm after screening and was selected for the experiment (Table I). The pectinolytic activity of *B. lentus* in this study is somewhat greater than *Bacillus* species. This variation in pectinolytic activity produced by these species may be due to differences in their genetic make-up. Ibrahim et al.[9] observed similar result on *Bacillus* spp. screened for amylase production.

The effect of temperature on the activity of pectinase is shown in Figure 1. From the figure, the optimum temperature for pectinase production by *Bacillus lentus* was 45 °C, with a reducing concentration of 322.5 mMol/L. Further increase in temperature resulted in a decrease in pectinolytic activity. Pectinase activity was stable. Similar results were also obtained by Qureshi et al.,[3] who also observed 45 °C as the best temperature for Pectinase production. When temperature is altered below or above the optimum the activity is decreased or becomes denatured.

The pH of the cultivation medium is an important factor in the production of pectinases for it influences the sort and content of those enzymes produced by bacteria. Figure 2 shows the effect of pH on pectinase activity of *Bacillus lentus*. The optimum pH for pectinase production was observed at an acidic pH of 4 with a reducing sugar concentration of 976.61 mMol/L. A slightly near neutral pH

Table I. Result of pectinase activity of the identified *Bacillus* species.

Bacterial species	Diameter of Zones of Clearance (mm)
Bacillus lentus	80.0
Bacillus lentus	70.0
Bacillus laterosporus	65.0
Bacillus laterosporus	30.0
Micrococcus varian	00.0

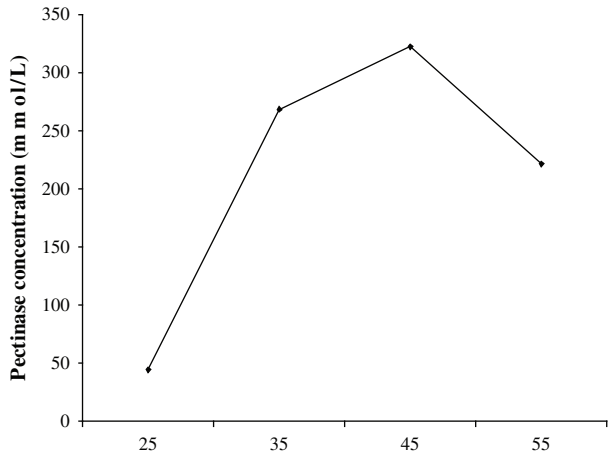

Fig. 1. Effect of temperature on pectinase production by *Bacillus lentus* isolated from waste mango fruit.

(4.8) has been reported by Vries et al.[10] and Tangaswamy et al.[11] This implies that pectinase holds commercial value for industries that carry out their operations at acidic pH. The least activity was displaced at pH 6 with an activity of 165.32 mMol/L. This result is in contrast to the findings of Kashyap et al.,[12] and Farooqui,[13] who recorded maximum activity at pH 8.0 and pH 6.5 respectively.

Figure 3 shows the effect of fermentation period on Pectinase production by *Bacillus lentus* at days 1, 2, 3 and 4. Pectinase activity increased progressively and attained a peak activity on the third day. Pectinase production reached maximum at day 3 with activity of 252.42 mMol/L. Further increase in incubation after 3 days did not show any significant increase in Pectinase production rather it decreased. Thus, optimum time of Pectinase synthesis was the third day after inoculation. The reduction in pectinase production after 3 days hours might be the result of change in pH during fermentation, denaturation or decomposition of enzyme due to interaction with other components of medium and depletion of nutrients in

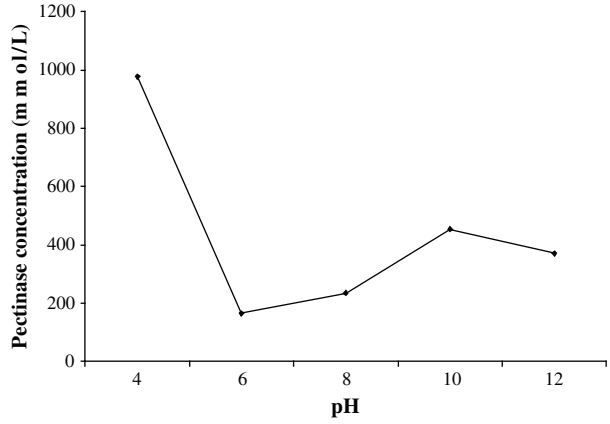

Fig. 2. Effect of pH on pectinase production by *Bacillus lentus* isolated from waste mango fruit.

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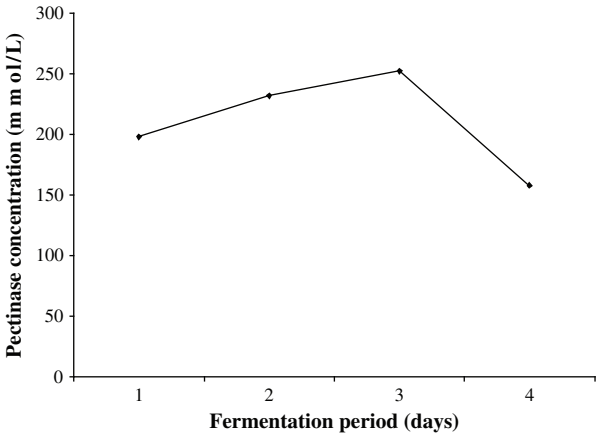

Fig. 3. Effect of fermentation period on pectinase production by *Bacillus lentus* isolated from waste mango fruit.

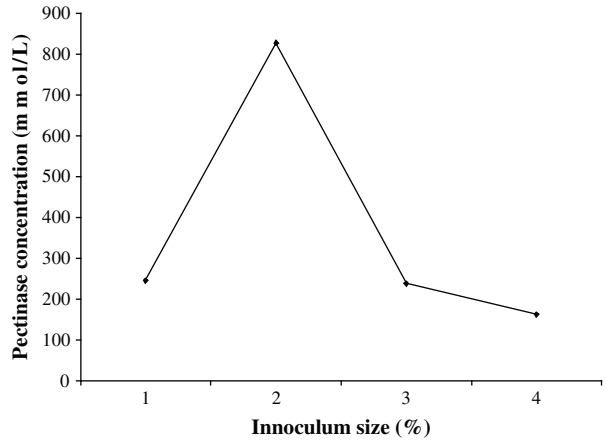

Fig. 5. Effect of innoculum size on pectinase production by *Bacillus lentus* isolated from waste mango fruit.

the medium.[3] The current result is the same with that of Thangaswamy et al.,[11] who reported peak activity at the first three days. Production of enzymes is usually initiated during the log phase of the growth and reaches maximum levels during the initial stationary phase.[14] Even though the extra pectinase enzymes are produced from log phase to initial stationary phase, within the phase the production may vary.

The effect of substrate concentration is shown in (Fig. 4). Pectinase activity was optimum at 3% substrate concentration with an activity of 829.03 mMol/L while substrate concentration of 4% gave the lowest Pectinase activity of 187.90 mMol/L (Fig. 4) with a reducing sugar value of 827.42 mMol/L.

Pectinase activity was found to be optimum at 2% inoculums size with activity of (827.42 mMol/L (Fig. 5). When the inoculums size was altered above or below 2%, the activity decreased. Decrease in pectinase activity with further increase in inoculum might be due to clumping of cell which could have reduced sugar and oxygen uptake rate, and also enzyme release and may be probably be due to limiting nutrients at higher inoculum size.[15]

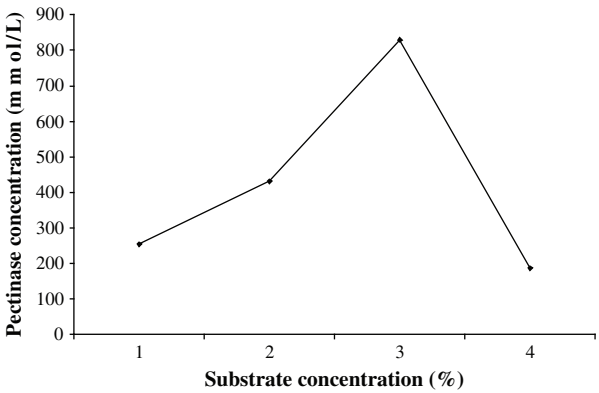

Fig. 4. Effect of substrate concentration on pectinase production by *Bacillus lentus* isolated from waste mango fruit.

6. CONCLUSION

The study shows that *Bacillus lentus* from waste mango fruit can be used to produce pectinase when cultured on *Parkia biglobosa* pulp as substrate and the optimum conditions for pectinase production are at a temperature 45 °C, pH 4, 2% inoculum size and 3% substrate concentration for 3 days incubation period. Thus, *Parkia biglobosa* pulp can be harnessed at low concentration for large scale *Bacillus* pectinase production.

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