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Pectinase Production by *Bacillus megaterium*, *Bacillus bataviensis*, and *Paenibacillus* sp. Isolated from Decomposing Wood Residues in the Lagos Lagoon

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ABSTRACT

Three wood decomposing bacteria isolated from the Lagos lagoon, *Bacillus megaterium*, *Bacillus bataviensis* and *Paenibacillus* sp. were screened for their pectinase producing abilities using pectin as substrate under submerged fermentation (SMF) conditions. The results showed that all three isolates produced appreciable pectinolytic activities. *Paenibacillus* sp. showed the highest pectinase activity when compared with the other two isolates. The optimum pH for pectinase activity for both *B. megaterium* and *B. bataviensis* was 8.0 while it was 6.5 for *Paenibacillus* sp., *B. bataviensis*, and *B. megaterium* showed optimum pectinase activity at 60°C and *Paenibacillus* sp. at 40°C. Metal ions such as Na^+ and K^+ improved the activity of pectinase produced by the three isolates when compared to the effect of Zn^{2+} and Mn^{2+}. The molecular weights of the enzymes were also estimated by gel filtration as 29,512 da, 32,359 da, and 25,119 da for *Paenibacillus* sp., *B. megaterium* and *B. bataviensis* respectively. The study has provided a platform for further investigation into the biochemical characterization of the enzyme, and optimization of culture conditions to scale up pectinase production for commercial exploitation.

Keywords: *Pectinase, Peanibacillus sp., Bacillus megaterium, Bacillus bataviensis*

INTRODUCTION

Pectinases constitute a unique group of enzymes which catalyze the degradation of pectin polymers present in plant cell walls. It is a high molecular weight polysaccharide, primarily made up of α (1,4) linked D-galacturonic acids and are found in the middle lamella and primary cell wall of higher plants [1]. Pectic substances are ubiquitous in the plant kingdom and their efficient utilization could enhance the economic competitiveness of bioconversion processes intended to compete with conventional industrial processes [2]. A significant interest in the degradation of pectic substances has been generated which is evident from a vast range of industrial applications such as degumming of bast fibers [2], treatment of alkaline pectic waste water and extraction, clarification and depectinization of fruit juices. Pectinases are one of the upcoming enzymes of commercial importance and about 10% of enzymes produced are pectinases.

Several microbes are capable of using pectin as carbon and energy sources by producing a vast array of enzymes in different environmental niches [3] and therefore have been exploited for the production of pectinases. This is because microorganisms are not influenced by climatic and seasonal factors and can be subjected to genetic and environmental manipulations to increase yield [4]. Although the major sources of acidic pectinases are fungi, alkaline pectinases are produced from alkalophilic bacteria mainly, Bacillus spp. Submerged fermentation (SMF) has been successfully used in pectinase production by bacteria [5]. It is a well-developed system used in industrial scale to produce a large variety of microbial metabolites. Submerged fermentation is preferred over solid-state fermentation because of the latter's restricted applications in processes using unicellular organisms, its low amenability to regulation and the ensuing frequently unsatisfactory reproducibility of the results [6]. In the present study, pecti-

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nase producing abilities of bacterial strains from decomposing wood were assessed and screened with a view to optimizing the process to enhance maximum production by these autochthonous bacteria.

MATERIALS AND METHODS

Source of microbial strains

Bacterial strains used in this study were isolated from decomposing sawdust dumped into the Lagos lagoon, Nigeria as described by [7] and identified by 16SrRNA gene amplification and sequencing.

Medium composition and culture conditions

Bacillus megaterium, *Bacillus bataviensis*, and *Paenibacillus* sp. isolated from decomposing wood in the Lagos Lagoon were grown at 30°C for 48 hours in 100 mL of yeast extract pectin (YEP) medium which contained pectin (0.5g), yeast extract (0.1 g), NH_4Cl (0.715 g), KCl (0.075 g), $Na_2HPO_4.12H_2O$ (0.45 g), KH_2PO_4 (0.63 g) and $MgSO_4$ (0.025 g). The pH of the medium was adjusted to 7.2 before sterilization at 121°C for 15 minutes. Cells were harvested by centrifugation at 5000 rpm with 115 (generation time of 25 minutes), 72 (generation time of 40 minutes) and 27 (generation time of 105 minutes) generations for *B. megaterium*, *B. bataviensis*, and *Paenibacillus* sp. respectively. The supernatant was thereafter assayed for pectinase activity.

Protein determination and enzyme assay

The protein content in eluted fractions was determined by the colorimetric method using bovine serum albumin as the standard [8]. Readings were carried out in a spectrophotometer at 660 nm. Polygalacturonase activity was determined by estimating the amount of reducing sugar released under assay conditions. This was achieved using arsenomolybdate method [9, 10]. The amount of galacturonic acid released per mL per minute was calculated from a standard curve. One unit of pectinase activity was defined as the amount of enzyme which liberated 1 µM galacturonic acid per minute.

Enzyme purification by gel filtration chromatography

The crude enzyme was purified to homogeneity by chilled ethanol (60%) precipitation and column chromatography. The partial purification of enzyme was carried out by adding chilled ethanol to crude enzyme and keeping it for overnight incubation. The precipitates thus obtained were spun at 15,000 rpm for 15 minutes at 40°C. The pellet was then dissolved in minimum volume of assay buffer (sodium-citrate or tris HCl) at pH 7.2. It was then centrifuged at 2,000 rpm for 20 minutes at 4°C to obtain a viscous sample. The precipitated enzyme was filtered through 0.22 µm filter and 2ml of the filtered precipitated enzyme was loaded on gel-permeation column and fractions collected. All eluted fractions were estimated for enzyme activity and absorbance at 280 nm. The specific activity of purified enzyme may also be compared to that of the crude enzyme.

Effect of temperature

The temperature of the Lagos Lagoon ranged from 25-30°C. The effect of temperature on the activity of the enzyme was carried out for each isolate by adding 0.25ml of 2% pectin in 0.1 mL acetate buffer to the crude enzymes and assaying at varying temperatures between 25°C and 80°C for the different isolates.

Effect of pH

The Lagos Lagoon has a pH ranging from 6.53-7.7. Thus, effect of pH optimum on pectinase activity was measured for each isolate at a fixed assay temperature at various pH values ranging from 3.0-9.5. In each case, the following pH buffer solutions were used following the assay method described by [11]; 0.1 M citrate buffer (pH 3.0-5.0), 0.1 M phosphate buffer (pH 6.0-8.0) and 0.1M borate buffer (pH 9.0). Pectin (0.5 mL, 2%) was added to each extract and 0.5 mL of a different buffer was added in each case and assayed for enzyme activity.

Effect of metal ions

The Lagos Lagoon is characterized by pollution with urban waste and untreated industrial effluents, hence the need to evaluate the effect of metal ions on pectinase produced by organism found there. The pectinase activity of each extract was determined with different metal ions using the chloride salt solutions of the following metals Na^+, K^+, Mn^+, and Zn^{2+}. Each metal ion (0.1 mL) was added into 0.25 mL of 2% pectin in 0.1 M acetate buffer, pH 6.0. The mixture was then incubated at 40°C for 10 minutes and assayed for enzyme activity.

Determination of molecular weight

The molecular weight of the enzyme was estimated by gel filtration and extrapolated from the standard curve of protein standards used (aldolase, egg albumin and bovine serum albumin).

RESULTS AND DISCUSSION

In this study, optimum temperatures between 40°C and 60°C were obtained for the three isolates with *Ba-*

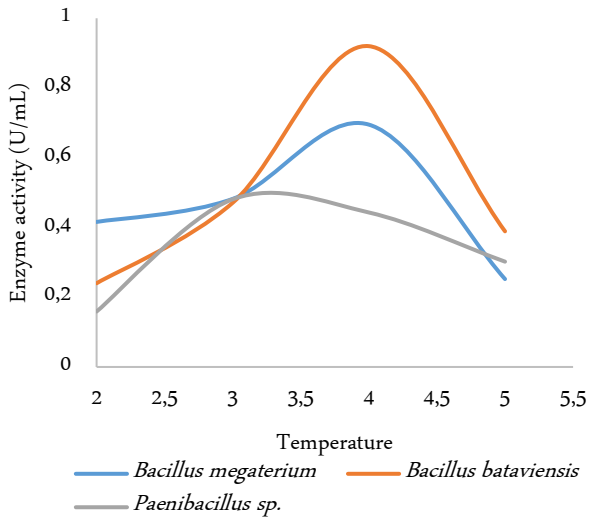

Figure 1. Effect of temperature on pectinase production

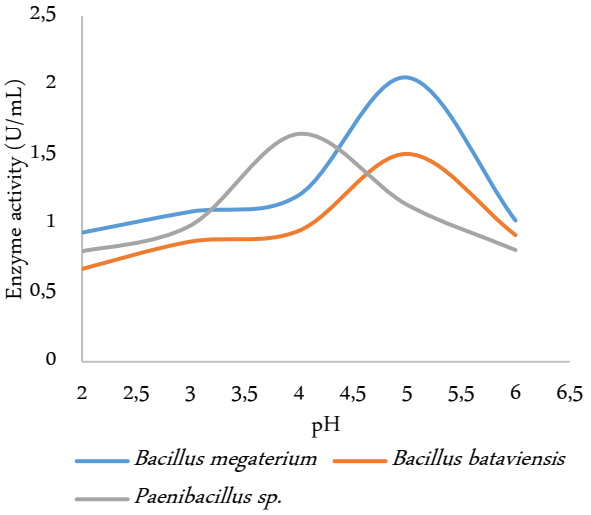

Figure 2. Effect of pH on pectinase production

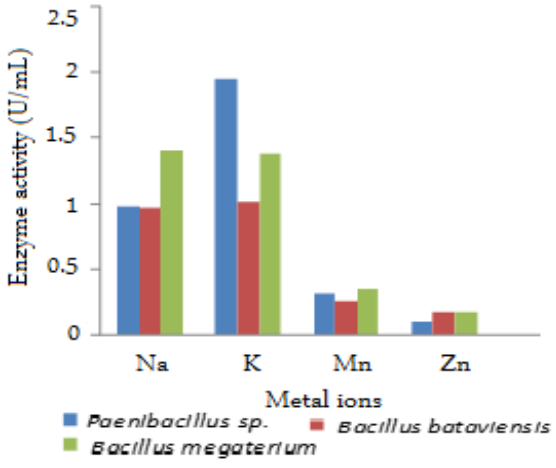

Figure 3. Effect of metal ions on pectinase production

cillus bataviensis and *Bacillus megaterium* showing highest activity at 60°C and *Paenibacillus* sp. at 40°C (Figure 1). Enzymes elaborated at thermophillic temperatures were more heat stable than those elaborated at mesophillic temperatures [12]. The optimum pH for pectinase activity was observed to be 8.0, 8.0 for both *B. megaterium* and *B. bataviensis* while it was 6.5 for *Paenibacillus* sp. (Figure 2). These results indicate that the pectinase enzymes in these bacterial strains are largely thermostable and alkaline in nature. These properties make the enzymes potentially suitable for various industrial processes that employ high temperatures and pH. Different optimum activities have been reported for different pectinases from different sources. *Bacillus subtilis* is known to degrade polygalacturonic acid with optimal activity at pH 10.0 and temperature of 65°C [13]. The metal ions play an important role on the activities and stability of enzymes. The Lagos Lagoon is characterized by the presence of heavy metals because of industrial effluents discharged into the water body. Although these metals are toxic on their own when ingested by humans, they do not affect the pectinase produced by these organisms and are therefore safe for industrial use. Pectinase activity was enhanced by cations including Na^+ and K^+ at their optimal concentrations beyond which they were inhibitory. The enzyme was however inhibited by Zn^{2+} and Mn^{2+} ions at all concentrations employed (Figure 3). This correlates with the finding from a research that Zn^{+2} inhibited the pectinase activity of *Aspergillus flavus* MTCC7589 at 1.0 mmol/L concentration and Torimiro and Okonji (2013) that K^+ enhanced the pectinase activity of *Bacillus cereus* [14]. However, this is contrary to pectinase enzyme from Bacillus cereus, *B. subtilis*, *and Bacillus stearothermophilus* in which Mn+2 and Zn+2 ions were found to enhance its activity [15]. This suggests that the requirement of metal ions for the pectinase activity vary depending upon their sources. The molecular weight of isolated pectinase enzyme from *B. bataviensis*, *B. megaterium* and *Paenibacillus* sp. corresponds to 32,357da, 25,119da and 29,512da. This is in agreement with pectinase from Bacillus cereus GC Subgroup A with a molecular weight of 38,304.27da [16].

CONCLUSION

This study made a successful attempt to determine if the three isolates from the Lagos lagoon were potential candidate organisms that will be industrially useful in producing pectinase enzymes. Although it has been reported that most *Bacillus* spp. are producers of pectinase [17], little or no work has been done on the tropical isolates used in this study that were obtained from decom-

posing wood residues in the polluted Lagos Lagoon. Hence, we have broadened our scope and expanded our options as all three isolates were confirmed to produce significant amounts of pectinase enzymes. This has provided a platform for further investigation into the biochemical characterization of the enzymes, as well as carrying out optimization studies for their commercial exploitation.

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