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Full Length Research Article

OPTIMIZATION OF BACTERIA PECTINOLYTIC ENZYME PRODUCTION USING BANANA PEEL AS SUBSTRATE UNDER SUBMERGED FERMENTATION

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ABSTRACT
The investigation was based on optimizing the cultural conditions of bacteria pectinolytic enzymes indigenous to soil of banana peel dump site. Optimization of cultural conditions was carried out by changing the physico-chemical environment such as pH, temperature, inocula size and substrate concentration of the production medium by testing for one factor at a time. Highest pectinase activity was observed at pH 6.0 which gave 1.455±0.095 U/mL and 1.695±0.01 U/mL for *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106 respectively. The optimized temperature for better yield was obtained at 37^{0}C with 0.955±0.006 U/mL for *Bacillus subtilis* TYg4-3 and 1.098±0.012 U/mL for *Bacillus amyloliquefaciens* SW106. Lactose yielded 1.655±0.046 U/mL and NH_4Cl 1.603±0.005 U/mL activity for *Bacillus subtilis* TYg4-3, while maltose yielded 1.343±0.062 U/mL and KNO_3 1.075±0.077 U/mL activity for *Bacillus amyloliquefaciens* SW106. *Bacillus subtilis* TYg4-3 produced best at 72 h of incubation giving 1.275±0.006 U/mL and at 36 h incubation for *Bacillus amyloliquefaciens* SW106 giving 1.345±0.033 U/mL activity. *Bacillus subtilis* TYg4-3 yielded 2.083±0.008 U/mL and 2.015±0.036 U/mL at 1.5% and 2.0% of inoculum size and substrate concentration respectively. *Bacillus amyloliquefaciens* SW106 produced best enzyme activity at 2.0% of inoculum and substrate concentration and gave 2.193±0.175 U/mL and 2.256±0.067 U/mL enzyme activities respectively.

Keywords: Banana, Pectinolytic, Submerged fermentation

INTRODUCTION

A large volume of waste is released from industries that make use of agricultural raw materials in their production processes. This leads to increase in clean-up problems from the environment, which in turn become breeding ground for pathogens, causing diseases such as cholera, malaria, and a host of other diseases. Martin *et al.* (2004) emphasized the increasing energy demand in the use of renewable agricultural and industrial wastes, as their disposal poses environmental problems. At present, through controlled biological degradation of food wastes by microbes, valuable compounds such as enzymes, citric acids and other raw materials for medical and industrial uses are obtained from these wastes (Magdy, 2011).

Pectinases are referred to as enzymes that attack pectin and depolymerize it by the process of hydrolysis and trans-elimination as well as by de-esterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin (Ceci and Loranzo, 2008). Pedrolli *et al.,* (2009), also defines pectinases as a large class of enzymes that split pectic polysaccharides of plant tissues into simpler molecules like galacturonic acids. Of the entire production of industrial enzymes, pectinase production accounts for 10%, with its application in juice and wine production (Semenova *et al*., 2006). For a long period of time, microorganisms have been used for pectinases production (Janani *et al*., 2011; Reddy and Sreeramulu, 2012). As reported by Jayani *et al.* (2005), microbial pectinases account for 25 % of the global food enzyme sales. Many microorganisms such as bacteria, yeasts and moulds have been found to be capable of producing pectinases (Saranjaj and Naidu, 2014).Several conventional industrial processes over the years have used pectinases for plant fiber processing, treatment of wastewater from industries containing pectinaceous material, textile, tea and coffee industries, oil extraction, purification of viruses and paper manufacturing (Jayani *et al.,* 2005). The two main fermentation methods used in pectinase production are the solid state fermentation (SSF) (Soccol and Larroche, 2008) and the submerged fermentation (SmF) (Rangarajan *et al*., 2010).

Many filamentous fungi like *Aspergillus niger, Aspergillus. Awamori, Mucor piriformis, Penicillium restrictum, Trichoderma viride,* and *Yarrowia lipolytica* have been used in both submerged as well as solid state fermentation for production of various industrially important products such as citric acid, ethanol etc. Fungi like *Aspergillus niger*, *Penicillium expansum, Aspergillus oryzae*, which have a GRAS status (generally regarded as safe) by United States Food and Drugs Administration (USFDA) are employed in food industry.

Acidic pectic enzymes used in fruit juice industries and wine making often come from fungal sources, especially from *Aspergillus niger* (Sunil *et al*., 2015). It has been shown that, high number of negative charges and association with ions is enhanced when pectins are demethylated. Thus, it can behave as a weak cation exchange resin and depending on the pH conditions, chelate toxic ions or make available minerals in the gut (Khan *et al.,* 2013). The splitting of the methyl group from pectins by pectinases to short chain fatty acids such as acetate, propionate and butyrate are more easily fermented by intestinal bacteria. Apart from the protection it offers the bowel against inflammatory diseases, it also modulates the release of gut hormones that control insulin release and appetite (Tolhurst *et al.,* 2012). The primary short chain fatty acid to reach the systematic circulation from the liver is acetate and thus, pectinases added to pectins find application in different food products for probiotic purpose (Khan *et al.,* 2013).

MATERIALS AND METHODS

Sample collection

Soil sample from banana waste dump site was collected at a depth of 8cm deep at Ipata Market, Ilorin, Kwara State into a sterile polythene bag and transported to the laboratory for analysis. Serial dilutions were prepared up to 10^{-6}. Diluention plates were prepared in triplicate, overnight growth culture plates were sub-cultured subsequently to obtain pure culture. Pure isolates were stored at 4^{o}C in the refrigerator for further studies.

Plate assay/screening

Pectinase screening agar medium (PSAM) was screened using Khairnar *et al.* (2009) method. A zero point five (0.5mL) inoculum of overnight pure culture was inoculated into PSAM and incubated for 7days at 37^{o}C. After 7days incubation, the plates were flooded with iodine solution and incubated at room temperature for 15minutes. Then, a clearance zone was measured and the plate with maximum diameter was taken as best producer and used for further studies.

Determinative Fermentation screening

All the isolates were screened for production and absorbance taken at 570nm. Results were compared with plate assay screening and isolates with the highest spectrophotometer reading was used for optimization of pectinolytic enzyme.

Identification of selected isolate

The selected bacterial isolate that showed the highest pullulanase activity was identified based on the morphological and biochemical parameters as described in the Bergey's Manual of Determinative Bacteriology (Bergy, 2000). Molecular identification based on 16sRNA was carried out on the selected isolate to confirm their identity.

Molecular Identification Using the 16s rRNA PCR Techniques

Molecular identification based on 16srRNA was also done on the selected bacterial isolates (PPB_E and PPB_F). The procedure for molecular identification included extraction of genomic DNA, PCR amplification and purification, nucleotide sequencing and blasting ((Pitcher *et al.,* 1989). Samples were taken to the Bioscience centre of the International Institute of Tropical Agriculture (IITA), Nigeria.

Substrate (Banana Peel) Preparation and Proximate Analysis

Waste substrate (banana peel) collected from Ipata market were peeled and dried in the hot air oven at 80^{0}C for 48h; the peels were ground and sieved to obtain fine particles in powdered form. Proximate analysis was carried out on the substrate to determine its percentage crude protein, crude lipid, moisture content (MC), total ash, crude fibre and carbohydrate.

Inoculum Preparation

A loopful of overnight culture was inoculated into prepared peptone water and incubated at 37^{0}C for 24 h.

Pectinase assay

Enzyme assay was carried out according to the method described by Kumar *et al*. (2012). Commercial pectin was used as substrate and pectinase activity was measured by estimation of glucose by DNSA method. Standard graph prepared by concentration of standard glucose solution. Unit of enzyme activity was defined as the amount of enzyme that releases 1µmol of glucose per minute according to standard curve. One millilitre of crude enzyme was added to 3mL sodium acetate buffer (pH 5.0) containing 1% pectin and then incubated at 37^{0}C for 1hour. 0.5mL DNSA reagent was then added to each tube and each tube was heated in boiling water bath for 10minutes. It was then allowed to cool to room temperature and absorbance taken at 570nm. The absorbance was taken over 8 minutes and the pectinase activity in unit was calculated as:

$$\text{Pectinase activity (U/mL)} = \frac{\text{change in absorbance at 570nm/time}}{0.01 \times \text{V}}$$

Where V= final reaction volume

Enzyme Production under Optimized Conditions

Production medium contained in 100mL of distilled water: banana waste- 3%; sucrose- 1g; KNO_3- 0.06g; KH_2PO_4- 0.02g; K_2HPO_4- 0.05g; KCl- 0.05g; $MgSO_4$- 0.01g; $MnSO_4$- 0.001g (was adjusted to pH 6.0 using 1M H_2SO_4). The medium was sterilized at 121^{o}C for 15minutes. One millilitre of prepared inoculum was then inoculated and incubated for 3days under agitation. The fermentation medium was then decanted with whatmann filter paper and the filtrate centrifuged at 7000rpm for 15minutes. Absorbance was read at 570nm. The filtrate was used as crude enzyme source for further studies (Kumar *et al*., 2012).

Effect of incubation time on pectinase production

Fermentation medium was inoculated with 1mL inoculum anset for 5 days. The effect of incubation time on enzyme production was carried out for a period of 5 days at 24, 36, 48, 72, 96 and 120 h. Enzyme activity was determined by DNSA method (Kumar *et al*., 2012).

Effect of temperature on pectinase production

This was carried out to determine the best temperature at which the organism can produce better activity. Temperature was studied at 25^{0}C, 37^{0}C, 45^{0}C, 55^{0}C and 65^{0}C. Enzyme activities were determined by DNSA method (Kumar *et al*., 2012).

Effect of pH on pectinase production

The effect of pH on enzyme production was studied at seven different pH ranges 3.5, 4.5, 5.5, 6.0, 7.5, 8.5. Solutions of 1M H_2SO_4 and 1M NaOH were used to adjust the pH (with HANNA HI 83141 pH-meter). The fermentation medium was incubated at 37^{0}C for 72 h. Enzyme activities were determined by DNSA method (Kumar *et al*., 2012).

Effect of inoculums size on pectinase production

Fermentation medium was inoculated with different concentrations of inoculum (1%, 1.5%, 2%, 2.5%, 3% and 3.5%). All the experiments were carried out in 500mL Erlenmeyer flask containing 100 mL of basal medium. After fermentation, the basal medium was analyzed for enzyme activity using the DNSA method (Kumar *et al*., 2012).

Effect of different carbon sources on pectinase production

Different carbon sources; glucose, sucrose, maltose, lactose, starch at 0.5%w/v were used to determine the best source for enzyme production. The pH of fermentation medium was adjusted to pH 6.0. After fermentation, enzyme activities were then determined for each carbon source (Kumar *et al*., 2012).

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Effect of different nitrogen sources on pectinase production

The selected bacterial isolates were given different nitrogen sources such as yeast extract, urea, KNO_3, NH_4Cl and $NaNO_3$ at concentration of 0.5%w/v in the fermentation medium. The pH was set at 6.0 and to allow for fermentation. Enzyme activities were then determined for each nitrogen source using the DNSA method (Kumar *et al*., 2012).

Statistical Analyses

The data were analyzed on the average of three replicates obtained from independent determinations. Statistical analyses of these averages was analysed using one way analysis of variance (ANOVA) and was carried out with IBM SPSS version 20 software at 95% significance level and Microsoft Excel 2007 version. Charts were drawn for all values of analysis using the Microsoft Office Excel 2007 version.

RESULTS

Isolation and Screening of Bacterial Isolates

Nine bacteria were isolated from soil of banana waste dump site. They include: *Corynebacterium* sp., *Streptomyces* sp., *Micrococcus leutus*, *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus cereus, Lactobacillus bulgaricus* and *Klebsiella pneumoniae*. The cultural, morphological and biochemical characteristics of the isolates are presented in Table 1. Plate 1 shows clearance zone (hydrolysis) of selected bacterial isolate on pectinase screening agar.

Table 2 presents the zone of hydrolysis of the different isolates while, Table 3 presents the fermentation pattern for pectinase production for all bacterial isolates with agitation. The molecular characterization of selected bacteria isolates is presented in Table 4. The proximate composition of substrate used and enzyme activity of pectinase by selected isolates from glucose graph is presented in Tables 5 and 6 respectively.

Table 1: The Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates from Soil sample of banana peel dump site.

ISOLATES	Isolate A	Isolate B	Isolate C	Isolate D	Isolate E	Isolate F	Isolate G	Isolate H	Isolate I	Control
Colonial characteristics	Circular, raised, smooth, cream, irregular	Yellowish, raised, irregular edge, shiny	Yellow colony, flat, shiny spread	Dull, raised, entire, smooth, spread	Cream, hard, dull, flat, irregular edge	Creamy White, dull, irregular edge	White, raised, round	Flat, spread, regular, crenated edge	Creamy white, flat	-
Morphological characteristics	Rod chain	Cluster chain	Cocci and cluster chain	Cocci	Rod chain	Rod chain	Rod chain	Rod chain	Cocci chain	-
Gram stain reaction	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-
Catalase	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-
Urease	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-
Citrate	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-
Oxidase	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-
Glucose	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-
Lactose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-
Maltose	+ ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-
Triple sugar iron	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-
Motility	+ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-
Tentative identity	*Corynebacterium* sp.	*Streptomyces* sp	*Micrococcu leutus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Bacillus amyloliquefaciens*	*Bacillus cereus*	*Lactobacillus bulgaricus*	*Klebsiella pneumoniae*	-

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Plate 1: clearance zone (hydrolysis) of different bacterial isolate on pectinase screening agar medium supplemented with 0.2% commercial pectin and flooded with iodine solution.

Table 2: size of zones in mm by pectinase producing isolates in well plate assay

Isolate Name	Diameter of zone of Clearance (mm)	Colony diameter (mm)	Clearance zone (mm)
Corynebacterium sp.	12	2	10
Streptomyces sp.	18	13	05
Micrococcu leutus	-	-	-
Pseudomonas aeruginosa	21	16	05
Bacillus subtilis	36	11	25
Bacillus amyloliquefaciens	32	14	18
Bacillus cereus	28	14	14
Lactobacillus bulgaricus	15	08	07
Klebsiella pneumoniae	-	-	-

Table 3: Fermentation pattern for pectinase production for all bacterial isolates with agitation

Isolate Name	Enzyme activity (U/mL)
Corynebacterium sp.	1.374 ± 0.002^{f}
Streptomyces sp.	1.07 ± 0.002^{e}
Micrococcus leutus	0.559 ± 0.005^{a}
Pseudomonas aeruginosa	0.902 ± 0.004^{c}
Bacillus subtilis	1.466 ± 0.011^{g}
Bacillus amyloliquefaciens	1.845 ± 0.004^{h}
Bacillus cereus	1.356 ± 0.003^{f}
Lactobacillus bulgaricus	0.978 ± 0.033^{d}
Klebsiella pneumonia	0.659 ± 0.001^{b}

Values presented are means and standard deviation of duplicates.

Molecular identification of selected bacterial isolates from soil sample of banana peel dump site

The result of molecular identification on selected isolates revealed the strain level of the isolates to be *Bacillus subtilis* strainTYg4-3 and *Bacillus amyloliquefaciens* strain SW106 using 16srRNA sequence.

Table 4: Molecular identification of selected Bacteria isolates

Isolates	Isolate Gene bank Homolog	Number of Bases	Identity (%)	Accession Number
PPB_E	*Bacillus subtilis* strain TYg4-3	479	99	HQ333017.1
PPB_F	*Bacillus amyloliquefaciens* strain SW106	539	99	JX203229.2

KEY: PPB_E- Pectinase producing bacteria $_E$; PPB_F- Pectinase producing bacteria $_F$

Table 5: Proximate composition of banana peel

ANALYSIS	VALUES OF BANANA PEEL (%)
Crude Protein	7.328 ± 0.155^{a}
Crude lipid	11.375 ± 0.177^{c}
Moisture content	10.062 ± 0.078^{b}
Total ash	16.418 ± 0.116^{d}
Crude fibre	21.276 ± 0.723^{e}
Carbohydrate	33.541 ± 0.198^{f}

Values presented are means and standard deviation of duplicates.

Effect of Optimization Conditions of Enzyme Production

Effect of incubation time on enzyme production

Bacillus subtilis TYg4-3 was found to produce the highest enzyme activity at 72 h of fermentation with 1.275±0.006U/mL while *Bacillus amyloliquefaciens* SW106 produced highest at 48 h of fermentation yielding 1.345±0.033U/mL enzyme activity. This is shown in Figure 1.

Effect of pH on enzyme production

The effect of different pH on selected isolates is shown in the Figure 2. *Bacillus subtilis* TYg4-3 produced enzyme maximally at pH 6.0 yielding 1.455±0.095U/mL and *Bacillus amyloliquefaciens* SW106 produced enzyme maximally at pH 6.0 yielding 1.695±0.01U/mL enzyme activity.

Effect of temperature on enzyme production

The effect of temperature on enzyme production is presented in

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Figure 3 Pectinase activity of different bacteria showed that *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106 produced the highest pectinase activity at 37^{0}C yielding 0.955±0.006U/mL and 1.098±0.012U/mL enzyme activity respectively.

Effects of carbon sources on enzyme production

In Figure 4, lactose was found to be the best source of carbon for the isolates in the production of pectinase using banana peel as substrate. Lactose yielded 1.655±0.046U/mL enzyme activity when incubated at 37^{0}C for *Bacillus subtilis* TYg4-3while maltose yielded 1.343±0.062U/mL enzyme activity for *Bacillus amyloliquefaciens* SW106.

The effects of different nitrogen sources on pectinase production are shown in Figure 5.

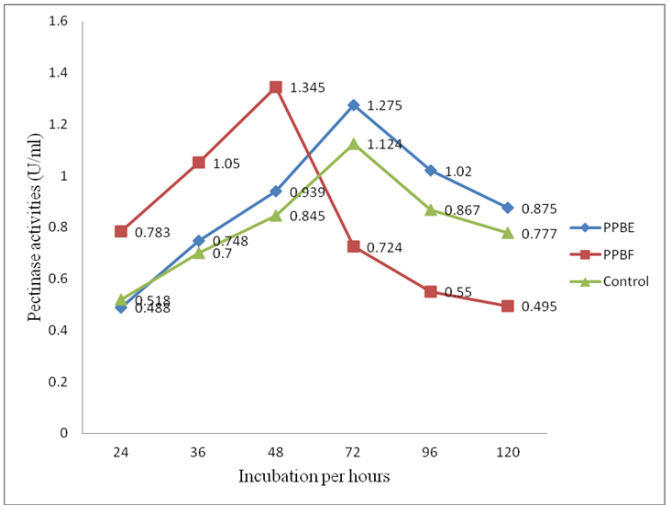

Figure 1: The pectinase activity of bacterial isolates incubated at different time interval (h) incubated at 37^{0}C.
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

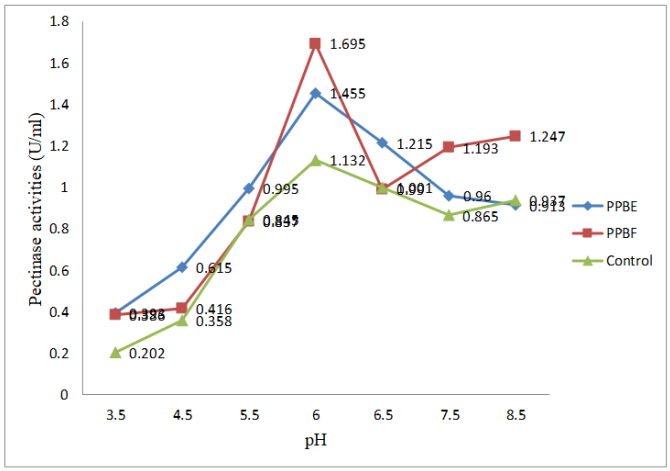

Figure 2: Pectinase activities of bacterial isolates with corresponding pH values incubated at 37^{0}C
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

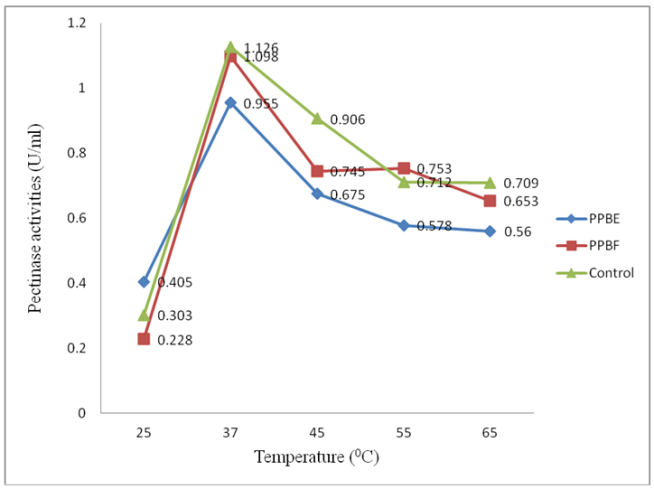

Figure 3: Pectinase activity of bacterial isolates at different temperatures.
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

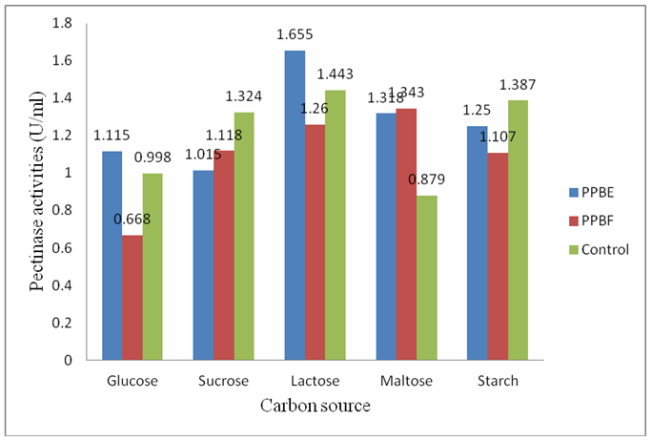

Figure 4: Pectinase activity of different bacterial isolates to different carbon sources incubated at 37^{0}C
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

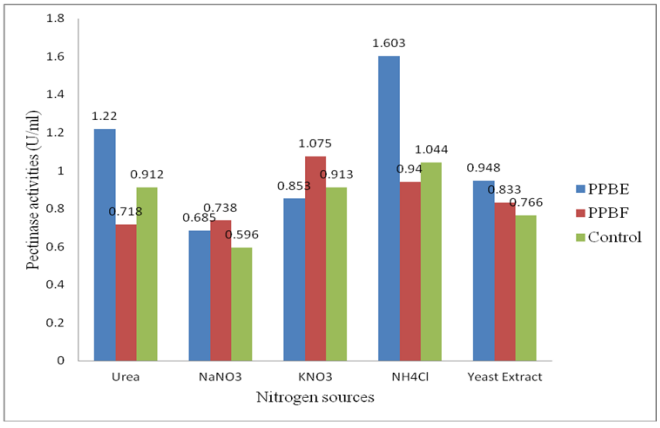

Figure 5: Pectinase activity of bacterial isolates to different nitrogen sources incubated at 37^{0}C
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

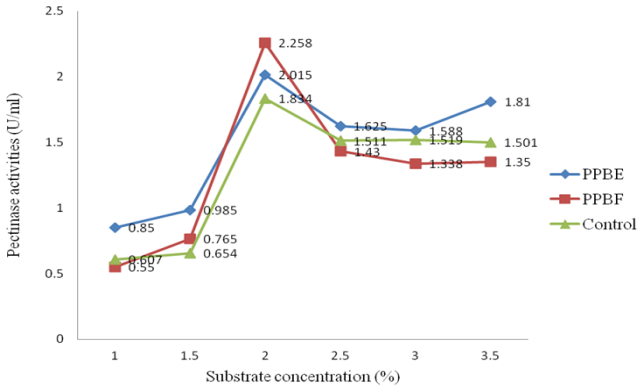

Figure 6: The pectinase activity of bacterial isolates to different substrate concentrations incubated at 37^{0}C
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

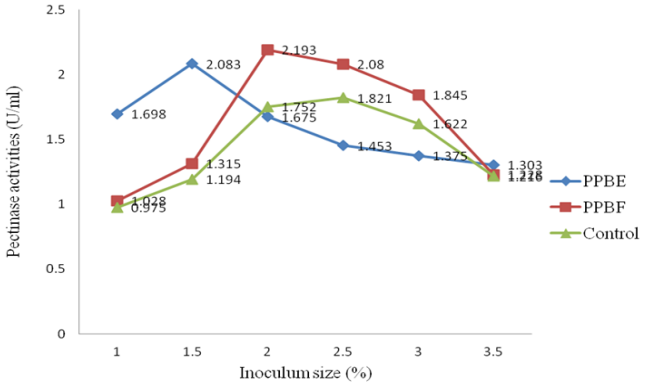

Figure 7: The pectinase activities of bacterial isolates with respective to inoculums size incubated at 37^{0}C
Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

Table 6: Enzyme Activity of Pectinase by selected isolates from glucose graph

PPB$_E$ using pectin (U/mL)	PPB$_E$ using banana peel (U/mL)	PPB$_F$ using pectin (U/mL)	PPB$_F$ using banana peel (U/mL)
1.590	1.270	1.535	1.518

Key: PPB$_E$- *Bacillus subtilis* TYg4-3
PPB$_F$- *Bacillus amyloliquefaciens* SW106

DISCUSSION

In this study, 9 bacteria were isolated from the soil of banana peel dump site and these include: *Corynebacterium* sp., *Streptomyces* sp., *Micrococcus leutus*, *Pseudomonas aeruginosa, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus cereus, Lactobacillus bulgaricus* and *Klebsiella pneumoniae*. These organisms were optimized for improved production and yield of pectinase enzyme. Many scientists have preferred the same location for isolation of pectinase producing microbes. Venkata *et al.* (2013) isolated bacteria from dump yards of Bangalore market. They isolated 3 bacterial species *Bacillus licheniformis, Bacillus cereus,* and *Staphylococcus aureus.* From the study of Kashyap *et al.* (2000), they isolated soil bacteria, *Bacillus subtilis* DT7 which has been found to produce significant amounts of an extracellular pectinase which was subsequently characterized as pectin lyase. Mukesh *et al.* (2012) produced pectinase from *Bacillus subtilis* MFW7 but used cassava waste for production. Patil *et al.* (2012) also isolated pectinolytic bacteria from carrot waste.

The substrate analysis presented in Table 5 showed that the substrate has a very high carbohydrate content of 33.541±0.198 in percentage, this was followed by crude fibre with percentage composition of 21.276±0.723. Other constituents found were low, with the least got from crude protein with percentage composition of 7.328±0.155. These analyses corroborate with the work of Sahoo *et al.* (2014) they reported higher carbohydrate and fibre content. Also, according to Lonsane *et al.* (1985), lower moisture content was found to cause reduction of nutrients solubility of the substrate, low degree of swelling and high water tension.

Laboratory scale optimization of different conditions for enhanced enzyme production was carried out by classical one factor at a time approach (OFAT).

The effect of incubation time on fermentation on pectinase activity with banana peel as substrate as depicted in Figure 3. For *Bacillus subtilis* TYg4-3, the enzyme activity rose from 24 h of fermentation yielding 0.488±0.007U/mL; 36 h it yielded 0.748±0.004U/mL; 48 h the yield was 0.939±0.062; and at72 h the yield 1.275±0.006U/mL; then fell at 96 h the yield was 1.02±0.002U/mL and at 120 h it was 0.875±0.075U/mL. For the *Bacillus amyloliquefaciens* SW106, at 24, 36, 48, 72, 96 and 120 h of incubation gave 0.783±0.012, 1.05±0.055, 1.345±0.033, 0.724±0.001, 0.55±0.05 and 0.495±0.01U/mL respectively. The highest enzyme activity was obtained with 72 h of fermentation and yielded 1.275±0.006U/mL for *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106 had the highest production at 48 h of fermentation yielding 1.345±0.033 U/mL. Afterwards incubation beyond 72 h resulted in a decreased enzyme activity, which might be due to depletion of nutrients available causing a stressed microbial physiology, eventually resulting in an inactivation of enzyme (Flores *et al.,* 1997). The effect of incubation on enzyme production was significantly different at $p< 0.05$. Maximum yield of pectinase (40 U/mL) was obtained after 72 h of incubation from *Bacillus* cereus isolated from soil by Nithisha *et al.,* (2016).

In Figure 2, it depicted the effects of pH on the production of pectinase enzyme. Enzyme activities of different isolates were tested at pH range from acidic to alkaline (3.5 – 8.5). It was observed that *Bacillus subtilis* TYg4-3 had the maximum enzyme activity at pH 6.0 with yield of 1.455±0.095 U/mL and *Bacillus amyloliquefaciens* SW106 had 1.695±0.01 U/mL at pH 6.0. Effects of pH on enzyme peoduction are statistically different at $p< 0.05$. This suggests that the pH of the optimizing condition influenced the growth and activity of microorganism and hence influenced enzyme activity. The pH at which enzymes were produced best differs and specific with microorganisms. This concur with the work of Kaur *et al.* (2016) they observed highest pectinase activity of 1.6 U/mL from *Bacillus cereus* at pH 6.0 and Laha *et al.,* (2014), they recorded highest enzyme activity at acidic pH 5.0. 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 (Roosdiana *et al.,* 2013; Helen *et al.,* 2013). From this study, it is noted that *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106 were capable of producing polygalacturonase better at pH range of 5.5 to 8.5 and optimum pH of 6.0 using banana peel as substrate. The highest pectinase

activity (1.6 IU/mL) was got at pH 6.0 from *Bacillus* species. Isolated from vegetable waste soil (Kaur *et al.,* 2016).

The effect of temperature on the production of this enzyme was also studied and the maximum pectinase titres were produced at 45^0C and 65^0C respectively for *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106. At 37^0C incubation, *Bacillus subtilis* TYg4-3 yielded 0.955±0.006 U/mL of enzyme while *Bacillus amyloliquefaciens* SW106 gave 1.098±0.012 U/mL at 37^0C (Figure 3). Values obtained are statistically different at $p< 0.05$. The incubation temperature was regarded as one of the most important factors affecting enzyme production, yield and stability. Maximum activity of enzymes at optimum incubation temperature might be due to the faster metabolic activities and increase in protein contents and extracellular enzyme production in the culture supernatant. At very low or reduced temperatures, membranes tends to solidify and high or increased temperatures damage microorganisms by denaturing enzymes, transport carriers and other proteins thus lowering enzyme activity indicating the mesophilic nature of the microorganism (Willey *et al.,* 2008). This observation is supported by the work of Kumar *et al.* (2012) that studies the production and optimization of pectinase from *Bacillus* sp. MFW7 and high production of pectinase (1.6I U/mL) was observed at temperature 35^0C. Also, Nithisha *et al.* (2016) studied the optimization of pectinase from *Bacillus* sp. and reported maximum enzyme activity of 40 U/mL at optimum temperature 35^0C.

Carbon sources have been regarded as one major factor affecting enzyme yield. Different carbon sources were used in order to determine the carbon source most suitable for enzyme production using banana peel as substrate. Supplementing carbon sources in the form of carbohydrates led to increase in enzyme production. Carbon sources used in this study included starch, maltose, lactose, sucrose and glucose. These are supplemented at 0.5%w/v into the fermentation medium and allowed for incubation for 72 h in a shaker incubator regulated at 37^0C. From the present study, it has been observed that lactose was a better source of carbon yielding 1.655±0.046 U/mL for *Bacillus subtilis* TYg4-3 followed by maltose, starch, glucose and sucrose yielding 1.318±0.071, 1.25±0.05, 1.115±0.035 and 1.015±0.015 U/mL respectively. For *Bacillus amyloliquefaciens* SW106, it has also been pointed out that maltose is a good carbon source for the organism using banana peel as substrate yielding 1.343±0.062 U/mL enzyme activity (Figure 4). The effects of carbon sources are statistically different at $p< 0.05$. Prakash *et al.* (2014) in their study reported highest pectinase production with lactose and glucose. Kumar *et al.,* (2012) asserted that 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 (Kumar *et al.,* 2012). Jayani *et al*. (2010) reported citrus pectin and xylose as best carbon source for the pectinase production by *Bacillus sphaericus.*

Different nitrogen source was supplemented into the culture medium at 0.5%w/v. These nitrogen sources include ammonium chloride (NH_4Cl), urea, sodium nitrate ($NaNO_3$), potassium nitrate (KNO_3) and yeast extract. Enzyme activity was conducted and assayed on individual nitrogen source. For the *Bacillus subtilis* TYg4-3, ammonium chloride and urea were found to give highest nitrogen source yielding 1.603±0.005 U/mL and 1.22±0.031 U/mL respectively. The least activity was observed for $NaNO_3$ giving 0.685±0.005 U/mL pectinase activity. For the *Bacillus amyloliquefaciens* SW106, KNO_3 and NH_4Cl was a good source of nitrogen producing 1.075±0.077 U/mL and 0.94±0.075 U/mL respectively. Sodium nitrate gave the smallest activity at 0.738±0.012 U/mL. The inorganic source like NH4Cl was found to stimulate the pectinase production (Kumar *et al.,* 2012). Prakash *et al*. (2014) also reported pectinase production in different organic sources and reported that peptone and yeast extract were better than other nitrogen sources. The effects of nitrogen sources are statistically different at $p< 0.05$.

The effect of concentrations of substrate used (banana peel) were also studied for their enzyme activity. This was done to establish the concentration at which substrate used produced the highest activity. Different concentrations of substrate from 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5% were supplemented into the fermentation medium and observed for their activities. For *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106, it was observed that the highest concentration of substrate were found at 2.0%w/v giving 2.015±0.036 U/mL and 2.256±0.067 U/mL respectively, and least activity was found at 1.0%w/v of substrate concentration for both microorganisms giving 0.85±0.031 U/mL and 0.55±0.001 U/mL respectively (as shown in Figure 6).

The data presented in Figure 7 show the effect of concentration of inoculums size on enzyme activity. The fermentation medium was supplemented with inoculums of different sizes 1.0%, 1.5%, 2.0%, 2.5%, 3.0% and 3.5% respectively. It was observed that the pectinase from Bacillus subtilis TYg4-3 has the highest activity of 2.083±0.008 U/mL at 1.5% w/v inoculum size and the least activity of 1.303±0.097 U/mL at 3.5% w/v inoculum size while pectinase from *Bacillus amyloliquefaciens* SW106 has the highest activity of 2.193U/mL at 2.0% w/v inoculum size. Thus, increase in the size of inoculum ultimately led to a decrease in the activity of the enzyme.

For *Bacillus subtilis* TYg4-3, the maximum productivity of pectinase achieved by optimized conditions such as temperature of 45^0C, pH 6, 72 h incubation time, 1.5% inoculum size, 2.0% substrate concentration, nitrogen source (NH_4Cl), lactose as carbon source was 2.011 U/mL while the highest productivity achieved for *Bacillus amyloliquefaciens* SW106 by optimized conditions such as temperature of 65^oC, pH 6, 48h incubation time, 1.5% inoculum size, 2.0% substrate concentration, nitrogen source (KNO_3), Maltose as carbon source was 2.076 U/mL.

Conclusion

From the above investigation, it can be deduced that, *Bacillus subtilis* TYg4-3 and *Bacillus amyloliquefaciens* SW106 were able to grow and produce appreciable amount of pectinase using banana waste and could be considered as potential candidate for industrial applications.

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