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Full Length Research Paper

Production characteristics and properties of cellulase/ polygalacturonase by a *Bacillus coagulans* strain from a fermenting palm-fruit industrial residue.

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A *Bacillus coagulans* strain isolated from palm fruit husk was tested for abilities to hydrolyse plant structural polysaccharides through the depolymerising activities of carboxymethylcellulase and polygalacturonase. The two enzymes were produced using solid substrate fermentation. Both had a working pH range of 4-9 with an optimum pH of 6.0 for the carboxymethylcellulase and 7.0 for the polygalacturonase. The respective enzymes remained active when allowed to stand at 27°C for 1 h over a wide pH and temperature range maintaining maximum activity at an optimum temperature of between 50°C and 60°C respectively. The Carboxymethylcellulase still retained full activity after being allowed to stand at 60°C for 10 min while the polygalacturonase retained full activity at 80°C for 5 min and had 50% activity at 70°C at 30 min. All enzymatic activities were fully inhibited by Mercury ions at 1.0 mM concentration. The carboxymethylcellulase had a molecular weight of between 116 and 205 KDa and another at 6.5 KDa. The molecular weight recorded for the polygalacturonase was 6.5 KDa. Polygalacturonase activity was enhanced by all surfactants applied while that of the cellulase was slightly enhanced by SDS and more by higher concentrations (0.5-5.0%) Tween-80.

Key words: Physiological study, *Bacillus coagulans,* Carboxymethylcellulase, Polygalacturonase, Palm fruit husks.

INTRODUCTION

Plant structural polysaccharides, made up of complex, strongly bound micro fibrils of crystalline cellulose, paracrystaline cellulose and hemicellulose fibrils, abound in nature (Davidson et al., 2004; Pordesimo et al., 2005). Cellulose, an important plant polymer has been found to structurally exist in crystalline molecules containing thousands of glucose residues (Rose and Bennett, 1999). Repeating units of cellulose is known to form a rigid structure due to close packing and the hydrogen bonding in the cellulose sheets producing cellulose crystallites (Meyers, 1992; Li and Gao, 1997; Rose and Bennett, 1999). The association of the cellulose molecules with the paracrystaline cellulose and other polymers result in the formation of microfibrils which, when tightly layered, make up the cell wall. The microfibrils, crystalline and paracrystaline core is surrounded by hemicellulose. After cellulose, this is the second most abundant plant polysaccharide in nature which serves as a storage component in some seeds. Hemicellulose is a branched polymer containing a mixture of hexoses (glucose, galactomannans) and pentoses (xylose, arabinose) which cross-link with other individual microfibrils and structural polysaccharides.

Microbial cultivation under solid state fermentation process is a procedure by which large biomass can be effectively harnessed to yield other advantageous by-products (Crueger and Crueger, 1997). The hydrolysis of plant components pose a big problem because of the complex bonding within the structural matrix as earlier defined. However, many plant pathogenic and non-plant pathogenic microorganisms produce cellulose and hemi-cellulose-degrading enzymes which assist in plant cell

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colonization, invasion and degradation. Cellulases hydrolyse both crystalline and paracrystaline cellulose structures while polygalacturonases cleave the α-1,4-gly-cosidic bonds between two galacturonic residues (Alani et al., 2005; Ajayi et al., 2007). Residues from industrial agricultural practices abound throughout the different seasons experienced in the Tropics. In Nigeria, many such residues are continually generated but not adequately disposed of thus generating environmental pollution problems.

Nigeria is one of the leading producers of palm-oil and the palm-fruit residues generated have been mainly enlisted to augment the strength of wood-fire, dumped as refuse or used as manure/or in composting (Oyenuga, 1968). The ability of a microorganism to contribute in a more effective degradation of palm-fruit husks through the array of enzymes produced is described in this work. This present investigation reports the production characterristics and properties of the hydrolytic cellulase and polygalacturonase of *Bacillus coagulans* from palm-fruit husk.

MATERIALS AND METHODS

Materials

All reagents used were by Sigma-Aldrich, St Louis, Missouri, USA.

Sample collection

The fibrous palm-fruit husk residue, a by-product of palm-oil extraction process, was obtained from its dump site at Bodija in Ibadan Metropolis in the South-western region of Nigeria. This was used as substrate for microbial isolation, cultivation and enzyme production.

Isolation and culture methods

The *Bacillus* strain was isolated from fermenting palm-fruit husk by direct plating of ten-fold serial dilutions of samples (Harrigan and MacCance, 1966). Isolate selection was a consequence of the production of large, clear zones on both cellulose and polygalacturonic acid agar plates. The bacterium was identified according to standard cultural, biochemical and physical characteristics. Experimental isolate was maintained on a nutritive and supportive agar medium containing 0.1% carboxymethyl cellulose or 0.1% Polygalacturonic acid, and stored at 4℃ in a cold room.

Screening for enzymes production

Cellulose agar plates were prepared using the method of Andro et al. (1984). The *Bacillus* isolate was stabbed on the solidified agar and allowed to incubate for 2 days to express cellulose depolymerisation through cellulase production into its surrounding medium. The plate was stained with 0.1% Congo red (pH 7.0) and counterstained with 1.0 M NaCl for 15-20 min. The zone of polymer hydrolysis was apparent as a clear area in the otherwise red CMC Congo red stained background. Polygalacturonase production was screened for by the method of Marcia et al. (1999). The agar plate containing this medium was stabbed with the isolate and incubated for 2 days. The plate was flooded with Iodine-Potassium iodide solution to detect clearing zones against a dark-brown background (Fernandes-Salomao et al., 1996).

Physiological responses of *Bacillus* strain to changes in environmental conditions

A culture broth of the *Bacillus* isolate was serially diluted out (Harrigan and MacCance, 1966) up to a colony count of 10^6, inoculated into sterile nutrient broth and incubated at varied temperature range between 27℃ and 55℃. For the estimation of bacterial responses to pH variations, the pH of the broth medium was adjusted using 0.1 M citrate phosphate buffer to ranges of 4.0 and 9.0, inoculated with the *Bacillus* and incubated at 37℃. The effects of metal ion additions as either inhibitors or promoters were monitored by adding 1.0 mM of any of the metal salts to the culture broth and incubated for 4 days. In all cases, the absorbance of the test broth, as index of microbial growth, was measured at 600 nm using a spectrophotometer, (Beckman DU 520, Beckman Instruments, Fullerton CA, USA).

Production of cellulases and polygalacturonases in Submerged Fermentation and Solid-State (SSF)

The polygalacturonase-producing submerged fermentation medium of Marcia et al. (1999), with and without mechanical agitation, was used. The liquid medium containing (w/v) concentrations of 0.14% $(NH_4)_2SO_4$, 0.6% K_2HPO_4, 0.20% KH_2PO_4, 1.0% polygalacturonic acid and 0.01% $MgSO_4.7H_2O$ at pH 6.0 was inoculated in 125 ml Erlenmeyer flasks and incubated at 37℃ on a rotary Orbit shaker bath, (Lab-line instruments, Illinois, USA) at 150 rpm for 60 h. Bacterial cellulase was produced using submerged fermentation in flasks containing 25 ml of medium according to the method of Li and Gao (1997), with the following composition (%, w/v): 0.02 $MgSO_4.7H_2O$; 0.075 KNO_3; 0.05 K_2HPO_4; 0.002 $FeSO_4.7H_2O$; 0.004 $CaCl_2.2H_2O$; 0.2 Peptone with agar 1.5; and 0.5 Cellulose was used with and without mechanical agitation. These were inoculated with a 10^6 cfu/ml of the *Bacillus* strain, incubated with agitation (150 rpm) at 37℃ for 60 h, after which the biomass was separated by centrifugation at 10,000 rpm.

The SSF was carried out in 250 ml Erlenmeyer flask containing 5 g of palm-fruit husk wetted with 10 ml of a basal mineral salt medium with the following composition: 1% $(NH_4)_2SO_4$; 0.06% K_2HPO_4; 0.05% KH_2PO_4; 0.04 mg $CuSO_4.5H_2O$ and 0.02% $MgSO_4.7H_2O$. The set-up was steam-sterilized at 121℃, inoculated with a 10^6 cfu/ml culture and incubated at 37℃ for 96 h. After inoculation, the fermented material was mixed with eight volumes its size of sterile distilled water, mechanically agitated at 150 rpm for 30 min and filtered under vacuum to separate the solid-liquid phase. The cell-free enzyme filtrate was harvested after cold-centrifugation (4℃) at 10,000 rpm for 15 min and used to evaluate the cellulose and polygalacturonic acid-degrading enzymatic activities.

The effect of incubation time; pH and medium agitation were tested to determine optimized cellulase and polygalacturonase production. The enzymatic culture medium was prepared in 0.1 M citrate-phosphate buffer within ranges of 4.0 and 9.0. Cultivation was in 125 ml Erlenmeyer flasks with 25 ml of medium and the submerged fermentation culture mechanically agitated on a rotary shaker at 150 rpm. Samples were withdrawn at 24 h intervals and used to determine the extent of enzyme production.

Enzyme assays

Carboxymethylcellulase: The modified method of Robson and Chambliss (1984) was used. The activity was determined by pre-

paring 1 ml of a 1.0% (w/v) carboxymethylcellulose in 0.1 M citrate-phosphate buffer (pH 6.0), and mixing with 1.0 ml of the enzyme sample. The mixture was incubated at 50°C for 30 min and reducing sugar measured by the dinitrosalicylic acid method (Miller, 1959). One unit of cellulase activity was defined as the amount of the enzyme which catalyses the release of 1 nM equivalent of glucose/ml under the specified assay conditions.

Polygalacturonase: The polygalacturonase activity was assayed for using the reducing sugar method of (Miller, 1959). The reaction mixture containing 0.8 ml of 1% polygalacturonic acid in 0.2 M citrate-phosphate buffer at pH 6.0 and 0.2 ml of culture supernatant, was incubated at 50°C for 30 min. Absorbance was determined at 540 nm. One unit of polygalacturonase activity (U) was defined as the amount of enzyme which releases 1 µmol of polygalacturonic acid per second from 1 ml enzyme fluid under the specified assay conditions.

Physicochemical properties of the enzymes

Cellulase and polygalacturonase enzymes were characterized by determination of the effects of pH, temperature, cations, surfactants and different substrate concentrations on their activities. In all determinations the residual enzyme activities were measured as earlier described.

1. Thermal stability: This was determined by pre-incubating enzyme solutions at the desired temperature (27, 40, 50, 60, 70, 80, 90 and 100°C) for different time intervals (5, 10, 15, 30 and 60 min). The enzymes so treated were applied in assay procedures.
2. Optimum temperature: The polygalacturonase and carboxymethylcellulose saccharifying activities were carried out at temperature range of 27 to 80°C in reaction mixtures containing 0.1 M citrate-phosphate buffer (pH 6.0) and the relevant substrate.
3. Optimum pH: Enzyme activities was measured at various pH ranging from 4.0- 9.0. One millilitre each of the adjusted pH buffer was introduced into test tubes containing 1 ml enzyme samples and incubated for 1 h.
4. pH stability: The pH stabilities of the enzymes in the quantities as above was also examined after incubating for 24 h at 30°C.
5. Effect of cations and surfactants on enzyme activity: One millilitre each of these solutions was introduced into test tubes containing 1 ml enzyme samples and incubated for 1 h. Chemicals used were $MgCl_2$, 10 mM; KCl, 20 mM; $CaCl_2$, 10 mM; $CoCl_2$, 10 mM; $HgCl_2$, 1.0 mM. SDS, Tween 80 and Triton X-100 were employed at 0.05, 0.1, 0.5, 1.0 and 5.0% (w/v) concentrations.
6. Substrate concentration: One millilitre of Increasing concentrations (0.2, 0.4, 0.6, 0.8 and 1.0% w/v) of carboxymethyl cellulose and polygalacturonic acid were dissolved in 0.1 M citrate-phosphate buffer at pH 6.0 and added to 1.0 ml enzyme samples. The reducing sugar released was monitored using the DNS Procedure (Miller, 1959). A reciprocal of the quantity of reducing sugar recorded was plotted against a reciprocal of the substrate concentration.
7. Protein estimation: The protein concentration within all enzyme preparations was tested for using bovine serum albumin as standard (Lowry et al., 1951).

Partial purification and polyacrylamide gel electrophoresis

Enzymatic broths were subjected to successive purification steps using Ammonium sulphate within 0-80% saturation, dialysis followed by column chromatography through a Bio-Rad resin. Fractions of the purified microbial cellulases and polygalacturonases which exhibited highest activities were pooled together and taken through electrophoretic separation using SDS-PAGE.

Denaturing electrophoresis (Laemmli, 1970) on 12% polyacrylamide slab minigels (MiniProtean II, Bio-Rad®) amended with 0.05% CMC in 0.5 M Tris-HCl and 1 M glycine buffer pH 8.8 was used to determine carboxymethylcellulase protein fractions. The electrode tank contained Tris-glycine buffer pH 8.8 like that used in the gel. Samples in 20 µl quantities were loaded into electrophoretic wells and electrophoresis done at room temperature using a constant current of 200 mA per gel for 2 h. A 13% polyacrylamide SDS-PAGE gel with 0.05% polygalacturonic acid was, however, used for polygalacturonase determination.

RESULTS

Screening for cellulase and polygalacturonase-producing bacterium

Of all the bacterial isolates obtained, 43% were *Bacillus* species. The *Bacillus* isolate PFH N7 was selected because it expressed the highest cellulase (33 mm) and polygalacturonase (27 mm) zones of clearing. Taxonomically, the isolate was identified as a Gram positive; motile rod; spore former, aerobic, catalase positive but oxidase negative and was able to ferment esculin, with a deep black uniform supernatant and an obvious black precipitate. Being indole production negative, positive for starch and casein hydrolysis as well as levan production, it was identified as *Bacillus coagulans* strain PFH N7.

Physiological characteristics

Since production of microbial enzymes is greatly influenced by the cultural conditions employed during microbial culturing, the *Bacillus* isolate PFH N7 expressed maximum growth at 37°C and pH 5.0. Metal ions such as magnesium, calcium and iron boosted microbial proliferation.

Production and assay of microbial enzymes obtained from SmF and SSF experiments

Influence of increase in time and mechanical agitation of medium at 150 rpm enhanced the production of both the cellulase and polygalacturonase of *B. coagulans* (Table 1).

The highest enzymatic activities for CMCase and polygalaaacturonase were realized from production broth adjusted to pH 7.0. The values from the submerged fermentation methods, with and without mechanical agitation, are represented in Table 1. Incubation of palm-fruit husks with *B. coagulans* isolate in a solid substrate fermentation system resulted in the expression of enzymatic activity values (2.685 *n*kat and 3.0 µg/ml), much higher than from unagitated submerged fermentation (0.865 *n*kat and 1.618 µg/ml) showing increases of 210.4% for CMCase and 85.5% for polygalacturonase respectively (Figure 1).

Table 1. Effect of incubation time and agitation on the production of cellulase and polygalacturonase by *B. coagulans* from fermented palm-fruit husk in submerged fermentation medium at pH 7.0.

Incubation Time (Hours)	Enzyme type/ Treatment			
	Carboxymethyl cellulase (*n*Kat)		Polygalacturonase (µgPGA/ml/sec)	
	Unagitated	Agitated	Unagitated	Agitated
0	0	0	0	0
24	0.354 ± 0.004*	0.345 ± 0.006	1.466 ± 0.004	1.593 ± 0.004
48	0.574 ± 0.005	0.723 ± 0.006	1.44 ± 0.013	1.616 ± 0.007
72	0.865 ± 0.004	0.967 ± 0.006	1.619 ± 0.004	1.768 ± 0.037
96	0.672 ± 0.004	0.882 ± 0.005	1.525 ± 0.004	1.737 ± 0.008

*values are means of duplicate determinations ± SD.

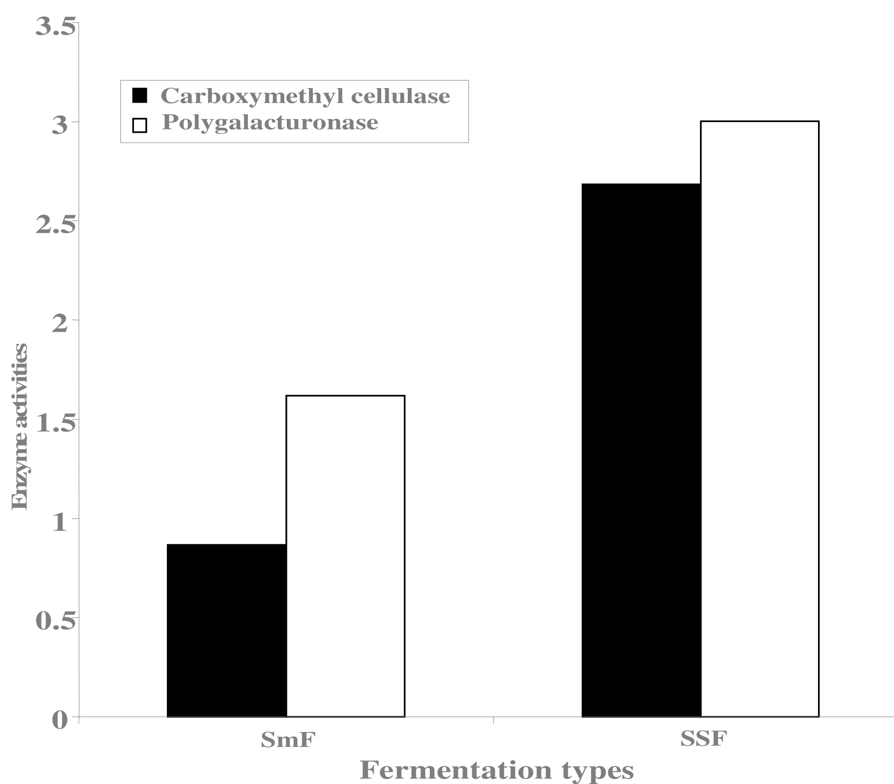


Figure 1. Comparison of the enzymatic activities of carboxymethyl cellulase and polygalacturonase obtained through unagitated submerged fermentation and solid substrate fermentation.

Mechanical of both the cellulase agitation of medium enhanced the production and polygalacturonase of *B. coagulans.* Production temperature for cellulase and polygalacturonase in culture media was found to be at 37°C (Figure 2). PGA-ase production peaked at 37°C, however, the values recorded at 27, 45, 55 and 65°C were lower (66.3, 55.6, 51 and 48.6% respectively). Optimum cellulase was produced at 37°C and gradually reduced through 27, 45, 55 and 65°C respectively with residual activities of 69.3, 38.6, 29.3 and 11.1%.

Effect of temperature and pH changes on enzyme activities

While the CMCase and polygalacturonase remained active when incubated at different temperature ranges, they maintained maximum activity at an optimum temperature of between 50 and 60°C respectively. In Figure 3, *B. coagulans* isolate strain PFH N7 showed full polygalacturonase activity for up to 30 min at 60°C, after which, it experienced a sharp drop. The enzyme was

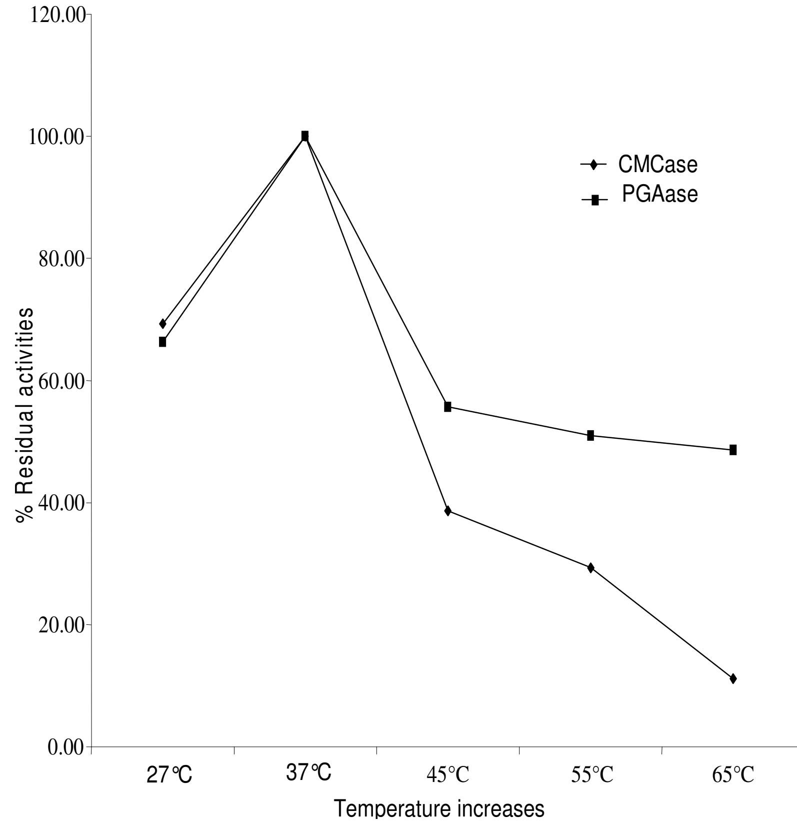


Figure 2. Influence of incubation temperature on the production of *B. coagulans* cellulase and polygalacturonase.

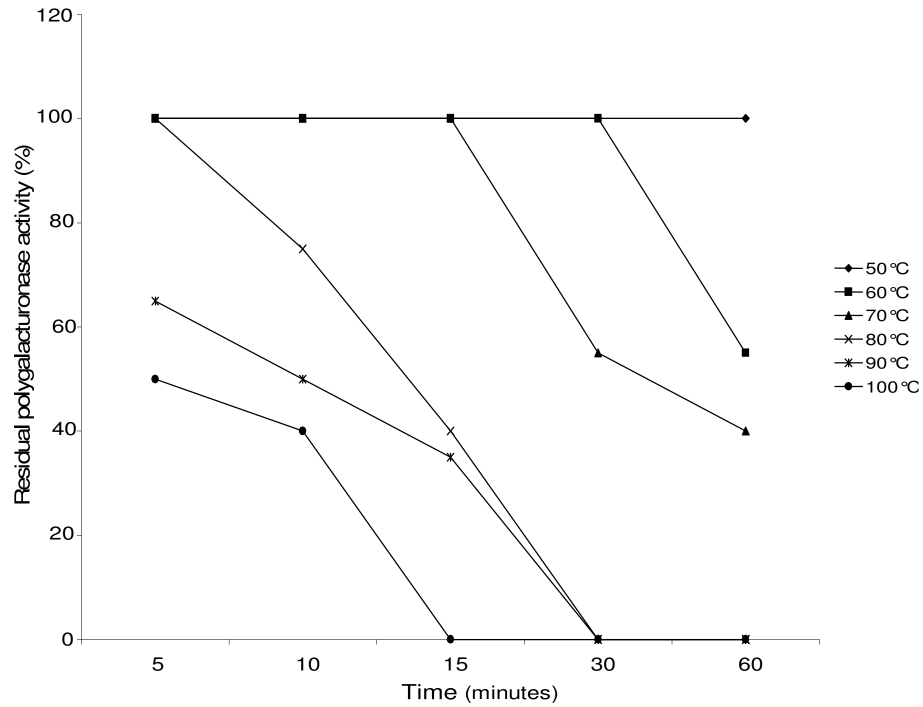
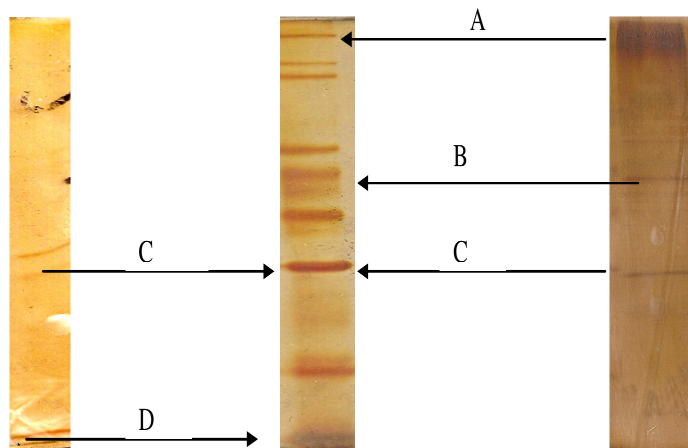


Figure 3. Effects of temperature increases with increasing time regimes on the enzymatic activities of *B.coagulans* polygalacturonases.

Plate 1. SDS/PAGE of *B. coagulans* carboxymethylcellulase, a wide range marker and polygalacturonase enzymatic fluid respectively.



A (~115-205kDa).
B (~55kDa).
C (~36kDa).
D (~ 6.5kDa).

stable for 15 min at 70℃, while at 90℃ for same 15 min, 35% activity still remained. The least polygalacturonase enzyme activity was recorded at 100℃ and 10 min (40%). After this, no activity was recorded. Between 80 and 100℃, no activity was recorded after the enzyme had been incubated for 30 min. The half-life of *B. coagulans PFH*N7 PGA-ase was recorded at 70℃ and 30 min.

Figure 4 shows the carboxymethylcellulase enzymatic activity of *B. coagulans* after being subjected to different incubation temperatures. Full activity was recorded from 27℃ through 60℃ for 10 min. Values at 70 and 80℃ were the same between 5 and 30 min. Half-life was reached beyond 90 and 100℃ at 10 min. Throughout the ranges considered, the activity strain *PFH*N7 was best observed at pH 7.0 to 9.0

The influence of pH on the stability of polygalacturonase activity of *B. coagulans* strain *PFH*N7 was best observed at pH 7.0 to 9.0. Throughout the ranges considered, the activity gradually increased recording peak activity at pH 8.0 (1.51 µg/ml), closely followed by that at pH 9.0 and pH 7.0 (Figure 5). The responses of *B. coagulans* cellulase to pH influences showed that the carboxymethylcellulase activity possessed a relatively broad pH, being highest at 6.0, and progressively reducing in the direction of alkalinity (Figure 6).

Effect of surfactant additions on enzyme activities

With polyoxyethylene sorbitan mono-oleate (Tween 80), polyethylene glycol p-isooctylphenyl ether (Triton X-100) and sodium dodecyl sulphate (SDS) in the reaction mixture, it was observed that lower quantities of SDS increased carboxmethylcellulase activities of *B. coagulans* (Figure 7), while higher surfactant concentrations yielded values (0.868 and 0.844 *n*kat) respectively very close to and lower than that of the were recorded with the addition of all surfactant types than that of the control (0.863*n*kat). Higher polygalacturonase activities' (Figure 8).

No residual cellulase and polygalacturonase activities were detected with the addition of 1 mM $HgCl_2$ solution, while with Ca^{2+} increase of 103% and 117% respectively was recorded with Li^+ and K^+ yielding 94 and 85% activity. Activity of *B. coagulans* polygalacturonase was 100% by Co^{2+} but K^+ and Li^+ resulted in activity levels of 94.7 and 89.5% respectively.

Effects of substrate concentration increases

For the polygalacturonase of *B. coagulans,* the K_m was 28.1 $mgml^{-1}$ while the V_{max} was 0.79 U/sec. However, the K_m and V_{max} of the bacterial carboxymethylcellulase was found to be 0.65 mg/ml and 1.36 U/sec respectively.

Enzyme purification

Results of cellulase chromatography showed two enzymatic activity peaks from fractions 23 and 27 having activities of 0.85*n*Kat and 1.07 *n*Kat corresponding to 0.47 and 0.52 mg of protein. The polygalacturonase had peak activity of 0.62 µgPGA/ml and 0.32 mg/ml protein. SDS-PAGE of the cellulase showed two distinct bands at between 116 and 205 KDa. The polygalacturonase protein had a molecular weight of approximately 6.5 KDa.(Plate 1).

DISCUSSION

To our knowledge, this is the first report of the production and purification characteristics of a *B. coagulans* cellulase and polygalacturonic acid-degrading activities from a spontaneously fermenting palm-fruit husk industrial residue. After the preliminary isolation work revealed a predominance of *Bacillus* species among the bacteria flora, subsequent enzymatic screening confirmed the ability of the *B. coagulans* strain to degrade structural palm-fruit husk fibres through the production of pale hydrolytic zones on cellulose and pectin solid agar media. This evidence serves as a strong indication of the hydrolytic capabilities of the *Bacillus* isolate contributing effectively to the degradation of the pectin and cellulose structural units present in the plant husk. Robson and Chambliss (1984) also reported the characterization of a *Bacillus* strain with endoglucanase activity.

Optimization of enzyme production by various environmental factors caused an increase of enzymatic activities.

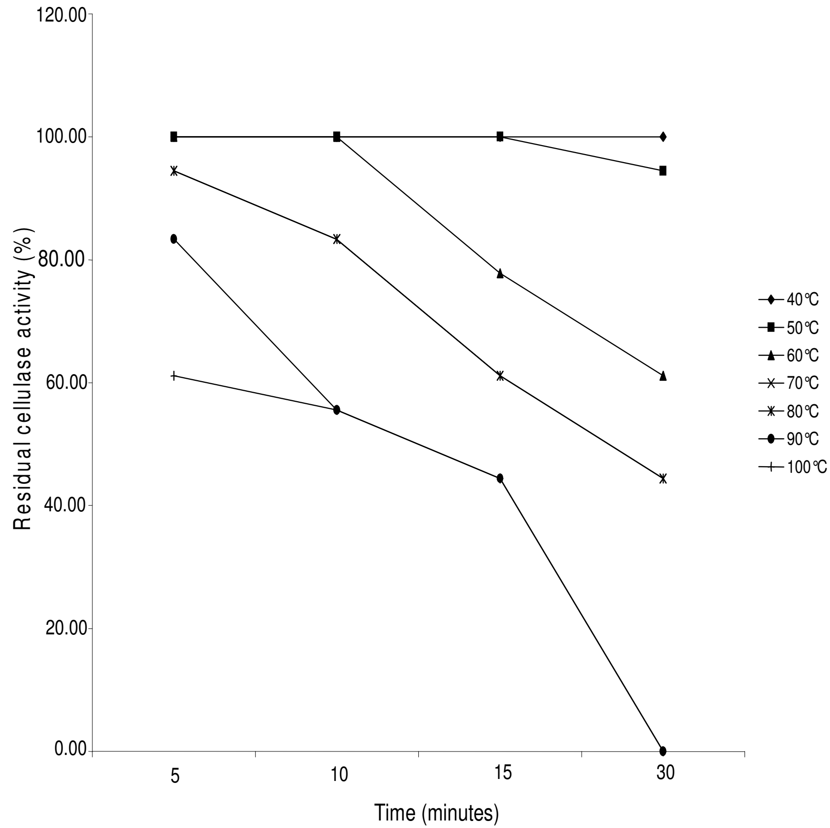


Figure 4. Effects of temperature increases with increasing time regimes on the enzymatic activities of *B.coagulans* cellulases.

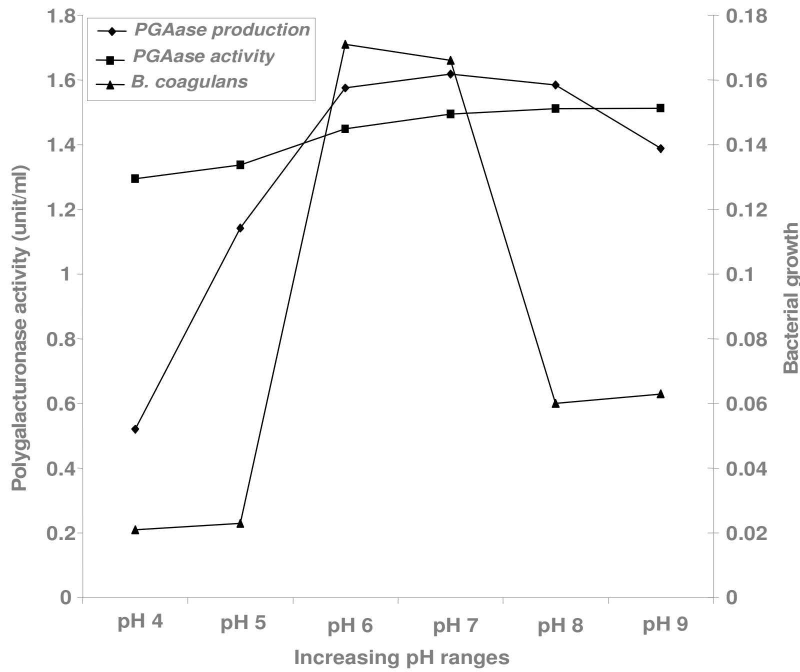


Figure 5. Polygalacturonase production and activities of *B. coagulans* enzymatic fluid.

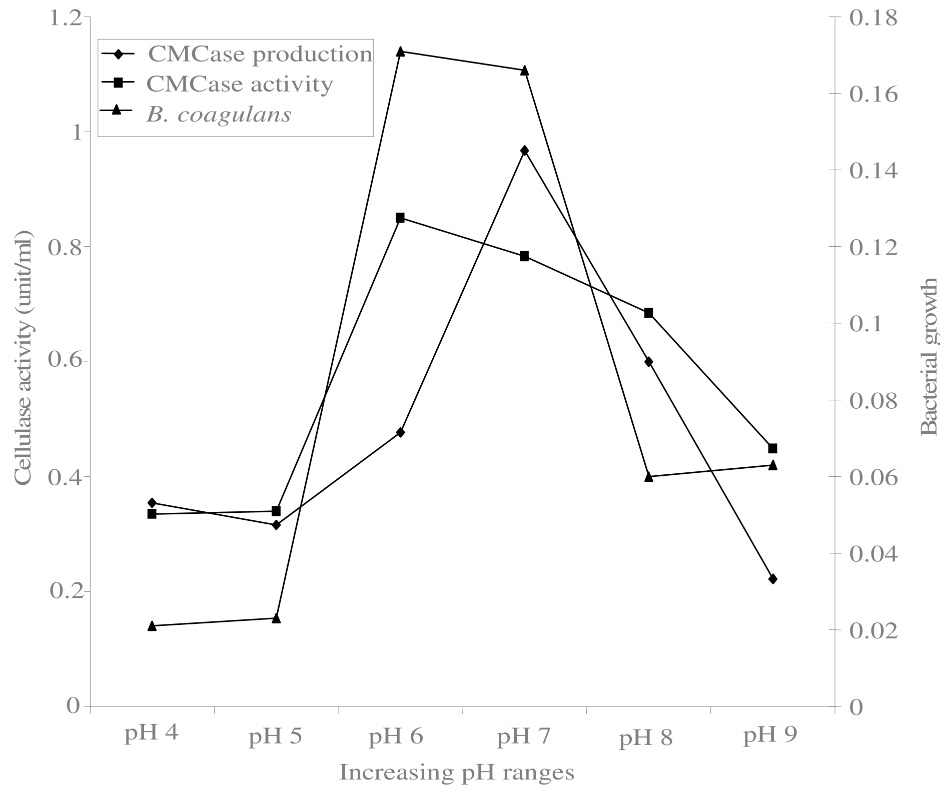


Figure 6. Carboxymethylcellulase production and activities of *B. coagulans* enzymatic fluid.

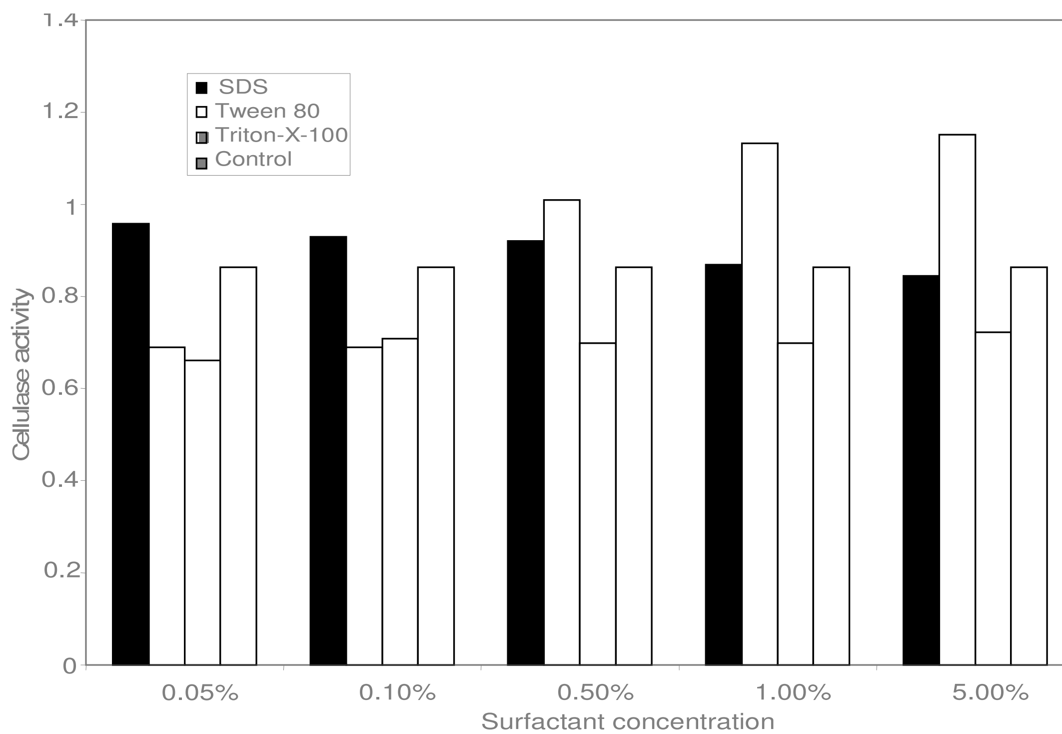


Figure 7. Effects of surfactant additions on carboxymethylcellulase activities of *B. coagulans* isolate *PFH*N7.

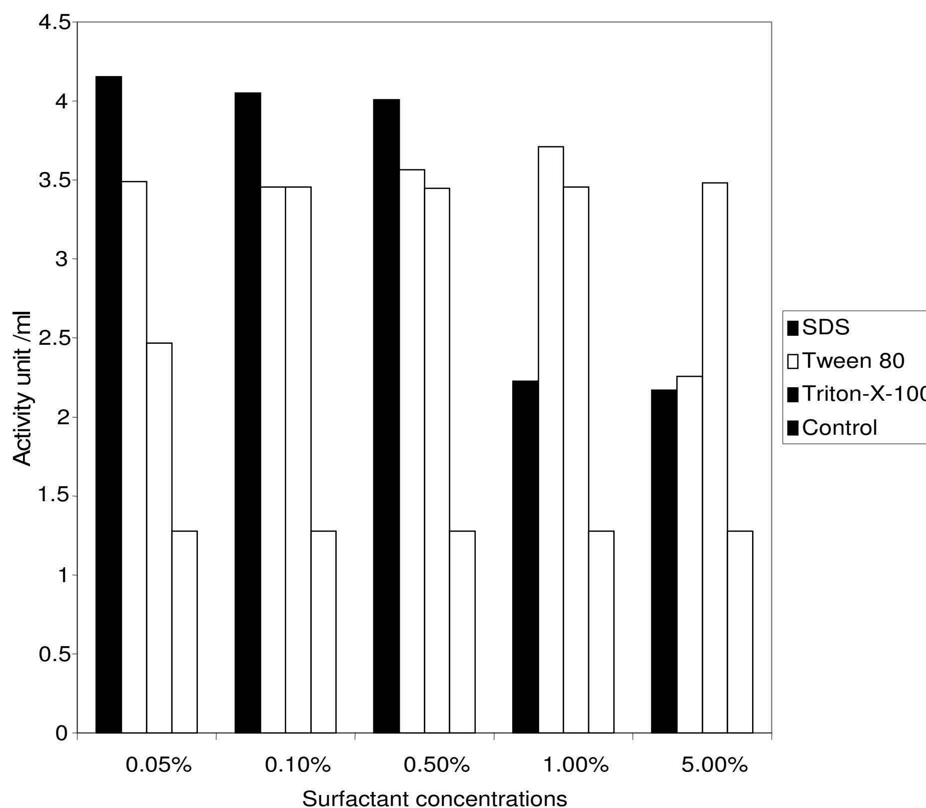


Figure 8. Effects of surfactant additions on the polygalacturonase activities of *B. coagulans* isolate *PFH*N7.

Mechanical shaking increased medium aeration and permitted better contact between the substrate and the microorganism causing significant differences in favour of the quantity of enzyme produced in an agitated system. Submerged substrate fermentation techniques have been widely used in the production of cellulase and other enzymes (Haltrich et al., 1996; Kim et al., 1997). Chahal et al. (1996), Haltrich et al. (1996), Jecu, (2000) have also suggested that the solid state fermentation is that enzymes using agricultural waste materials due to its lower capital investment and reduced operating cost. Solid substrate fermentation system elucidated higher enzyme yield.

Temperature and pH were found to be important parameters that influenced enzyme production and activities. High *B. coagulans* cellulase and polygalacturonase titres were still attained at a temperature and pH different from that for optimal microbial growth and enzyme activities. At either highly acidic or alkaline pH, cellulase and polygalacturonase production was high, but lower than that recorded at pH values between 6.0 and 8.0. The effect of pH on the activities of cellulase and polygalacturonase by the *Bacillus* isolate was very gradual especially from pH 6 to alkaline ranges. Nakai et al. (1987), reported cellulaseswith alkaline optimum pH from alkalophilic *Bacillus* strains. The enzymes in this work were active in a large spectrum of pH and temperature conditions. An indication of the importance of temperature and pH especially as they affect growth, enzyme activities and metabolic pro-cesses in fermentation process was also suggested by Krishna (2005) and Gulati et al. (2007). The cellulase and polygalacturonase of this *Bacillus* isolate expressed enzymatic activities at all pH regimes employed in the work (4.0-9.0) and showed it to be pH-tolerant. Chadha et al. (2004) described a *Rhizopus pussillus* strain with a broad pH range of activity. Thus, the enzymes described in this work share properties with many cellulases and polygalacturonases from other microbial sources and may thus find applications in various industrial processes.

Surfactant additions, especially to the polygalactorunase greatly boosted enzymatic activities. With increase in surfactant concentrations, the cellulase activity increased with Tween-80 and reduced with SDS. Triton-X-100 repressed carboxymethylcellulase activities. These surfactant activities could support enzymatic stability and possibly prevent enzyme inactivation.

Since enzymatic metal-ion activation was best with Ca^{2+} while other molecules used did not significantly enhance the expression of maximum activity, it is possible that the addition of Ca^{2+} is a requirement for the expression of full enzymatic activities (Figure 9).

Higher substrate affinity was recorded by the polygalacturonase of *B. coagulans* while the speed of enzymatic

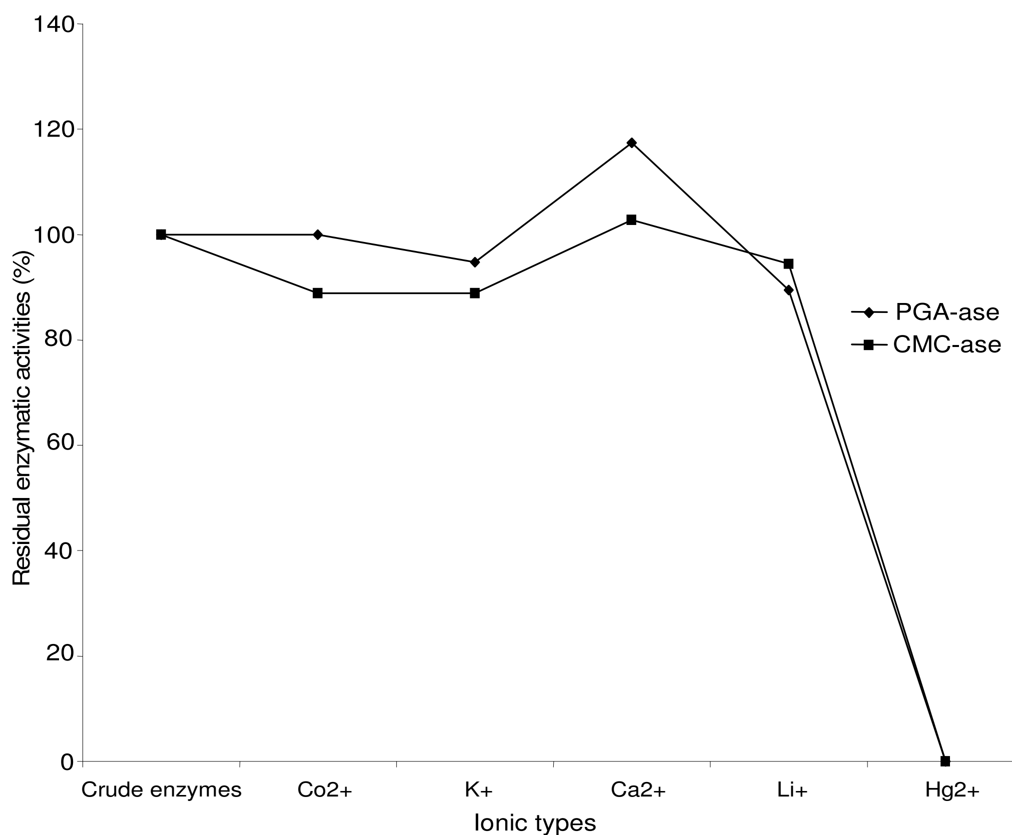


Figure 9. Cationic effects upon cellulase and polygalacturonase enzymes.

Table 2. Purification steps and yield of the polygalacturonase and carboxymethylcellulase of *B. coagulans* from fermenting palm-fruit husk.

Enzyme type	Concentration method	Total protein (mg)	Total activity (nKat)	Specific activity (nKat/mg)	Yield (%)
CMCase	Crude extract	311.58	96.73	0.31	100.00
	Ammonium sulphate	279.64	68.42	0.24	70.73
	Column chromatography	247.70	44.82	0.18	46.34
Polygalacturonase	Crude extract	381.44	127.80	0.34	100.00
	Ammonium sulphate	351.50	99.71	0.28	78.02
	Column chromatography	217.76	59.60	0.27	46.64

reaction was faster in the cellulase (Table 2). An increase in substrate concentration made more binding sites available for the enzymes to adhere to and the rate at which product formation would be achieved therefore would be faster (Dixon and Webb, 1971). The synergistic activities of both enzymes would contribute to enhance more effective degradation of the structural polysaccharides within the palm-fruit husk matrix. The polygalacturonase specific activity and yield decreased as concentration steps increased, relative to the crude The same trend was recorded for the carboxymethylcellulase. While the molecular weight of an attractive alternative process to produce microbial the polygalacturonase was low (~6.5 kDa), the cellulase had a higher weight (~36, 55 and 205 kDa). Niture et al. (2001) and Mohamed et al. (2006) have described low and medium ranged molecular weight microbial cellulases. It was unique that this micro-organism possessed both very high and low molecular weight hydrolytic enzymes which were confirmed to possess plant structural depolymerising activities.

Conclusion

The study of both the physiological and physicochemical characteristics of *B. coagulans* along with its cellulase

and polygalacturonase indicates that enzyme production is governed by different factors. These enzymes can be further researched for their application in upgrading poultry layer feed (Odeniyi et al., unpublished data), and can contribute beneficially when applied in industries such as poultry and animal feeds, etc. A cost-effective means of enzyme production has also been confirmed through the use of industrial palm-fruit husk as substrate. The physiological characteristics of this *B. coagulans* are expected to have important applications in these various industries.

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