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SCREENING OF BACTERIAL STRAINS FOR BETA-MANNANASES PRODUCTION IN SOLID STATE FERMENTATION

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ABSTRACT
This study was carried out to screen bacterial strains of agricultural wastes origin for β-mannanases production in solid state conditions. The eight bacterial strains obtained from stock culture were screened for mannanase production in solid state condition. The strains with different codes were classified as *Klebsiella edwardsii* 1A, *Bacillus substilis* BS, *K. edwardsii* 2B, *K. edwardsii* X1, *K. edwardsii* X5, *K. edwardsii* X4, *K. edwardsii* X3 and *B. polymyxa* BP. Solid substrate fermentation was carried out in Erlenmeyer flask using Mandels and Weber's medium as the moistened agent. Beta-mannanase activity was determined by dinitrosalicylic acid method, while protein was determined by Lowry method. In the screening exercise conducted, all the 8 bacterial strains displayed mannanase activity which ranged 87.958 to 103.200 U/ml, while protein content ranged from 4.347 to 9.722mg/ml with the highest mannanase activity and protein content lied on isolate 1A. The optimal β-mannanase activities was achieved at 18 hrs of incubation for bacterial strains 1A and BP, 24 hrs for BS, X1 and X4, 30 hrs for 2B and X5, while X3 exhibited two activity peaks which was at 18 and 36 hrs of incubation. The optimal fermentation time for bacterial growth estimation was obtained at 12 hrs for 1A, 2B and X1, 18 hrs for BS, X5, X4 and BP, while 24 hrs was the best fermentation time for X3. In this study, the screened bacterial strains evaluated for mannanase production from agro-wastes elaborated considerable mannanase activity and this could be exploited for economic uses.

KEY WORDS: Bacterial strains, beta-mannanase, solid state fermentation, screening

INTRODUCTION

Lignocellulose is the major structural component of plant cell walls and is mainly composed of lignin, cellulose and hemicellulose, and represents a major source of renewable organic matter. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003). Large amounts of lignocellulosic "waste" are generated through forestry and agricultural practices, paper pulp industries, timber industries and many agro-industries and they pose an environmental pollution problem. However, the huge amounts of residual plant biomass considered as "waste" can potentially be converted into various different value-added products including biofuels, chemicals, and cheap energy sources for fermentation, improved animal feeds and human nutrients. Lignocellulytic enzymes also have an significant applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture (Bhat, 2000; Sun and Cheng, 2002; Beauchemin *et al.*, 2003; Howard *et al.*, 2003).

There is a considerable interest in the biological degradation of lignocelluloses as the most abundant reusable resource in nature and its potential for industrial application (El-Naggar *et al*., 2006). The main carbohydrate constituents of lignocellulosic materials (cellulose, mannan, and xylan) consist of chains of β-1,4-linked pyranosyl units, which can be substituted in various forms. The β- 1,4-glycosidic bonds within the polysaccharide backbones are hydrolyzed by cellulases, mannanases, and xylanases. Cellulase can degrade beta-1,4-bond between glucose and glucose, mannanase can degrade beta-1,4-bond between mannose and mannose, xylanase degrade beta-1,4-bond between xylose and xylose (Sachslehner *et al.,* 1998). Various mannanases from *Streptomyces* sp. (Takahashi *et al*., 1984), *Bacillus subtilis* (Zakaria *et al*., 1998), *Sclerotium* (Athelia) *rolfsii* (Sachslehner and Haltrich, 1999), *Bacillus stearothermophilus* (Zhang *et al*., 2000), *Aspergillus awamori* (Kurakake and Komaki, 2001), *Trichoderma harzianum* (Ferreira and Filho, 2004) and *B. subtilis* WY34 (Jiang *et al*., 2006) have been purified and characterized, and some genes from *B. subtilis* and *B. stearothermophilus* encoding mannanases were also cloned, sequenced and expressed (Ethier *et al*., 1998). Endo β-D-mannanase (EC 3.2.1.78, mannan endo-1, 4-β-D-mannosidase) cleaves randomly within the-1, 4-β-D-mannan main chain of galactomannan, glucomannan, and mannan (McCleary, 1988). There has been an increasing interest in the potential application of β-mannanases in the industry, because these enzymes play an important role in the bioconversion of lignocellulosic materials. The coconut residue contains highly concentrated mannan, which can be hydrolyzed by mannan-degrading enzyme system to produce single-cell protein (Hossain *et al*., 1996). The other areas of applications include production of animal feed (Lee *et al*., 2005; Wu *et al*., 2005) and laundry detergents (Schäfer *et al*., 2002). Their effective role in pulp bleaching processes minimized the use of environmentally harmful bleaching chemicals in pulp and

paper industry (Cuevas *et al*., 1996). Mannanases have been used in the food industry for the extraction of vegetable oils from leguminous seeds and the clarification of fruit juices (Gubitz *et al*., 1996). The enzyme also can be used in the reduction of the viscosity of extracts during manufacturing of instant coffee, chocolate, and cacao liquor (Sachslehner *et al*., 2000) to lower the cost for subsequent evaporation and drying. In addition, mannanases have the potential for application in the pharmaceutical industry for the production of physiologically interesting oligosaccharides (Gubitz *et al*., 1996). Thus, β-mannanases have wide commercial applications in industries such as paper, pulp, food, feed, as well as pharmaceutical, and energy industries (Lee *et al*., 2005). This study was carried out to screen bacterial strains of agricultural wastes origin for β-mannanases production in solid state conditions.

MATERIALS AND METHODS

Materials and chemicals

The coconut residual cakes were collected from farm field in Akure, Ondo State, Nigeria and it was used as a carbon source for medium formulation. The residual were treated with petroleum ether and dried at 60^0C for 2 hr. After that, the residual were blended, milled and sieved to obtain uniform particle size of 0.5 mm. Locust bean gum was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Bacterial strains

Eight bacterial strains belonging to the general *Klebsiella* and *Bacillus* isolated from agricultural wastes were obtained from bank's stock cultures of Research Laboratory, Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria. The bacterial strains were maintained on locust bean gum containing agar plates and sub-cultured at regular intervals and stored at 4^oC in refrigerator on agar slants.

Screening for mannanase production

For the production of mannanase in solid state fermentation, the isolates were grown at 35^0C in 250 ml Erlenmeyer flasks containing 10 grams of the coarsely ground copra meal. Mandels and Weber's medium modified by El-Naggar *et al*. (2006) was used to adjust the moisture content from 50% to 80%.

Enzyme extraction

The solid state cultures were prepared by adding 10-fold (v/w) 0.1 M phosphate buffer (pH 6.8) and shaking (180 rpm) at 30^0C for 60 min. The solid materials were separated by centrifugation (6000 rpm, 15 min at 4^0C). The clear supernatant was used for enzyme assays (El-Naggar *et al*., 2006).

Enzyme assays

Mannanase activity was assayed in the reaction mixture composing of 0.5 ml of 50mM potassium phosphate buffer pH 7.0 and 1% Locust Bean Gum (LBG) with 0.5 ml of supernatant at 45^0C for 60 min (modified method of El-Naggar *et al*., 2006). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) (Miller, 1959). One unit of mannanase activity was defined as amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.

Protein estimation

Protein in the medium was determined by the method of Lowry *et al.* (1951) with Bovine Serum Albumin (BSA) as standard.

Effect of incubation period on mannanase production

Fermentation period is an important parameter for enzyme production. In this study, the fermentation experiment was carried out up to 42 hours and production rate was measured at 6 hours intervals. Mannanase assay was carried out according to standard assay procedures (El-Naggar *et al*., 2006).

Bacterial growth estimation

The bacterial density with increase in incubation time was determined by UV Spectrophotometer at 660nm (Mabrouk and El-Ahwany, 2008).

Statistical analysis

Data presented on the average of three replicates (±SE) are obtained from there independent experiments.

RESULTS AND DISCUSSION

Quantitative determination of β-mannanase in solid state fermentation

A total of eight bacterial strains belonging to the genera *Klebsiella* and *Bacillus* previously isolated from agricultural wastes were screened for β-mannanase in solid state fermentation. The strains with different codes were classified as *Klebsiella edwardsii* 1A, *Bacillus substilis* BS, *K. edwardsii* 2B, *K. edwardsii* X1, *K. edwardsii* X5, *K. edwardsii* X4, *K. edwardsii* X3 and *B. polymyxa* BP. In the quantitative determination of β-mannanase activity, all the 8 bacterial strains displayed mannanase activity between 87.958 to 103.200 U/ml, while protein content ranged from 4.347 to 9.722mg/ml with the highest mannanase activity and protein content lied on isolate 1A (Table 1).

TABLE 1: Mannanase production by bacterial isolates in solid state fermentation

Source	Isolates	Final pH	Mannanase activity (U/ml)	Protein content (mg/ml)	Yield (U/g)
PAP	1A	5.78^{a}±0.05	103.200^{e}±0.96	9.722^{g}±0.15	10.320^{c}±0.10
CS	2B	5.81^{ab}±0.03	91.650^{c}±0.96	7.361^{d}±0.17	9.165^{a}±0.10
FCN	X3	6.67^{c}±0.12	91.250^{c}±0.13	6.014^{c}±0.00	9.125^{a}±0.11
	X1	7.47^{f}±0.10	95.833^{d}±0.17	4.347^{a}±0.01	9.583^{b}±0.18
	BS	5.92^{b}±0.02	88.889^{b}±0.11	8.333^{f}±0.02	8.889^{a}±0.05
RB	BP	7.13^{e}±0.02	88.889^{b}±0.11	4.903^{b}±0.21	8.889^{a}±0.07
FCN	X4	6.97^{d}±0.02	91.667^{c}±0.10	8.097^{e}±0.240	91.667^{e}±0.11
	X5	5.89^{b}±0.10	87.958^{a}±0.62	7.222^{d}±0.10	87.958^{d}±0.56

Values are presented as Mean±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05).

CS=Cotton seed, **PPP**=Potato peels, **FCN**= Fermented coconut, **PAP**= Pineapple peels **CP**= Cassava peels, **RB**=Rice bran

Variation in protein content generated by each of the strains could be attributed to the production of variety of enzymes (amylases, cellulases, protease and xylanases) apart from the enzyme been examined in this study. Besides that, the protein from bacterial cells and metabolites might also interfere with mannanase production causing variation in protein contents by the strains (Khan and Husaini, 2006).

Beta-mannanase activity in different fermentation periods

The optimization of the time course is of prime importance for mannanase biosynthesis by bacteria (Ray *et al*., 2007). Since fermentation duration is crucial, it is also important to find out the optimum period for mannanase production. Some organisms are reported to produce maximally in the log phase of growth, whereas some at their stationary phase (Ray *et al.,* 2007). Data presented in figure 1 to 8 showed the effect of different incubation periods on mannanase activity, protein content, productivity and growth by the screened bacterial strains. From the results, it was found that mannanase activities revealed its best production at 18 hrs of incubation for bacterial strains 1A and BP, 24 hrs for BS, X1 and X4, 30 hrs for 2B and X5, while X3 exhibited two activity peaks at 18 and 36 hrs of incubation. In terms of mannanase productivity, it was observed that it's followed the same pattern with activity obtained from each of the bacterial strains. This shows that there was relationship between mannanase activity and productivity per hour. Protein content increased with increase in fermentation period. The optimal protein content were obtained at 18 hrs for BS, X5 and BP, while 24 hrs were observed to be best fermentation time for protein production by bacterial strains 1A, 2B, X1, X4 and X3. Figure 1 to 8 also revealed bacterial growth estimation, the optimal incubation time obtained were 12 hrs for 1A, 2B and X1, 18 hrs for BS, X5, X4 and BP, while 24 hrs was the best fermentation time for X3. Generally, there was an increase in mannanase activity, protein content, productivity and bacterial growth with increase in fermentation periods and beyond the optimal conditions a decline was observed. The decrease observed in these parameters might be due to the depletion of nutrients and accumulation of other by-products like proteases in the fermentation medium initiating autolysis of cells (Meenakshi *et al*., 2010; Malik *et al*., 2010).

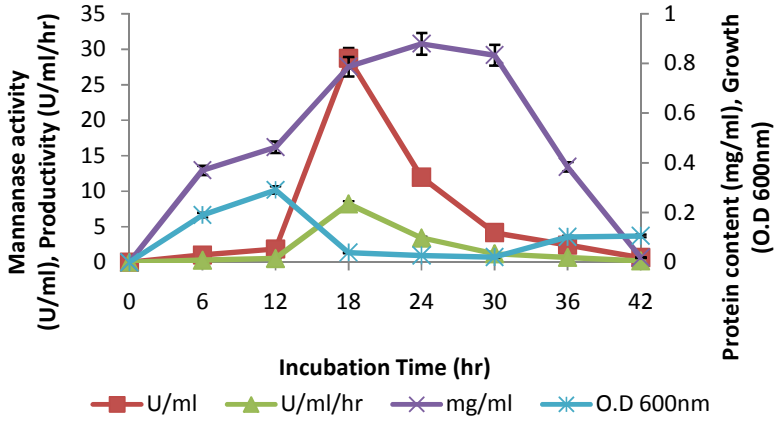

FIGURE 1: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *Klebsiella edwardsii* 1A

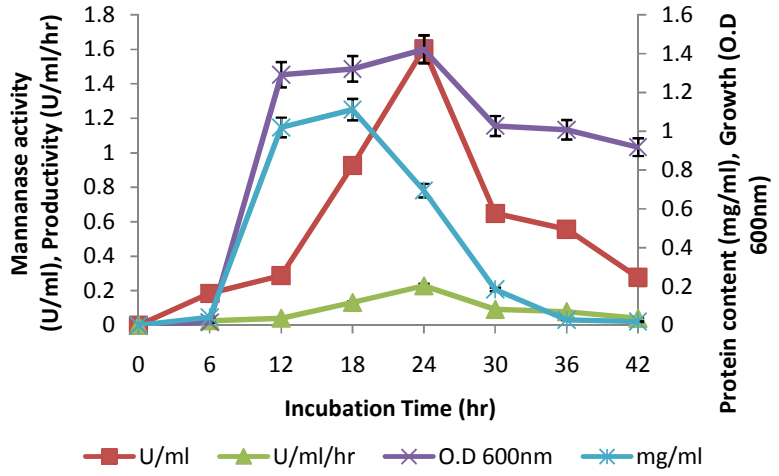

FIGURE 2: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *Bacillus subtilis* BS

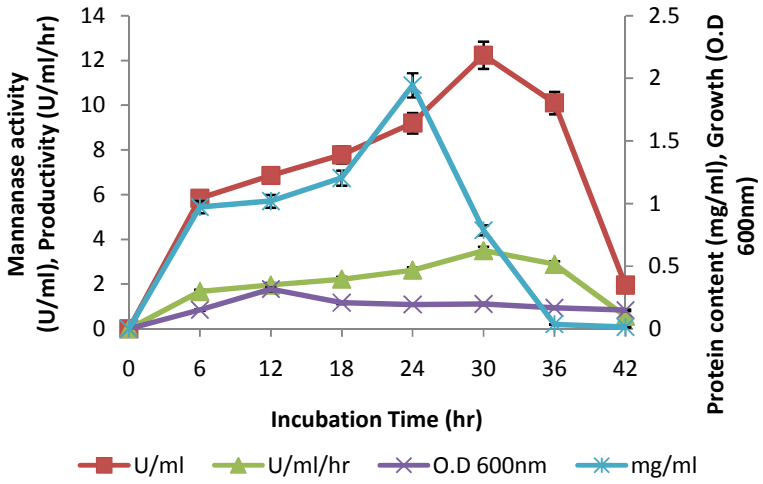

FIGURE 3: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *K. edwardsii* 2B

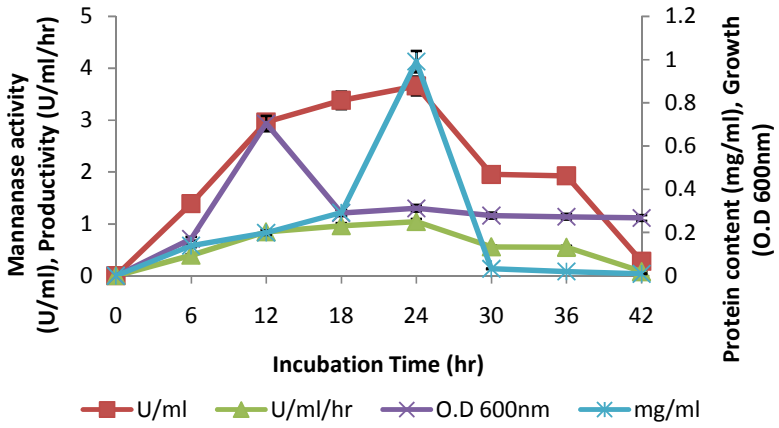

FIGURE 4: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *K. edwardsii* X1

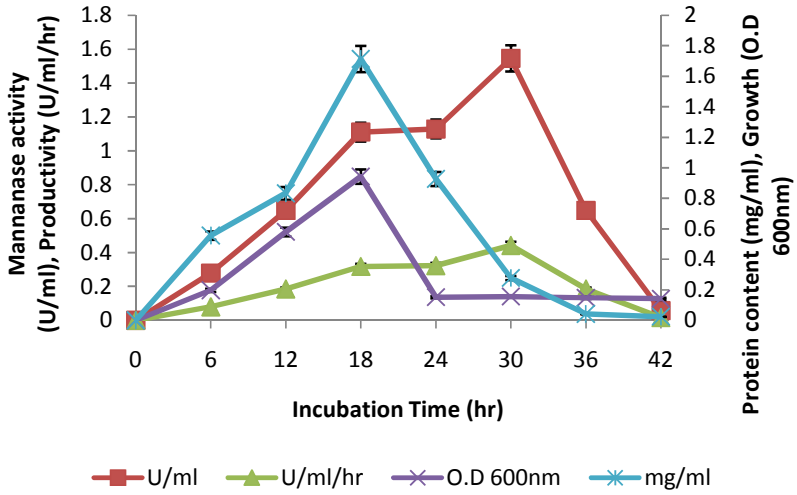

FIGURE 5: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *K. edwardsii* X5

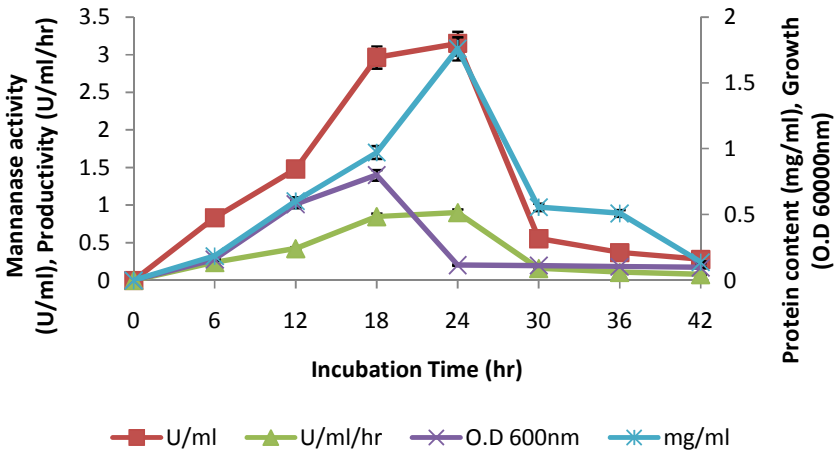

FIGURE 6: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *K. edwardsii* X4

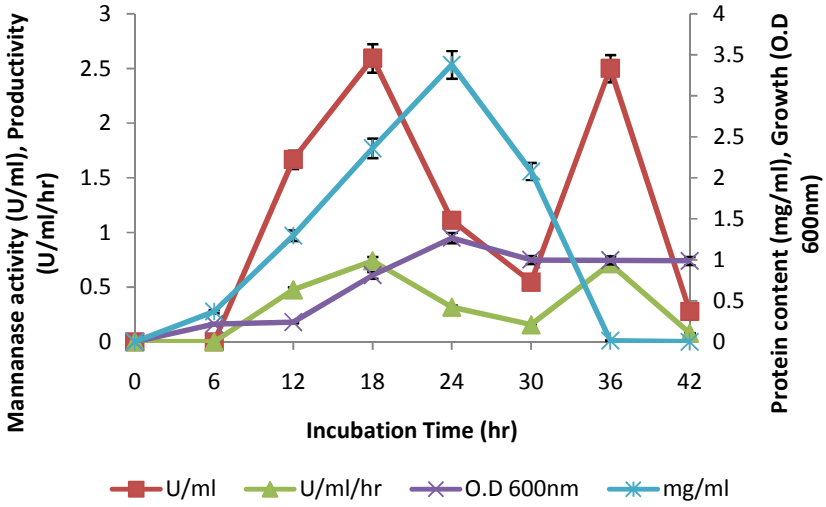

FIGURE 7: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *K. edwardsii* X3

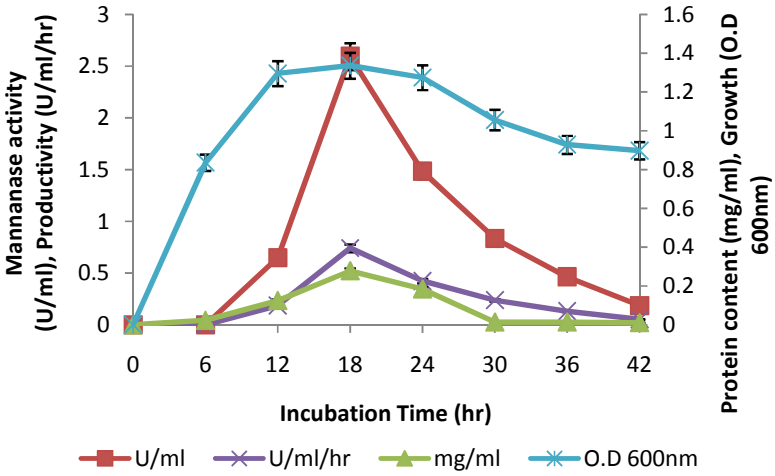

FIGURE 8: Time course profile of mannanase activity, protein content, productivity and bacterial growth by *B. polymyxa* BP

CONCLUSION

In view of results obtained, we were able to establish that agricultural wastes accommodate mannanase producing bacteria which could be exploited commercially at bioreactor level for industrial production of mannanase for any industrial application. This study recommended that the isolates should be subjected to optimization study to fully harness the potential in them to scale up production of mannanase in SSF.

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