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Screening of Bacterial Strains of Agricultural Waste Origin for Beta-mannanase Production and Optimization of Process Parameters

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BJA and DJA managed the analyses of the study and the literature searches. The authors read and approved the first manuscript.

Original Research Article

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ABSTRACT

Aims: This study was carried out to screen bacterial strains of agricultural wastes origin for β-mannanase production and optimization of culture conditions.
Study Design: The first experiment, bacterial strains were screened for β-mannanase production. In the second experiment, the best incubation time was determined. In the third experiment, different agricultural wastes were screened. In the fourth experiment, different nitrogen sources were screened. In the fifth and sixth experiments described the effect of different pH values and incubation temperatures on β-mannanase production. The best moisture content was determined in the seventh experiment, while in experiment eight; effect of different inoculum concentrations was evaluated.
Place and Duration of Study: Microbiology Research Laboratory, Federal University of Technology, Akure, Nigeria between September 2011 and March 2012.
Methodology: Bacterial strains were screened and β-mannanase production from optimization studies was conducted on Locust Bean Gum. Enzyme activity was determined by dinitrosalicylic acid method.
Results: Out of the sixteen bacterial strains screened, *Klebsiella edwardsii* designated 1A

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was selected as the most potent in producing enzyme of high activity and it was therefore selected for further studies. Pineapple peels were found to be the most effective carbon source with a highest β-mannanase activity of 8.533±0.08U/ml. Ammonium nitrate (NH_4NO_3) was obtained to be the best nitrogen source out of all the nitrogen sources screened. The best moisture content was obtained at 1:11 (ratio of substrate to salt solution). Inoculum concentration of 1.0% (v/v) yielded highest β-mannanase activity of 15.833±0.01U/ml. Addition of simple carbon sources to medium containing LBG caused a catabolic repression of β-mannanase synthesis.
Conclusion: The optimal culture conditions obtained from this study will help to standardize the requirements for optimum β-mannanase production using cheaper substrates.

Keywords: Screening; optimization; solid state fermentation; β-mannanase; Klebsiella edwardsii 1A.

1. 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 [1]. 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 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 [2,3].

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 [4]. 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 β-1,4-bond between glucose and glucose, mannanase can degrade β-1,4-bond between mannose and mannose, xylanase degrade beta-1,4-bond between xylose and xylose [5]. Various mannanases from *Streptomyces* sp. [6], *Trichoderma reesei* [7], *Sclerotium* (Athelia) *rolfsii* [8], *Bacillus stearothermophilus* [9], *Aspergillus awamori* [10], *Trichoderma harzianum* [11], *Penicillium italicum* [12], *Aspergillus niger* [13], *P. oxalicum* [14] and *B. subtilis* WY34 [15] have been purified and characterized, and some genes from *B. subtilis* and *B. stearothermophilus* encoding mannanases were also cloned, sequenced and expressed [16]. Among these enzymes, 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 [17].

The biotechnological potential of mannan-hydrolysing enzymes, in particular the mannanases has been demonstrated within various industries. Industrially useful mannanase have recently attracted attention due to their role in the pulp and paper industry

to remove the hemicelluloses from pulps [18] and in pulp bleaching processes. This positive role has minimized the use of environmentally harmful bleaching chemicals in the pulp and paper industry [19]. Mannanases have potential application in animal feed production [20,21,22] and laundry detergents [23]. Bioconversion of agriculture waste containing mannan-based polysaccharides into valuable products such as animal feeds also required microorganisms capable of producing mannan degrading enzymes. Mannanases are also used for the extraction of vegetable oils from leguminous seeds and the clarification of fruit-juices in the food industry [24]. They are useful in reducing the viscosity of extracts during manufacture of instant coffee, chocolate and cacao liquor [25] to lower the cost for subsequent evaporation and drying [26]. Mannanases are potentially used in the pharmaceutical industry for the production of physiologically interesting oligosaccharides [3]. This study was carried out to screen bacterial strains of agricultural wastes origin for β-mannanases production and optimization of process parameters in solid state fermentation.

2. MATERIALS AND METHODS

2.1 Commercial Substrate and Chemicals

Locust Bean Gum (LBG) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2 Sample Sources

Nine agricultural wastes (pineapple peels, cassava peels, yam peels, groundnut shell, orange peels, potato peels, wheat bran, palm kernel cake and rice bran) were collected from farm fields, domestic sources and local market. The samples were blended and milled to obtain uniform particle size of under 0.5mm using sieve.

2.3 Bacterial Strains

Sixteen bacterial strains of *Klebsiella edwardsii* isolated from different agricultural wastes were obtained from stock culture of Research Laboratory, Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria. The bacterial strains were maintained on LBG containing agar plates and sub-cultured at regular intervals and stored at 4ºC in refrigerator on agar slants.

2.4 Screening of Bacterial Strains for β-mannanase Production

The bacterial isolates were screened for β-mannanase production under solid state fermentation. Enzyme production was performed in 250ml Erlenmeyer flask containing 110 ml of enzyme producing medium as subsequently described. For the screening of bacterial strains, 10g of LBG was suspended in mineral salt solution at moisture level of ratio 1 to 11 (10g of substrate to 110ml of mineral salt solution=9.09g of the substrate in 100ml mineral salt solution) [27]. The medium composition was as followed: 9.09% LBG, 0.1% peptone, 0.1% yeast extract, 0.2% $NaNO_3$, 1.4% KH_2PO_4, 0.06% $MgSO_4.7H_2O$, and 1% inoculums, pH 6.8. The flasks were incubated at 37°C for 24h at static condition. The crude enzyme was prepared by adding 10-fold (v/w) 0.1M phosphate buffer (pH 6.8) and shaking (180rpm) at 30ºC for 60min. The solid materials and bacterial cells were separated by centrifugation (6000rpm, 15min at 40ºC). The clear supernatant was used for enzyme assays and soluble protein determination.

2.5 Enzyme Assay

Mannanase activity was assayed in the reaction mixture composing of 0.5ml of 1% LBG prepared in 50mM potassium phosphate buffer (pH 6.8) and 0.5ml of the supernatant at 45ºC for 60min (modified from [4]). The control tube contained the same amount of substrate and 0.5ml of the supernatant previously heated at 100ºC for 15min. Both the experimental and control tubes were incubated at 45ºC for 60min. At the end of the incubation period, tubes were removed from the water bath (Lamfield Medical England Model DK-600), and the reaction was terminated by addition of 2ml of 3, 5- dinitrosalicylic acid (DNSA) reagent per tube. The tubes were incubated for 5 min in a boiling water bath for colour development and were cooled rapidly. The optical density was measured at 540nm to determine reducing sugars [28]. One unit of manganese activity was defined as amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.

2.6 Protein Determination

The amount of protein liberated in the fermentation media was evaluated according to the method designed by Lowry et al. [29] using Bovine Serum Albumin (BSA) as a standard.

2.7 Optimization of Culture Conditions for β-manganese Production by *K. Edwardsii* 1A

2.7.1 Effect of incubation period on β-mannanase production

In this study, fermentation was carried out up to 120h and mannanase activity was measured at 6h intervals. The enzyme was assayed according to the standard assay procedure [28].

2.7.2 Effect of different carbon sources on β-mannanase production

Effects of various carbon compounds namely: yam peels, wheat bran, groundnut shell, palm kernel cake, cassava peels, pineapple peels, potato peels, rice bran and orange peels were used in this study in comparison with LBG (commercial substrate). 10g of LBG suspended in mineral salt solution at moisture level of ratio 1 to 11 (10g of substrate to 110ml of mineral salt solution=9.09g of substrate in 100ml mineral salt solution) used for the screening of bacterial strains was substituted by equal amount of each carbon source. The enzyme was assayed according to the standard assay procedure [28].

2.7.3 Effect of different nitrogen sources on β-mannanase production

Evaluation of selected nitrogen sources was carried out to determine the appropriate nitrogen source for β-mannanase production by the *K. edwardsii* 1A. The fermentation medium was supplemented with other nitrogenous compounds (NH_4Cl, NH_4NO_3, yeast extract, whey, peptone, soybeans, urea and locust beans) at 0.2% level, replacing the prescribed inorganic nitrogen source (sodium nitrate) of the fermentation medium [12].

2.7.4 Effect of different sugar supplementation on β-mannanase production

Various sugars (0.2% w/v) were added to the enzyme production medium containing LBG in order to evaluate its induction or repression effect on mannanase production was tested [30].

2.7.5 Effect of pH and temperature on β-mannanase production

The most suitable pH for β-mannanase production was determined by adjusting the initial pH of the culture medium at different levels in the range of pH 4.0 to 9.0 using NaOH or HCl (All adjustments were made before sterilization). In order to determine the effective temperature for β-mannanase production by *K. edwardsii* 1A, the fermentation was carried out at 25ºC, 30ºC and 35ºC, 40ºC and 60ºC.

2.7.6 Effect of moisture content on β-mannanase production

The determination of optimum moisture content for β-mannanase production was evaluated by adjusting the ratio of the substrate to salt solution to 1:11, 1:15, 1:25, 1:35 and 1:40 respectively [27].

2.7.7 The effect of inoculum concentration on β-mannanase production

The best inoculum concentration for β-mannanase production by *K. edwardsii* was determined by inoculating the fermentation media with different inoculum concentrations ranging from 0.5 to 4.0% (v/v).

2.8 Statistical Analysis

Data presented on the average of three replicates (±SE) are obtained from their independent experiments. Experiment data were subjected to ANOVA of SPSS programming. Duncan's multiple range tests was used to identify significant differences between means of treatments.

3. RESULTS AND DISCUSSION

3.1 Screening of Bacterial Strains for β-mannanase Production

All the strains of *K. edwardsii* isolated from different agricultural wastes were able to produce extracellular β-mannanase in solid state fermentation although with differences in the rate of enzyme production. The highest β-mannanase activity of 103.200±0.96U/ml was obtained with *K. edwardsii* coded 1A followed by *K. edwardsii* 2B with an activity of 91.720±0.21, while the lowest was recorded for *K. edwardsii* 1B (3.060±0.06) (Table 1). Protein content ranged from 9.722±0.15mg/ml to 0.926±0.01mg/ml with the highest protein content lied on isolate *K. edwardsii* 1A. Therefore, *K. edwardsii* 1A was selected for further studies [12]. The variation between the strains of *K. edwardsii* for β-mannanase production might be attributed to the source of isolation and slight variation in their genetic makeup. 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 β-mannanase production causing variation in protein contents by the strains [31,32]. Mannanase activity had been reported in a variety of bacterial strains [3,9,27,30,32] but few data are available on mannanase activity of *K. edwardsii*. Out eleven bacteria isolated from hot spring and screened for β-mannanase by Harnentis and Maria [33], isolate SM-1.4 displayed highest enzyme activity of 119.44U/ml.

Table 1. Screening of bacterial strains of *Klebsiella edwardsii* for β-mannanase production in solid state fermentation

Isolate codes	Mannanase activity (U/ml)	Protein content (mg/ml)
1A	103.200^{r}±0.96	9.722^{q}±0.15
1B	3.060^{a}±0.06	0.926^{a}±0.01
1D	19.720^{hi}±0.21	2.403^{h}±0.02
2B	91.650^{p}±0.96	7.361^{n}±0.17
2C	83.510^{m}±0.12	2.222^{g}±0.10
3A	16.940^{g}±0.20	2.083^{t}±0.05
4A	19.170^{h}±0.03	1.806^{d}±0.01
4B	4.440^{b}±0.20	3.194^{j}±0.02
5A	20.560^{j}±0.92	1.292^{bc}±0.02
6A	15.830^{t}±0.37	1.944^{e}±0.04
7A	20.000^{ij}±0.03	1.847^{d}±0.01
8B	33.333^{l}±0.25	1.806^{d}±0.00
9B	13.060^{e}±0.51	3.194^{j}±0.01
9E	10.000^{d}±1.02	1.389^{c}±0.02
10B	9.720^{d}±0.00	1.292^{bc}±0.02
11B	5.000^{c}±0.50	1.250^{b}±0.01

Means with the same superscript letters along the same column are not significantly different (P<0.05). 1A=Klebsiella edwardsii 1A, 1B=K. edwardsii 1B, 1D=K. edwardsii 1D, 2B=K. edwardsii 2B, 2C=K. edwardsii 2C, 3A=K. edwardsii 3A, 4A=K. edwardsii 4A, 4B=K. edwardsii 4B, 5A=K. edwardsii 5A, 6A=K. edwardsii 6A, 7A=K. edwardsii 7A, 8B=K. edwardsii 8B, 9B=K. edwardsii 9B, 9E=K. edwardsii 9E, 10B=K. edwardsii 10B, 11B=K. edwardsii 11B

3.2 Effect of Incubation Period on β-mannanase Production

The optimization of the time course is of prime importance for mannanase biosynthesis by bacteria [34]. Since fermentation duration is crucial, it is also important to find out the optimum period for mannanase production. Data presented in Fig. 1 shows the effect of different incubation periods on β-mannanase activity and protein content of *K. edwardsii* 1A. From the results, it was found that *K. edwardsii* 1A revealed its best β-mannanase activity at 18h of incubation, while maximum protein content was attained at 24h of fermentation. Generally, there was an increase in β-mannanase activity and protein content with increase in fermentation periods and beyond the optimal period a decline was observed. The decrease observed in β-mannanase activity and protein content might be due to the depletion of nutrients and accumulation of other byproducts like proteases in the fermentation medium initiating autolysis of cells [3,27]. Harnentis and Maria [33] reported highest mannanase activity at 24h of incubation for *Bacillus* sp. whereas; 36h of fermentation was observed to be the optimum incubation period for β-Mannanase production by *Bacillus subtilis* [34].

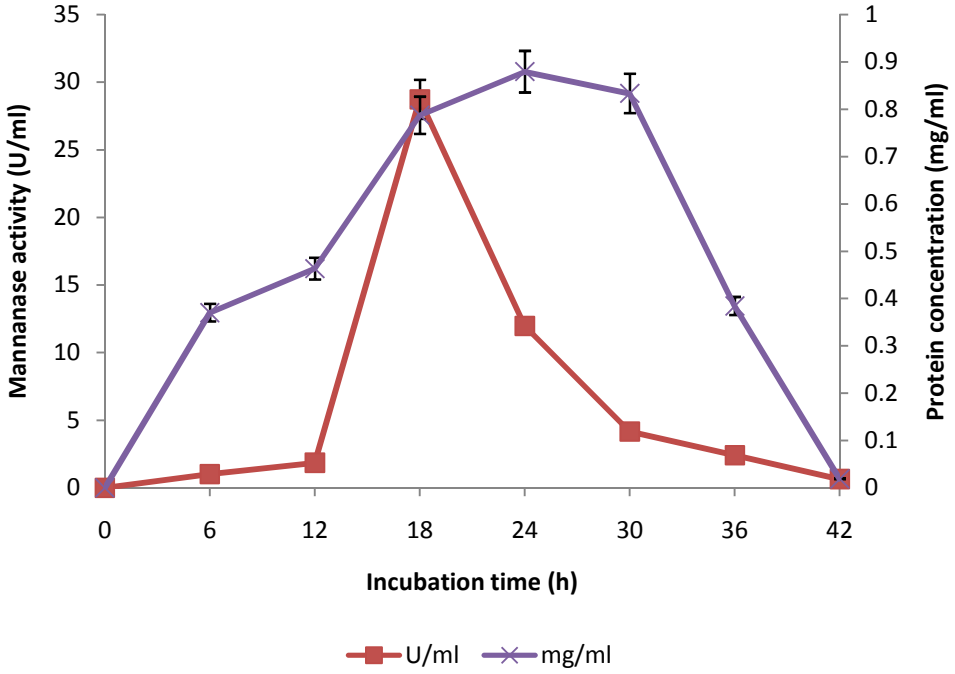

Fig. 1. Time course profile of β-mannanase activity and protein content by *Klebsiella edwardsii* 1A

3.3 Effect of Different Carbon Sources on β-mannanase Production

Substrate selection for enzyme production in a solid state fermentation (SSF) process depends upon several factors, mainly relating to substrate cost and availability and thus may involve screening several agro-industrial residues [35]. Table 2 shows that several types of agro-industrial by-products were evaluated as substrates for β-mannanase production by *K. edwardsii* 1A in comparison with LBG [12]. *Klebsiella edwardsii* 1A grew well on various raw materials of commercial potential with significant differences in the rate of β-mannanase production. The large variation in mannanase yield may be due to the nature of cellulose or hemicellulose, presence of some components (activators or inhibitors) in these materials and variations in the substrate accessibility [3,30]. Of all the substrates tested, pineapple peels were found to be the best substrate for β-mannanase production with maximum β-mannanase activity of 8.533±0.08U/ml. However, the value obtained for pineapple peels was significantly lower than the value obtained for LBG (12.333±0.01U/ml). It might be due to the fact that pineapple peels provided adequate amount of nutrients like proteins, carbohydrates, fats, fibers, ash, calcium, magnesium, phosphorous, potassium, sulphur, various amino acids and porosity for oxygen supply required by the organism for metabolic functions [3,36]. Potato peels was reported by Mabrouk and El Ahwany [30] to be the most effective carbon source for β-mannanase production, but it was not the case in our study.

Table 2. Effect of different carbon sources on β-mannanase production by *Klebsiella edwardsii* 1A

Carbon sources	Mannanase activity (U/ml)	Protein (mg/ml)
Yam peels	6.222^{d}±0.01	0.023^{a}±0.00
Wheat bran	4.294^{b}±0.03	0.556^{d}±0.00
Groundnut shell	2.439^{a}±0.02	0.833^{e}±0.00
Palm kernel cake	6.811^{f}±0.01	1.713^{g}±0.01
Cassava peels	5.994^{c}±0.07	0.926^{f}±0.01
Pineapple peels	8.533^{i}±0.08	0.278^{c}±0.01
Rice bran	6.728^{e}±0.01	0.042^{b}±0.00
Potato peels	7.922^{h}±0.01	0.023^{a}±0.00
Orange peels	7.133^{g}±0.00	0.278^{c}±0.01
LBG (control)	12.333^{j}±0.01	0.556^{d}±0.01

Means with the same superscript letters along the same column are not significantly different (P<0.05)

3.4 Effect of Different Nitrogen Sources on β-mannanase Production

In this study, different nitrogen sources were separately added to the fermentation medium at 0.2% concentration replacing sodium nitrate in minimal salt medium. Among the nitrogen sources tested; ammonium nitrate (NH_4NO_3) gave maximum β-mannanase activity of 1.533±0.00U/ml [12], while the lowest β-mannanase activity of 0.217±0.00U/ml was obtained for urea. However, 62.50% of the nitrogen sources tested had higher β-mannanase activity when compared with $NaNO_3$ (control) (Table 3). Highest β-mannanase activity obtained from NH_4NO_3 might be attributed to the ease of the organism to utilize nitrogen from it. Maximum enzyme activity was obtained with ammonium nitrate as a nitrogen source in a research carried out by Mabrouk and El Ahwany [30].

Table 3. Effect of different nitrogen sources on β-mannanase production by *Klebsiella edwardsii* 1A

Nitrogen sources	Mannanase activity (U/ml)	Protein (mg/ml)
Yeast extract	0.423^{c}±0.00	1.199^{d}±0.00
Whey	1.134^{g}±0.00	0.292^{a}±0.00
Peptone	0.248^{b}±0.01	0.940^{c}±0.00
NH_4Cl	0.887^{e}±0.01	1.384^{e}±0.00
Soybeans	0.963^{f}±0.00	2.232^{f}±0.00
Urea	0.217^{a}±0.00	0.514^{b}±0.00
Locust beans	1.273^{h}±0.00	3.255^{g}±0.38
NH_4NO_3	1.533^{i}±0.00	3.259^{h}±0.00
$NaNO_3$ (control)	0.607^{d}±0.00	3.393^{i}±0.00

Means with the same superscript letters along the same column are not significantly different (P<0.05)

3.5 Effect of Different Sugar Supplementation on β-mannanase Production

The effect of various sugars (0.2% w/v) supplemented to the enzyme production medium was investigated to evaluate its induction or repression effect on β-mannanase production [12] (Table 4). The highest activity was exhibited in medium in which no sugar was added (LBG control) and the association of additional different sugars with LBG was accompanied by severe inhibitory effects on enzyme production. Such results may be due to the catabolic

repression processes when easily assimilated carbon sources were added [30,37]. Similar results were observed in the research findings of Mabrouk and El Ahwany [30] when different sugars were supplemented to the enzyme production medium.

Table 4. Effect of different sugar supplementation on β-mannanase production

Sugars	Mannanase activity (U/ml)	Protein (mg/ml)
Maltose	16.852^{f}±1.11	0.139^{a}±0.00
Glucose	11.111^{e}±0.02	0.139^{a}±0.00
Mannose	1.389^{a}±0.00	0.556^{d}±0.00
Sucrose	9.444^{d}±0.13	0.370^{c}±0.00
Arabinose	2.500^{b}±0.00	0.324^{b}±0.00
Mannitol	3.333^{c}±0.00	0.787^{e}±0.01
Lactose	17.500^{g}±0.01	0.139^{a}±0.00
Galactose	11.111^{e}±0.00	0.139^{a}±0.00
LBG (control)	22.870^{h}±0.00	0.880^{f}±0.00

Means with the same superscript letters along the same column are not significantly different (P<0.05)

3.6 Effect of pH and Temperature on β-mannanase Production

The effect of initial pH (4.0-9.0) and incubation temperature on the culture for the biosynthesis of β-mannanase by *K. edwardsii* 1A was studied (Figs. 2 and 3). There was an increase in β-mannanase activity and protein content with increase in pH until the maximum activity of 309.074U/ml and protein content of 31.028mg/ml were reached at pH 6.0. Further increase in pH resulted in reduction of enzyme and protein biosynthesis by the organism. Enzyme production varies with changes in physical parameters such as temperature and pH of the production medium. Any change in these parameters induces morphological changes in microbes and in enzyme secretion [38]. The effect of pH is related to the growth and metabolic activities of the organism. A change in pH affects the ionization of essential active site amino acid residues that are involved in substrate binding and catalysis. The ionization of these residues may cause distortion of the active site cleft and hence may indirectly affect enzyme activity [38]. An optimum pH of 7.0 for *Bacillus amyloliquefaciens* [30]. *Bacillus* sp [33]. *Bacillus subtilis* ATCC3366 was reported [30,33,34]. The difference between our results and the previous reports might be related to the bacterial species.

To reveal the effect of different incubation temperatures on β-mannanase production by *K. edwardsii* 1A in solid state fermentation, experiments were conducted at 25ºC, 30ºC, 35ºC, 40ºC and 60ºC and β-mannanase activities were found to be 24.445, 41.110, 59.720, 55.000 and 22.102U/ml, respectively. Thus, maximum β-mannanase production was observed at 35ºC. Protein content obtained from different fermentation temperatures followed the same trend with enzyme activity, and this show that there was relationship between enzyme activities and protein contents. The influence of temperature on enzyme production is related to the growth of the organisms [39]. Incubation temperature is characteristic of an organism and profoundly affects the enzyme yield [40]. Different optimal temperatures for mannanase had been reported by many researchers. Mabrouk and Ahwany [30] reported 35ºC as the optimum temperature for mannanase production by *B. amyloliquefaciens*, Harnentis and Maria [33] reported 60ºC as the optimum incubation temperature for mannanase production by *Bacillus* sp, while Chin et al. [34] reported 55ºC as the optimum. Different optimal temperature reported by these workers suggested that enzyme production depends on strains variation of the microorganisms [41].

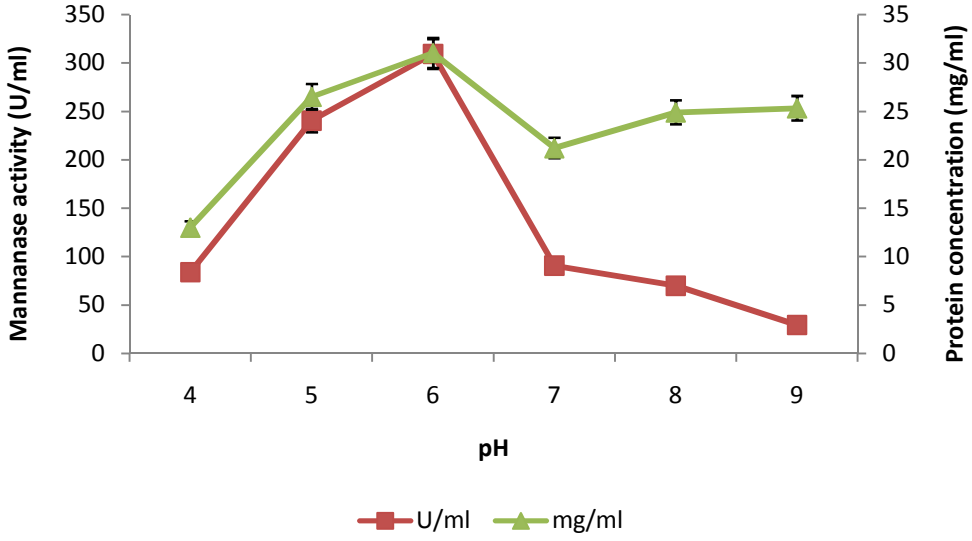

Fig. 2. Effect of pH values on β-mannanase production

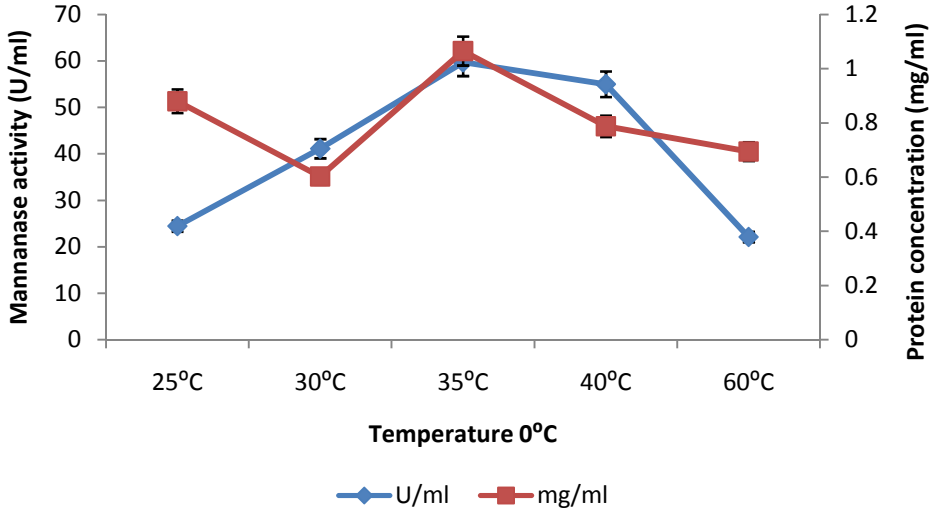

Fig. 3. Effect of incubation temperatures on β-mannanase production

3.7 Effect of Moisture Content on β-mannanase Production

The moisture content is an important factor that influences the growth and product formation in SSF [3,42]. Moisture is reported to cause swelling of the substrates, thereby facilitating better utilization of the substrate by microorganisms. The data presented in the Fig. 4 clearly indicates that the β-mannanase production by *K. edwardsii* 1A decreased with increase in moisture content from 1:11 to 1:40 with an optimum activity of 11.833U/ml obtained at 1:11 [12]. Protein content of *K. edwardsii* 1A decreased with increase in moisture content from

1:11 to 1:25, further increase in moisture content beyond this (1:25) resulted in an increase in protein yields. The decrease observed in enzyme activity with increase in moisture content might be due to decrease of inter-particle spaces which in turn caused decreased diffusion of nutrients [42].

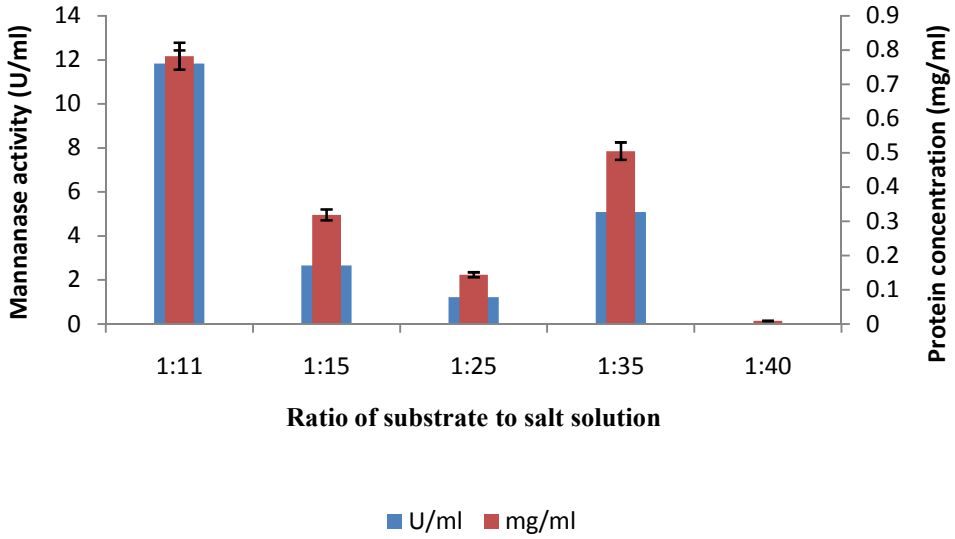

Fig. 4. Effect of moisture content on β-mannanase production

3.8 The Effect of Inoculum Concentration on β-mannanase Production

Inocula of different sizes (0.5 to 4.0%) introduced into fermentation media were tried out with respect to β-mannanase production (Table 5). The results indicate that enzyme production in the culture filtrate was affected by the inoculums concentration. The maximum mannanase yield and protein content (15.833±0.01) was noted when the cultured medium was provided with 1.0% inoculums [12]. An increase in the inoculum size to 1.0% would ensure increased mannanase yield by *K. edwardsii* 1A. However, after a certain limit, the competition for the nutrients resulted in a decrease of the metabolic activity of the organism. With optimum inoculum size, there was a balance between biomass synthesis and availability of nutrients that supports production of enzyme [30,43].

Table 5. Effect of inoculum concentration on β-mannanase production

Inoculum conc. (%v/v)	Mannanase activity (U/ml)	Protein concentration (mg/ml)
0.5	7.417^{b}±0.10	0.644^{a}±0.01
1.0	15.833^{d}±0.01	1.324^{c}±0.00
2.0	7.750^{c}±0.07	1.032^{b}±0.00
3.0	7.389^{b}±0.02	1.060^{b}±0.00
4.0	1.444^{a}±0.10	1.245^{c}±0.10

Means with the same superscript letters within the same column are not significantly different (p<0.05)

4. CONCLUSION

This study focuses on the screening of bacterial strains and optimization of culture parameters for the maximal production of crude extracellular β-mannanase from *K. edwardsii* 1A. Pineapple peels as carbon source, ammonium nitrate, 18h of incubation, 1:11 salt solution (ratio of substrate to salt solution) with initial medium pH of 6.0 at 35ºC were obtained to be optimal culture conditions for β-mannanase production by *K. edwardsii* IA. The optimal culture conditions obtained from this study will help to standardize the requirements for optimum production of β-mannanase using cheaper substrates; thereby contributing to better fish feed formulation incorporating plant ingredients, especially in the larval stages of fish fingerlings when the enzyme system is not efficient [44].

COMPETING INTEREST

Authors have declared that no competing interests exist.

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