Biotechnology Journal International

22(1): 1-8, 2018; Article no.BJI.6763
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Production of β-Mannanase by *Penicillium italicum* Subjected to Different Growth Conditions

O. O. Olaniyi[1*] and B. J. Akinyele[1]

[1]*Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.*

Authors' contributions

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

Article Information

DOI: 10.9734/BJI/2018/6763
Editor(s):
(1) Julius T. Dongdem, School of Biomedical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, UK.
(2) Dr. Joao Lucio Azevedo, Professor, Department of Genetics, University of Sao Paulo, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ / USP), Sao Paulo, Brazil.
(3) Dr. Rashedul Islam, Department of Biological Sciences, Inha University, South Korea.
(4) Faraj Barah, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.
(5) Dr. Eggehard Holler, Cedars-Sinai Medical Center, USA and University of Regensburg, Germany.
(6) Dr. En Tao Wang, Professor, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México and State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China.
(7) Kuo-Kau Lee, Professor, Department of Aquaculture, National Taiwan Ocean University, Keelung, 2, Pei-Ning Road, Taiwan.
Reviewers:
(1) I. O. Abdullahi, Ahmadu Bello University, Nigeria.
(2) A.O. Ajayi, University of Fort Hare, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/27055

Original Research Article

Received 06 September 2013
Accepted 26 May 2014
Published 05 November 2018

ABSTRACT

Aims: The process parameters affecting enzyme production were optimized to ascertain the best optimal conditions for β-mannanase production by *Penicillium italicum* in solid state fermentation.
Study Design: Four stages of experimental processes were designed for this study. The first experiment, samples were withdrawn after 24, 48, 72, 96, 120, 144,168 and 192 h incubation. In second experiment, the fermentation media were incubated at different temperatures. In third experiment, the effect of different pH values on β-mannanase production was evaluated, while the fourth experiment described the supplementation of surfactants in mineral salt solution for β-mannanase production.
Place and Duration of Study: Microbiology Research Laboratory, Federal University of Technology, Akure Nigeria between September 2011 and March 2012.

**Corresponding author: E-mail: microladit@gmail.com;*

Methodology: β-mannanase production was conducted using Locust Bean Gum (LBG) as the sole carbon source; moisten with mineral salt solution, and enzyme activity determined by dinitrosalicylic acid method, while protein content was determined by Lowry method.
Results: Maximum enzyme activity (146.389 U/ml) was observed after 72 h of incubation. Different surfactants were supplemented in the basal medium, and Sodium Dodecyl Sulfate (SDS) was observed to give the highest β-mannanase activity of 53.335 U/ml. Initial pH of the culture medium was optimized and a pH of 6.0 was found to support maximum enzyme activity (173.241 U/mg protein). The optimum incubation temperature was achieved at 35°C.
Conclusion: The results obtained provide information on optimal process parameters that might improve the yield of β-mannanase by *P. italicum* for better fish feed formulation, especially in the larval stages of fish fingerlings when the enzyme system is not efficient.

Keywords: β-mannanase; solid state fermentation; agrowastes; optimization; Penicillium italicum.

1. INTRODUCTION

Hemicelluloses are complete polysaccharides consisting of linear and branched chain in the cell walls of higher plants which are closely associated to the cellulose and lignin forming lignocelluloses biomass [1]. Mannan is the most abundant polysaccharide presented in softwood hemicelluloses. Manno-oligosaccharides are generated when mannan is hydrolysed by the combination of β-mannanase (EC.3.2.78), β-mannosidase (EC. 3.2.1.25) and β-glucosidase (EC.3.2.21) with debranching enzymes such as galactosidase (EC.3.2.1.22) and acetyl esterase (EC.3.1.1.6) [2,3,4].

Filamentous fungi are commonly used for enzyme production in solid state fermentation (SSF) since they have the ability to secrete large amount of protein into growth medium [5]. Solid state fermentation (SSF) can be defined as any fermentation process performed in the absence or near absence of free water, employing a natural substrate or an inert support [6]. It was reported that SFF technique is yet to be explored for mannanase production [7,8,9,10,11,12] despite its many advantages such as lower capital investment, improved product recovery and easy downstream processing etc. over the conventional submerged process [13,14].

Mannanase enzyme is important in paper industry including bioleaching pulp [12], waste bioconversion of biomass to fermentable sugars [15], increasing the quality of feed quality [16] and reduces viscosity of coffee extracts [17]. Major limiting factors on the commercial use of this enzyme are low activity and high cost of the available enzyme production. This has necessitated a renewed search for mannolytic organisms with novel mannanase properties and strategies for low cost-cost enzyme production. In search of viable mannolytic organisms, we isolated different mannolytic microfungi from agricultural wastes in Akure, Nigeria, in which *Penicillium italicum* gave highest activity under solid state fermentation [18,19]. In the present study, *P. italicum*, a promising candidate for mannanase production, was subjected to different growth conditions under solid state fermentation.

2. MATERIALS AND METHODS

2.1 Fungal Isolate

Penicillium italicum was obtained from bank's stock cultures of Research Laboratory, Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria. The organism was previously confirmed positive for mannanase activity by plate assay in our previous work [19]. The identity of this organism was confirmed using the method designed by Pitt and Hocking [20] on the bases of cultural characters (colour, shape of colony, surface and reverse pigmentation and texture of the colony) as well as microscopic structure (septate or nonseptate hyphae, structure of hyphae and conidia). The fungal isolate was maintained on Locust Bean Gum (LBG) containing agar plates and sub-cultured at regular intervals. It was incubated at 30±2°C until the entire plates were covered by active mycelium and stored at 4°C in refrigerator on agar slants.

2.2 Chemicals and Substrates

The selected agro-wastes (pineapple peels, cassava peels, yam peels, groundnut shell, orange peels, potato peels, wheat bran, palm kernel cake, fermented coconut and rice bran) utilized as carbon sources were procured from farm field, local market and domestic sources. The substrates were washed, sun-dried and

oven-dried at $70^{o}C$ with Model DHG Heating Drying Oven for a period of 2 h, sieved to 40 mm mesh size and stored in air tight transparent plastic containers to keep it moisture free. LBG was purchased from Sigma Chemicals (St. Louis, MO). All other chemicals were of analytical grade.

2.3 Mannanase Production

For the production of β-mannanase in solid state fermentation, the isolate was cultured at $30^{o}C$ in 250 ml Erlenmeyer flasks containing 10 grams of the coarsely ground copra meal. The substrate was suspended in 33 ml modified Mandels and Weber's medium [21]. This medium (moistening agent) contained the following ingredients (g/L): Peptone 2, yeast extract 2, $NaNO_3$ 2, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.5, KCl 0.5 and $FeSO_4.7H_2O$ traces. After sterilization at 121°C for 15 min, it was cooled and inoculated with 10 discs of 8 mm diameter of the organism from LBG culture plates using sterile cup borer. The flask was incubated at 30°C for 5 days at static condition.

2.4 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 $30^{o}C$ for 60 min. The solid materials and fungal biomass were separated by centrifugation (Centurion Scientific Limited) (6000 rpm, 15 min at $4^{o}C$). The clear supernatant was used for enzyme assays and soluble protein determination. Each treatment was carried out in triplicates and the results obtained throughout the work were the arithmetic mean of at least 3 experiments.

2.5 Enzyme Assays

Beta-mannanase activity was assayed in the reaction mixture composing of 0.5 ml of 1% LBG prepared in 50 mM potassium phosphate buffer pH 6.8 and 0.5 ml of supernatant at 45ºC for 60 min (modified from El-Naggar et al. [21]). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) [22]. One unit of mannanase 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 of Lowry et al. [23] using Bovine Serum Albumin (BSA) as the standard.

2.7 Optimization of Process Parameters

2.7.1 Effect of incubation period on β-mannanase production

In this study, the fermentation experiment was carried out up to 192 h and production rate was measured at 24 h intervals. Mannanase assay was carried out according to standard assay procedures [21].

2.7.2 Effect of incubation temperature on β-mannanase production

In order to determine the optimum temperature for β-mannanase production by *P. italicum,* the fermentation medium was incubated at 25, 30, 35 and 40°C for 5 days. After 5 days of cultivation at different incubation temperatures, mannanase assay was determined according to standard assay procedures [21].

2.7.3 Effect of pH on β-mannanase production

The most suitable pH of the fermentation medium was determined by adjusting the 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). After inoculation, the flasks were incubated under static condition for 5 days at $30^{o}C$. After 5 days of incubation, the culture was centrifuged to obtained supernatants which were used for mannanase assay and protein content determination according to standard assay procedures [21].

2.7.4 Effect of surfactants on β-mannanase production

The effect of surfactants (Tween-80, Tween-20 and SDS) was evaluated on the production of mannanase in solid state fermentation. This was carried out by supplementing fermentation media with each of the surfactant at 0.2% v/v level [24].

3. RESULTS AND DISCUSSION

3.1 The Effect of Incubation Period on Β-Mannanase Production

Mannanase activity in the culture increased regularly during the first 72 h of incubation and the highest mannanase activity (146.389 U/ml) was obtained at the 72 h (Fig. 1). At longer incubation periods, the activity decreased

gradually and after 168 h the mannanase activity was 46.8% lower than the activity obtained at 72 h of incubation. The highest productivity reached the maximum of 18.229 U/ml/h at 72 h incubation showing that there was relationship between mannanase activity and production of mannanase. The optimization of the time course is of prime importance for β-mannanase biosynthesis by fungi [21]. The decrease in the production of β-mannanase by *P. italicum* after 72 h of incubation might be due to the depletion of nutrients and accumulation of other by-products like proteases in the fermentation medium initiating autolysis of cells [25,26]. The β-mannanase activity and productivity reached the maximum of 41.1 U/ml (at 72 h) and 571.1 U/l/h, respectively, when *Penicillium ocittanis* was used as mannanase producer [27]. Beta-mannanase activity reached its peak at day 17 (3.166 U/ml) and day 8 (2.482 U/ml) for *Sclerotium rolfsii* and *Aspergillus niger* respectively [28].

3.2 The Effect of Temperature on β-Mannanase Production

The effect of different operating temperatures is shown in Fig. 2. The maximum β-mannanase activity was observed at 35°C (61.945 U/ml). At higher or lower temperatures, the enzyme activity in the culture media showed a lower value. Medium temperature plays an important role in enzyme production. The optimum temperature of 35°C obtained for β-mannanase production by *P. italicum* is identical to that reported previously [10]. However, the optimum temperature may varied in different fungi since 30°C was reported [29] for mannanase production by *Penicillium oxalicum*.

3.3 The Effect of Initial pH on β-Mannanase Production

Penicillium italicum showed maximal mannanase activity at pH 6.0, although significant levels of mannanase activities were recorded at other pH (Fig. 3). An increase in the initial pH of growth medium up to 6.0 increased the production of mannanase. Production declined upon further increases in pH beyond pH 6.0. Microbial enzymes synthesis at pH 4.0 and 9.0 agreed with the previous observation [30] that low or high pH values inactivate enzyme and may affect its production. The optimum pH for the growth of fungi has been reported to vary from one organism to another. The optimum pH 4.0 to 5.5 was reported for *A. terreus* [31]; pH 5.0 to 6.0 for *A. niger* [21] and pH 5.0 was reported for *Penicillium oxalicum* [29].

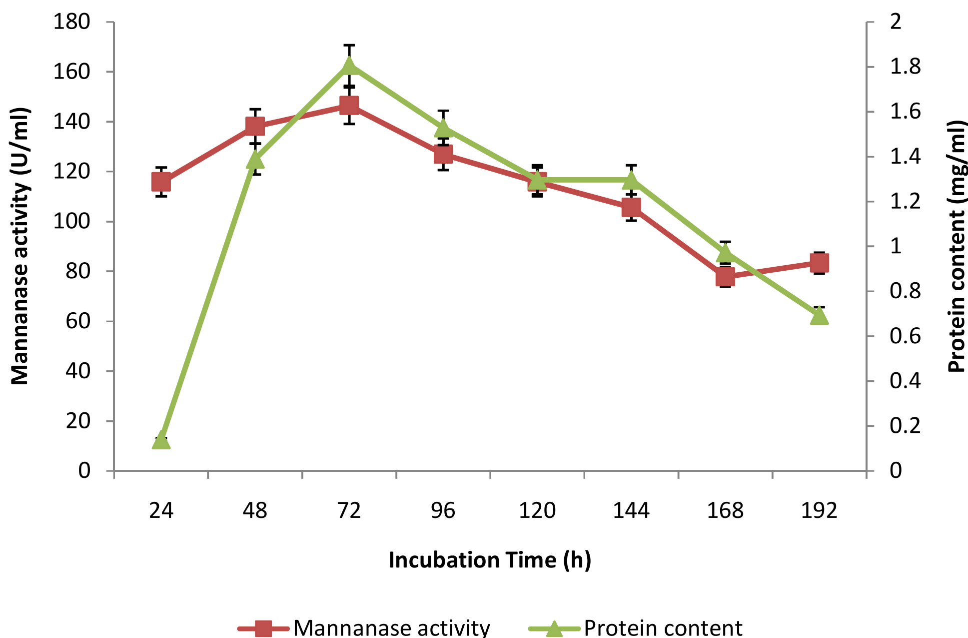

Fig. 1. Time course profile of β-mannanase activity and protein production of *P. italicum*

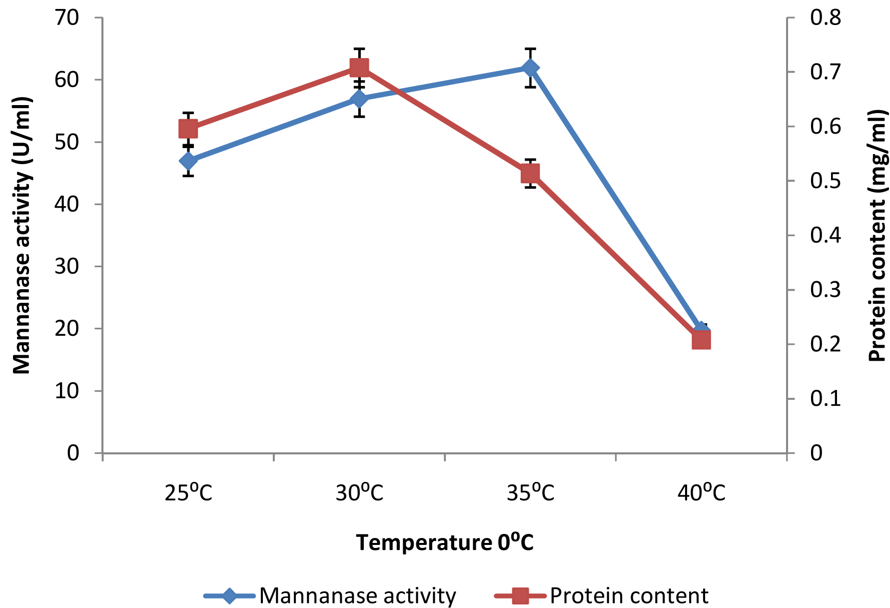

Fig. 2. Effect of different incubation temperature on β-mannanase activity and protein production of *P. italicum*

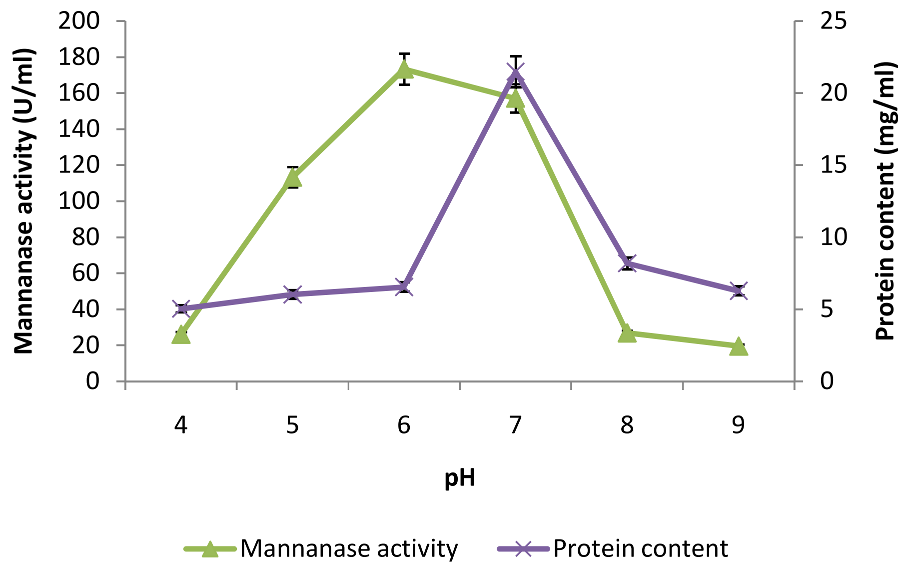

Fig. 3. Effect of different pH values on β-mannanase activity and protein production of *P. italicum*

3.4 The Effect of Surfactants on Β-Mannanase Production

The β-mannanase production was improved when basal media was supplemented with surfactants. The highest β-mannanase activity of 53.335 U/ml was achieved in the medium supplemented with SDS, while others had considerable activities (Fig. 4). Surfactants in the fermentation medium are known to increase the secretion of proteins and positively influence enzyme activity by increasing cell membrane permeability. Thus, induction in β-mannanase production in this study could be attributed to increase cell permeability which in turn facilitated high diffusion rate across the cell membrane [32].

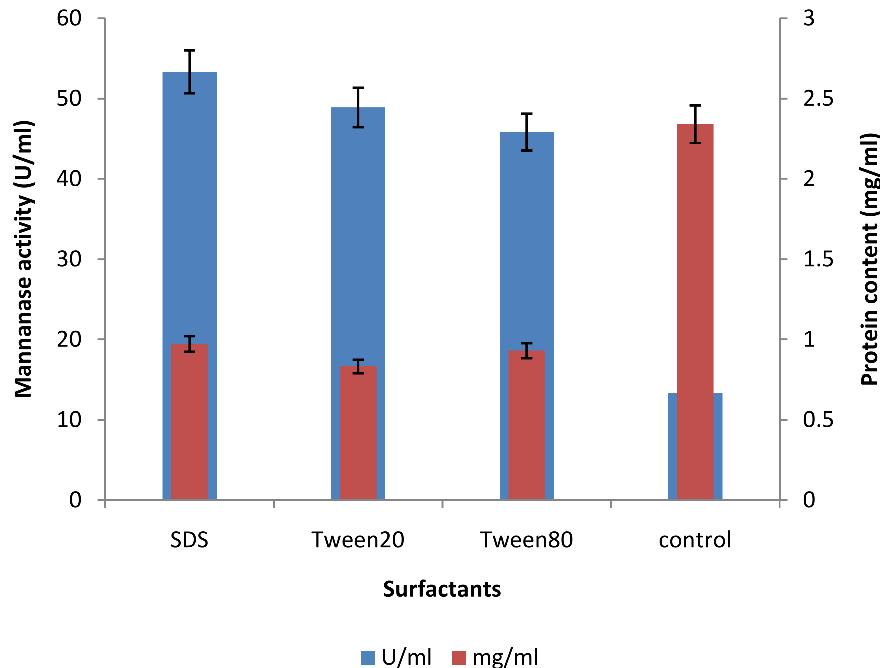

Fig. 4. Effect of surfactants on β-mannanase activity and protein production of *P. italicum*

4. CONCLUSION

The results obtained from this study provide information on optimal process parameters that might improve the yield of β-mannanase by *P. italicum* for better fish feed formulation, especially in the larval stages of fish fingerlings when the enzyme system is not efficient. The optimal growth factors for the production of β-mannanase were proposed at incubation period of 72 h, pH 6.0 and 35°C and supplementation of SDS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moreira LR, Filho EX. An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol. 2008;79:165-78.
2. Gubitz GM, Hayn M, Sommerauer M, Steiner W. Mannan-degrading enzymes from *Sclerotium rolfsii*: Characterization and synergism of two endo β-mannanases and a β-mannosidase. Biores Technol. 1996;58:127-35.
3. Singh S, Madlala AM, Prior BA. *Thermomyces lanuginosus*: Properties of strains and their hemicellulases. FEMS Microbiol Rev. 2003;27:3-16.
4. Petkowicz CLO, Schaefer S, Reicher F. The mannan from *Schizolobium parahybae* endosperm is not a reserve polysaccharide. Carbohydr Polym. 2007; 69:659-64.
5. Van Zyl PJ, Moodley V, Rose SH, Roth RL, Van Zyl WH. Production of the *Aspergillus aculeatus* endo-1,4-β-mannanase in *A. niger*. J Ind Microbiol Biotechnol. 2009;36:611-17.
6. Sabu A, Augur C, Swati C, Pandey A. Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. Process Biochem. 2006;41:575-80.
7. Noraini S, Vikineswary S, Daud MJ, Ibrahim A, Aman A, Sevagam P. Biosynthesis of β-mannanase from *Aspergillus niger* by solid substrate fermentation (SSF) using palm kernel cake as the basis of solid substrate. Proceedings of the international Seminar on Solid State Fermentation, October 4-6, 2004, Penang, Malaysia. 2004;175-6.
8. Ong LGA, Abd-Aziz S, Noraini S, Karim MIA, Hassan MA. Enzyme production and profile by *Aspergillus niger* during solid

substrate fermentation using palm kernel cake as substrate. Appl Biochem Biotechnol. 2004;118:73-9.
9. Regulapati R, Malav NP, Gummadi NS. Production of thermostable a-amylases by solid state fermentation-A review. Am J Food Technol. 2007;2:1-11.
10. Abd-Aziz S, Hung GS, Hassan MA, Karim MIA, Samat N. Indirect method for quantification of cell biomass during solid state fermentation of palm kernel cake based or protein content. Asian J Sci Res. 2008;1:385-93.
11. Norita SM, Rosfarizan M, Ariff AB. Evaluation of the activities of concentrated crude mannan-degrading enzymes produced by *Aspergillus niger*. Malaysian J Microbiol. 2010;6:171-80.
12. Jahwarhar IA, Noraini S, Wan MWY. Screening and optimization of medium composition for mannanase production by *Aspergillus terreus* SUK-1 in solid state fermentation using statistical experimental methods. Res J Microbiol. 2012;7(5):242-55.
13. Pandey A, Soccol CR, Rodriguez-Leon JA, Nigam P. General considerations about Solid-State Fermentation Processes. In: Solid State Fermentation in Biotechnology: Fundamentals and Applications, Pandey, A., Soccol, C.R., Rodriguez-Leon, J.A. and Nigam, P. (Eds.). Asiatech publishers, New Delhi. 2001;11-8.
14. Naveena BJ, Vishnu C, Altaf M, Gopal R. Wheat bran an inexpensive substrate for production of lactic acid in solid state fermentation by *Lactobacillus amylophilus* GV6. Optimization of fermentation conditions. J Sci Ind Res. 2003;62: 453-6.
15. Chandrakant P, Bisaria VS. Simultaneous bioconversion of cellulose and hemicellulose to ethanol. Crit Rev Biotechnol. 1998;18:295-331.
16. Marini AM, Yatim AM, Babji AS, Annuar BO, Noraini S. Evaluation of nutrient contents and amino acid profiling of various types of PKC. J Sci Technol Trop. 2006;2:135-41.
17. Hagglund P, Eriksson T, Collen A, Nerinckx W, Claeyssens M, Stalbrand H. A cellulose-binding module of the *Trichoderma reesei* β-mannanase Man5A increases the mannan-hydrolysis of complex substrates. J Biotechnol. 2003; 101:37-48.
18. Akinyele BJ, Olaniyi OO, Adetunji CO. Screening and optimization of nutritional conditions for mannanase production by *Penicillium italicum* LAD-A5 in solid state cultivation. E3 J Biotechnol Pharm Res. 2013;4(2):35-41.
19. Arotupin DJ, Olaniyi OO. Screening and identification of mannanase-producing fungi isolated from selected agricultural wastes. Brit Microbiol Res J; 2013. (In press).
20. Pitt JI, Hocking AD. Fungi and food spoilage, 2th Edition, Blacki Academic, London, U.K. 1997;414.
21. El-Naggar MY, Youssef SA, El-Assar SA, Beltagy EA. Optimization of cultural conditions for β-mannanase production by a local *Aspergillus niger* isolate. Int J Agri Biol. 2006;8(4):539-45.
22. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem. 1959;31:426-8.
23. Lowry OH, Rosebrough JN, Farr LA, Randali JR. Protein measurement with the Folin-Phenol reagents. J Biol Chem. 1951; 193:265-75.
24. Ulger C, Curakoglu C. α-Amylase production by *Bacillus substilis* and *B. amyloliquefaciens* in different PEG solutions. World J Microbiol Biotechnol. 2001;17:93-4.
25. Meenakshi M, Singh G, Bhalla A, Hoondal GS. Solid state fermentation and characterization of partially purified thermostable mannanase from *Bacillus* sp. MG-Biores. 2010;5(3):1689-701.
26. Malik S.K, Mukhtar H, Farooqi AA, Haq I. Optimization of process parameters for the biosynthesis of cellulases by *Trichoderma viride*. Pak J Bot. 2010;42(6):4243-51.
27. Blibech M, Ghorbel RE, Chaari F, Dammak I, Bhiri F, Neifar M, Chaabouni SE. Improved mannanase production from *Penicillium occitanis* by fed-batch fermentation using acacia seeds. Int Sch Res Network. 2011;2011:938347.
Available:http://dx.doi.org/10.5402/2011/938347
28. Abd-Aziz S, Ab-Razak NA, Musa MH, Hassan MA. Production of mannan-degrading enzaymes from *Aspergilllus niger* and *Sclerotium rolfsii* using palm kernel cake as carbon source. Res J Environ Sci. 2009;3(2):251-6.

29. Steiner J, Socha C, Eyzagiurre J. Culture conditions for enhanced cellulose production by a native strain of *Penicillium purpurogenum.* World J Microbiol Biotechnol. 1994;10:280-4.
30. Chantorn ST, Buengsrisawat K, Pokeseam A, Sombat T, Dangpram P, Jantawon K, Nitisinprasert S. Optimization of extracellular mannanase production from *Penicillium oxalicum* KUB-SN2-1 and application for hydrolysis property. Songklanakarin J Sci Technol. 2013;35(1): 17-22.
31. Pushelkar S, Rao KK, Menon K. Production of beta-glucosidase by *Aspergillus terreus.* Curr Microbiol. 1995; 30:255-8.
32. Sivaramakrishnan S, Gangadharan D, Madhavan K, Nampoothiri C, Soccol R, Pandey A. α-Amylase from microbial source. Food Technol Biotechnol. 2006; 44(2):173-84.

© 2018 Olaniyi and Akinyele; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27055