MANNANASE PRODUCTION BY CO-CULTURE OF *PENICILLIUM ITALICUM* AND *TRICHOSPORONOIDES OEDOCEPHALIS* IN SOLID STATE FERMENTATION

Oladipo Oladiti OLANIYI[*], Juliet Bamidele AKINYELE

Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria

Abstract

The study evaluated various process parameters for mannanase production using co-culture of *Penicillium italicum* and *Trichosporonoides oedocephalis* under solid state fermentation. Different fermentation parameters affecting enzyme production were optimized using one-factor-at-a-time approach. Mannanase production was conducted in mineral salt medium into which copra meal (defatted coconut residual cake) had been incorporated as the sole carbon source and enzyme activity was evaluated. Maximum mannanase activity (144.444 U/mL) was obtained after 96 h of incubation in solid state fermentation. Different agro-wastes were screened as carbon sources for enzyme production in comparison to locust bean gum (control). Among tested carbon sources, orange peels proved to be the best substrate for mannanase production. Ammonium nitrate was observed to be the best nitrogen source with highest activity of 84.444 U/mL out of all the nitrogen sources screened. Also, for enzyme production the optimum temperature and percentage moisture content were 30°C and 60%, respectively.

Keywords: mannanase, agro-wastes, process parameters, locust bean gum, carbon and nitrogen sources selection

Introduction

There is a considerable interest in the biological degradation of lignocelluloses as the most abundant reusable resources 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 (Sachslehner *et al.,* 1998; Lee *et al*., 2011). Various mannanases from *Streptomyces* spp. (Ademark *et al*., 1998; Takahashi *et al*., 1984), *Bacillus* spp (Phothichitto *et al*., 2006; Mabrouk and El Ahwany, 2008), *Sclerotium rolfsii* (Sachslehner *et al*., 1998), *Aspergillus awamori* (Kurakale and Komaki, 2001) and *Trichoderma harzianum* (Ferreira and Filho, 2004) have been produced, and some genes from *Bacillus subtilis* and *Bacillus stearothermophilus* encoding mannanases were also cloned, sequenced and expressed (Mendoza *et al*., 1995; Ethier *et al.,* 1998). The two most important and representative

[*]Corresponding author: microladit@gmail.com

Olaniyi, Akinyele: *Manannase production by co-culture* **Innovative Romanian Food Biotechnology** (2014) 14, 46-53
*of Penicillium italicum and Trichosporonoides
oedocephalis in solid state fermentation*

hemicelluloses are the hetero-1,4-β -Dxylans and the hetero-1,4-β -D-mannans. Endo -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 (Lee *et al*., 2011).

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. 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*., 2003). 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), production of animal feed (Lee *et al*., 2005; Wu *et al*., 2005) and laundry detergents (McCoy, 2001).

Their effective role in pulp bleaching processes minimized the use of environmentally harmful bleaching chemicals in pulp and paper industry (Meenakshi *et al*., 2010). Mannanases have been used in the food industry for the extraction of vegetable oils from leguminous seeds and the clarification of fruit juices (Mabrouk and El Ahwany, 2008). The enzyme also can be used in the reduction of the viscosity of extracts during manufacturing of instant coffee, chocolate, and cacao liquor (Francoise *et al*., 1996; 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 (Phothichitto *et al*., 2006).

Co-culturing had been reported by few researchers and established for other extracellular enzymes biosynthesis with no information on co-culture of potential strains for enhanced mannanase production. This had been reported to enhance enzyme production. In our earlier studies, *Penicillium italicum* and *Trichosporonoides oedocephalis* were screened using plate assay technique (Arotupin and Olaniyi, 2013) and thereafter cultural conditions were optimized, and

they were observed to give considerable mannanase activities. In this present study, these two fungal strains were co-cultured to see if it could enhance their mannanase production ability than when individual producer strain was used separately. The objective of the study is to evaluate the effect of variation of certain process parameters on extracellular mannanase biosynthesis yield in solid state fermentation.

Materials and Methods

Microorganisms

Penicillium italicum and *Trichosporonoides oedocephalis* strains (Arotupin and Olaniyi, 2013) previously confirmed to be positive for mannanase activity were obtained from the Research Laboratory, Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria. The fungal isolates were maintained on locust bean gum (LBG) agar plates and sub-cultured at regular intervals. They were incubated at 30 ±2°C until the entire plates were covered by active mycelium and stored at 4°C in refrigerator on agar slants.

Chemicals and substrates

The selected agro-wastes (yam peels, wheat bran, groundnut shell, palm kernel cake, cassava peels, pineapple peels, potato peels, rice bran and orange peels) utilized as carbon sources were procured from farm field, local market and domestic sources. The substrates were washed and oven-dried at 70°C with 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 (Iqbal *et al*., 2010). LBG was purchased from Sigma Chemicals (St. Louis, MO). All other chemicals were of analytical grade.

Molds cultivation and mannanase production

For the production of mannanase in solid state fermentation system, the molds strains were co-cultivated at 30°C in 250 mL Erlenmeyer flasks containing 10 grams of the coarsely ground copra meal (10g of substrate to 33 mL of mineral salt solution). Mandels and Weber's Medium modified by El-Naggar *et al*. (2006) was used to adjust the

moisture content from 20% to 80%. 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, pH 6.8. After sterilization at 121 °C for 15 min, two agar blocks each of the test organisms were used as inocula. The flasks were incubated at 30°C for 5 days at static condition.

Optimization of fermentation conditions

Effect of time of cultivation on mannanase production

The fermentation was carried out up to 192 h and enzyme production yield was measured at 24 h intervals. Mannanase assay was carried out according to standard assay procedures (El-Naggar *et al*., 2006).

Effect of agro-waste (carbon sources) on mannanase production

Effect of various carbon compounds orange peels, yam peels, wheat bran, rice bran, cassava peels, pineapple peels, potato peels, groundnut shell and palm kernel cake were evaluated comparing with LBG serving as a control. A quantity of 10 grams of each carbon sources were suspended in 33 mL Mandels and Weber's medium before inoculation of the strains and after culture inoculation (two agar blocks each of the test organisms), the flask were incubated for 5 days (El-Naggar *et al*., 2006).

Effect of different nitrogen sources on mannanase production

The appropriate nitrogen source for mannanase production by co-culture of *P. italicum* and *T. eodocephalis* was determined by supplementing the fermentation medium with different nitrogen sources (soya beans, locust beans, yeast extract, whey, peptone, urea and ammonium chloride) at 0.2% level, replacing the prescribed $NaNO_3$ of the fermentation medium (moistening agent) (El-Naggar *et al*., 2006).

Effect of temperature on mannanase production

In order to determine the optimum temperature for mannanase production by co-culture of *P. italicum* and *T. oedocephalis*, the fermentation medium was incubated at 25, 30, 35 and 40 °C for 5 days. After 5 days of cultivation at different incubation temperature, mannanase assay was determined according to standard assay procedure (El-Naggar *et al*., 2006).

Percentage moisture content of the semisolid-medium

The determination of optimum percentage moisture content for mannanase production was evaluated by adjusting the moisture content to 20, 40, 50, 60 and 80% with moisturizing agent (Mandels and Weber's medium). After moisture content adjustment, the fermentation flasks were inoculated with two agar blocks each of the test organisms and the flasks were incubated under static condition for 5 days at 30°C. After 5 days of cultivation, enzyme assay was carried out (El-Naggar *et al*., 2006).

Enzyme extraction

The solid state fermented medium was mixed by adding 10-fold (v/w) 0.1 M phosphate buffer (pH 6.8) and shaking (180 rpm) at 30°C for 60 min. The solid materials and fungal biomass were separated by centrifugation at 6000 rpm for 15 min at 4°C using refrigerated centrifuge (Model KBM-70, Centurion Scientific Limited, Germany). The clear supernatant was used as crude extract for enzyme assay and soluble protein determination.

Enzyme assay

Mannanase activity of supernatant collected at the end of each optimization step was determined using Spectrophotometer (Lab-Tech Digital Spectrophotometer, Germany) by the method of El-Naggar *et al.* (2006). The reaction mixture contained 1mL of 1% LBG dissolved in 50 mM phosphate buffer pH 6.8 and 1 mL enzyme solution. The control tube contained the same amount of substrate and 1 mL of the enzyme solution heated at 100°C for 15 min. Both the experimental and control tubes were incubated 40°C in a water bath (Lamfield Medical England Model DK-600) for 5 min. At the end of the incubation period, the reaction was terminated by the addition of 2 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube (Miller, 1959), after cooling. The activity of reaction mixture was measured against a reagent blank at 540 nm. The

Olaniyi, Akinyele: *Manannase production by co-culture* **Innovative Romanian Food Biotechnology** (2014) 14, 46-53
*of Penicillium italicum and Trichosporonoides
oedocephalis in solid state fermentation*

released mannose due to mannanase activity was determined by DNS method (El-Naggar *et al*., 2006). One unit of mannanase activity is defined as the amount of enzyme, which produced 1 mol/mL/min of mannose under standard assay conditions.

Statistical analysis

Each treatment was carried out in triplicates and the results obtained throughout the work were the arithmetic mean of at least 3 experiments. Data presented on the average of three replicates ($\pm$ SE) are obtained from their independent experiments. Experiment data was subjected to ANOVA of SPSS software (Microsoft Corporation, USA). Duncan s multiple range tests was used to identify significant differences between means of treatments (P$\leq$ 0.05).

Results and Discussion

Time course profile (Figure 1) for extracellular mannanase production was studied from 24 to 192 h of solid state cultivation. The production of enzyme increased with increase in fermentation period and reached maximum (144.444 U/ml) at 96 h of cultivation whereas in our previous studies when each of the organism was used separately highest mannanase activities of 146.389 U/mL at 72 h and 149.073 U/mL at 96 h were obtained for *P. italicum* and *T. oedocephalis* respectively. Subsequent increase beyond the optimum time (96 h) resulted into a decline in enzyme production. A decrease in activity is always found to be associated with depletion of nutrients or accumulation in the media of some metabolites with inhibition effect on microorganism (Ikram-Ul-Haq *et al*., 2005; Iqbal *et al*., 2010). In addition, the medium components were initially more susceptible to fungal metabolism and make a rapid rise in enzyme biosynthesis. But with prolongation of time of cultivation, the easily digestible portions of the substrate were completely hydrolyzed by microorganism which inhibited the enzyme secretion pathways (Gautam *et al.,* 2010). Other related factors, such as the nature of the microorganism and the physiological conditions of the media, are also considered to be important

during enzyme production (Narasimh, 2006; Malik *et al*., 2010). The highest protein content was also observed at 96 h of incubation to show that there was direct relationship between the protein content of the tested culture and the production of enzyme.
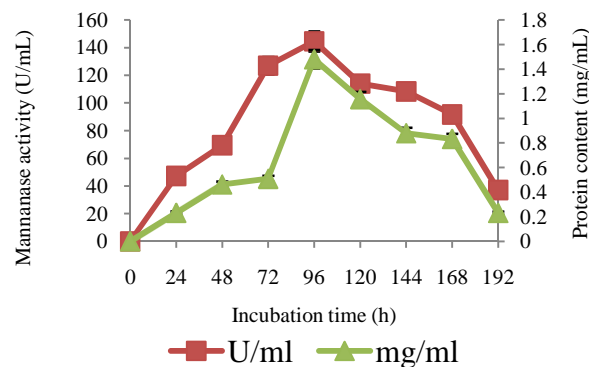
Figure 1. *Time course profile of mannanase production and protein content of co-culture of P. italicum and T. oedocephalis in solid state fermentation cultivation system*

Data presented in Table 1 shows that mannanase production by co-culture of *P. italicum* and *T. oedocephalis* was significantly influenced by the type of carbon source in the fermentation medium. Agro-wastes are available in large amounts and they have been used for the production of several enzymes (Mabrouk and El Ahwany, 2008; Howard *et al.*, 2003). Co-culture of *P. italicum* and *T. oedocephalis* grew well on various raw materials of commercial potential with significant differences in the rate of enzyme production in comparison with LBG (control). The large variation in mannanase activity 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. Among these, orange peels were found to be the most suitable substrate for mannanase production with an activity of 80.566 U/mL. In our previous studies with individual fungal strain, maximal activities of 136.111 U/mL and 102.778 U/mL were obtained for *P. italicum* and *T. oedocephalis* respectively cultivation on orange peels being the best carbon source screened. However, the yield of enzyme value obtained by using orange peels as carbon source was significantly lower than that of LBG (control)

(158.333 U/mL). The lower value obtained for orange peels in comparison with LBG might be due to the presence of inhibitor compounds like limonene. The higher value obtained for orange peels in comparison with others carbon sources (agricultural wastes) might be attributed to the fact that orange 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 (Bakri *et al.*, 2003; Javed *et al.*, 2006).

Table 1. Effect of different carbon sources on mannanase production by co-culture of P. italicum and T. oedocephalis in solid state fermentation cultivation system

Carbon sources (0.3g/mL)	Mannanase activity (U/mL)	Protein (mg/mL)
Orange peels	80.556[h]±0.00	1.482[e]±0.01
Wheat bran	9.167[d]±0.00	0.278[b]±0.00
Groundnut shell	1.111[a]±0.00	0.139[a]±0.00
Palm kernel cake	50.000[g]±0.12	1.389[d]±0.00
Cassava peels	20.833[e]±0.00	1.991[g]±0.00
Pineapple peels	43.056[f]±0.00	4.583[h]±0.00
Rice bran	3.056[b]±0.05	0.417[c]±0.00
Potato peels	4.815[c]±0.00	0.278[b]±0.00
LBG (control)	158.333[i]±0.03	1.667[f]±0.00

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

The effect of different nitrogen sources on the production of mannanase by co-culture of *P. italicum* and *T. oedocephalis* was evaluated using sodium nitrate as the sole nitrogen source (Table 2). Sodium nitrate (0.2% (w/v)) was replaced by equal amount of yeast extract, whey, peptone, ammonium chloride, soy beans, locust beans and ammonium nitrate, each at a time. Of the entire tested nitrogen sources, ammonium nitrate was observed to yield maximum mannanase activity (84.444 U/mL) followed by ammonium chloride and the lowest mannanase activity of 35.556 U/mL was recorded for soy beans. The use of sodium nitrate (control) as a nitrogen source caused a reduction in enzyme activity by 52.63% of that obtained with ammonium nitrate. However, all the nitrogen sources used had better mannanase

activity than sodium nitrate (control) except soy beans meal. In our previous studies with individual organism, maximal activities of 128.380 U/mL and 95.000 U/mL were obtained for *P. italicum* and *T. oedocephalis* on yeast extract and soy bean at 0.2% (w/v) respectively. The result obtained could be attributed to the fact that the ammonium nitrate provided both the ammonium ions for conidial cell growth and enzyme production (Mekala *et al.*, 2008).
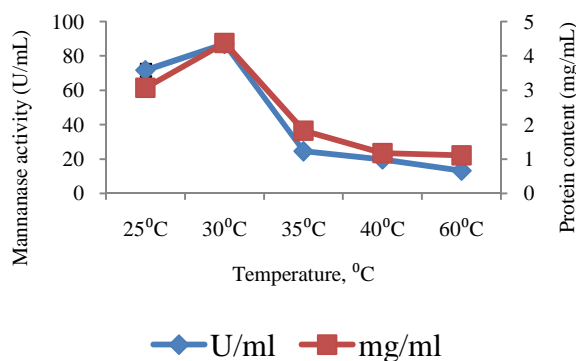
Table 2: Effect of different nitrogen sources on mannanase production by co-culture of P. italicum and T. oedocephalis in solid state fermentation cultivation system

Nitrogen sources 0.2% (w/v)	Mannanase activity (U/mL)	Protein (mg/mL)
Yeast extract	55.000[e]±0.01	0.926[e]±0.01
Whey	32.222[a]±0.01	0.324[a]±0.01
Peptone	35.833[c]±0.06	0.648[b]±0.01
NH4Cl	76.944[g]±0.08	1.806[g]±0.00
Soybeans	35.556[b]±0.08	0.833[d]±0.01
Locust beans	60.556[f]±0.03	0.741[c]±0.01
NH4NO3	84.444[h]±0.10	1.111[f]±0.01
NaNO3 (control)	40.000[d]±0.07	0.324[a]±0.00

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

To reveal the effect of incubation temperatures on mannanase production by co-culture of *P. italicum* and *T. oedocephalis* under solid state fermentation, experiments were conducted at 25°C, 30°C, 35°C and 40°C and the highest mannanase activity of 86.889 U/mL was achieved at 30°C. Thus, maximum mannanase activity was observed at 30°C (Figure 2). In our previous studies with individual fungal strain, maximal enzyme activities of 61.945 U/mL and 98.083 U/mL were obtained for *P. italicum* and *T. oedocephalis* at 35°C and 30°C respectively. The influence of temperature on enzyme production is related to the growth of the microorganisms (Ahmed et al. 2009). Hence, the temperature depends on whether the culture is mesophilic or thermophilic. Among the fungi, most enzymes production studies have been done with mesophilic fungi within the temperature range of 25-37 °C (Lu et al. 2003; Gautam et al. 2010).

Olaniyi, Akinyele: *Manannase production by co-culture* **Innovative Romanian Food Biotechnology** (2014) 14, 46-53
*of Penicillium italicum and Trichosporonoides
oedocephalis in solid state fermentation*

Abd-Aziz et al. (2008) used palm kernel cake as carbon source for the production of mannanase by *Aspergillus niger* FTCC 5003 with optimum mannanase activity obtained at 35°C. Different optimal temperatures were reported by few researchers and the differences were attributed to the strain variation of the microorganisms (Gautam et al. 2010).

Figure 2. *The effect of incubation temperature on mannanase activity and protein content by co-culture of
P. italicum and T. oedocephalis in solid state
fermentation cultivation system*

The moisture content is an important factor that influences the growth and product yield in solid state fermentation (Mrudula *et al*., 2011; Alam *et al*., 2005). Moisture is reported to cause swelling of the substrates, thereby facilitating better utilization of the substrate by microorganisms (Kim *et al*., 1985; Nagendra and Chandrasekharan, 1996). The data presented in the Figure 3 clearly indicates that the mannanase activity of co-culture of *P. italicum* and *T. oedocephalis* by cultivation in solid state fermentation cultivation system increased with increase in percentage moisture content from 20% to 60% with an optimum activity of 126.136 U/mL obtained at 60% (0.3g of substrate in 1mL of mineral salt solution). Any further increase in the percentage moisture content beyond 60% resulted in the decrease of enzyme yields which may be due to clumping of solid particles which results in the decrease of inter-particle space and decrease the diffusion of nutrients (Mrudula *et al*., 2011; Alam *et al*., 2005; Babu and Satyanarayana, 1996). In contrast, the low moisture content leads to the decrease solubility of nutrients present in the substrate thereby decreases enzyme yields.
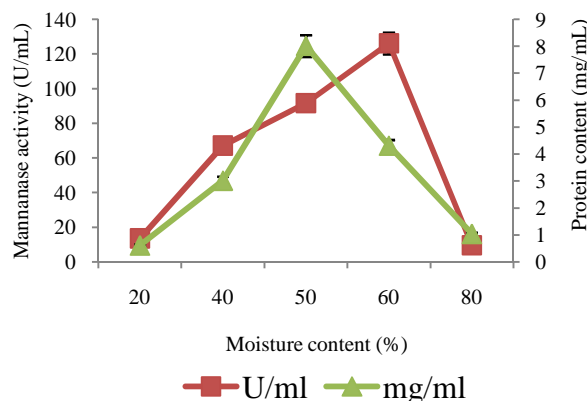
Figure 3. *The effect of percentage moisture content on
mannanase activity and protein content of co-culture of
P. italicum and T. oedocephalis in solid state
fermentation cultivation system*

Conclusions

In conclusion, fungal strains compatibility is the determining factor for successful mixed culture fermentation. In this study, it was observed that the co-culture of *P. italicum* and *T. oedocephalis* yielded lower mannanase activity in comparison with results obtained when individual microorganism was used separately. The incompatibility of mixed cultures might be due to their antagonistic effect. The optimal process parameters for the production of mannanase were proposed at incubation period of 96 hrs, orange peels, ammonium nitrate, 30°C and 60% moisture content.

References

Abd-Aziz S., Ong, L.G.A., Hassan M.A., Karim M.I.A. (2008) Process parameters optimization of mannanase production from *Aspergillus niger* FTCC 5003 using palm kernel cake as carbon source. *Asian Journal of Biotechnology*, 3(5), 297-307.

Ademark P., Varga A., Medve J., Harjunpa A.V., Drakenberg T., Tjerneld F. (1998) Softwood hemicellulosedegrading enzymes from *Aspergillus niger*: purification and properties of a β-mannanase. *Biotechnology*, 63, 199- 210.

Olaniyi, Akinyele: *Manannase production by co-culture* **Innovative Romanian Food Biotechnology** (2014) 14, 46-53
of Penicillium italicum and Trichosporonoides
oedocephalis in solid state fermentation

Ahmed S., Bashir A., Saleem H. (2009) Production and purification of cellulose degrading enzymes from a filamentous fungus *Trichoderma harzianum. Pakistan Journal of Botany*, 41, 1411-1419.

Alam M. Z., Muhammad N., Mahmat M. E. (2005) Production of cellulose from oil palm biomass as substrate by solid state bioconversion., *Advance Journal of Applied Science.* 2(2), 569-572.

Arotupin D.J., Olaniyi O.O. (2013) Screening and Identification of Mannanase-Producing Fungi Isolated from Selected Agricultural Wastes. *British Microbiology Research Journal*, 3(4), 635-644.

Babu K.R., Satyanarayana T. (1996) Amylase production by thermophilic *Bacillus coagulans* in solid state fermentation. *Process Biochemistry,* 30, 305-309.

Bakri Y., Jacques P., Thonart P. (2003) Xylanases production by *Penicillium canescens* 10-10c in solid state fermentation. *Applied Biochemistry and Biotechnology*, 108, 737-748.

El-Naggar M.Y., El-Aassar S.A., Youssef A.S., El-Sersy N.A., Beltagy E.A. (2006) Extracellular mannanase production by the immobilization of the locally isolated *Aspergillus niger*. *International Journal of Agriculture and Biology*, 8, 57-62.

Ethier H.M., Talbot G., Sygusch J. (1998) Gene cloning, DNA sequencing, and expression of thermostable β-mannanase from *Bacillus stearothermophilus*. *Applied Environmental Microbiology.* 64, 4428-4432.

Ferreira H.M., Filho E.X.F. (2004) Purification and characterization of a β-mannanase from *Trichoderma harzianum* strain T4. *Carbohydrate Polymer*, 57**,** 23-29.

Francoise M., Ghakis C., Dupont C., Morosoli R., Kluepfel D. (1996) Improved production of mannanase by *Streptomyces lividans*. *Applied Environmental Microbiology*, 62, 4656-4658.

Gautam S.P., Budela P.S., Pandey A.K., Jamaluddin A.M.K., Sarsaiya S. (2010) Optimization of the medium for the production of cellulase by the *Trichoderma viride* using submerged fermentation. *International Journal of Environmental Science*, 4(1), 656-665.

Hossain M.Z., Abe J., Hizukuri S. (1996) Multiple forms of β-mannanase from *Bacillus* sp. KK01. *Enzyme Microbial Technology*, 18, 95-98.

Howard R.L., Abotsi E., Jansen Van Rensburg E.L., Howard S. (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology*, 2, 602- 619.

Ikram-ul-Haq S., Muhammad M.J., Zafar S., Tehmina S. (2005) Triggering of beta-glucosidase production in *Trichoderma viride* with nutritional and environmental control. *Journal of Applied Science Research*, 2(11), 884-889.

Iqbal M.N.T., Muhammad A., Ishtiaq A., Shahbaz H. (2010) Media optimization for hyper-production of carboxymethylcellulase using proximally analysed agro-industrial residues with *Trichoderma harzianum* under SSF. *International Journal for Agro Veterinary and Medical Sciences*, 4(2), 47-55.

Javed M.M., Ikram U.H., Siddiq Z., Saleem T. (2006) Triggering of glucosidase production in *Trichoderma viride* with nutritional and environmental control. *Journal of Applied Science Research*, 2(11), 884-889.

Kim J.H., Hosobuchi M., Kishimoto M., Seki T., Ryu D.D.Y. (1985). Cellulase production by a solid state culture system. *Biotechnology and Bioengineering*, 27, 1445-1450.

Kurakake M., Komaki T. (2001) Production of β-mannanase and β-mannosidase from *Aspergillus awamori* K4 and their properties. *Current Microbiology*, 42**,** 377-380.

Lee J.T., Bailey C.A., Cartwright A.L. (2003) Beta mannanase ameliorates viscosity - associated depression of growth in broiler chickens fed guar germ and hull fractions. *Poultry Science*, 82, 1925-1931.

Lee J.T., Connor-Appleton S., Bailey C.A., Cartwright A.L. (2005) Effects of guar meal by-product with and without β-mannanase hemicell on broiler performance. *Poultry Science*, 84, 1261-1267.

Lee Y., Chandra M.R., Park I., Zhou Y., Kim K., Choi Y.L. (2011) Isolation, purification and

Olaniyi, Akinyele: *Manannase production by co-culture* **Innovative Romanian Food Biotechnology** (2014) 14, 46-53
of Penicillium italicum and Trichosporonoides
oedocephalis in solid state fermentation

characterization of a thermostable β-mannanase from *Paenibacillus* sp. DZ3. *Journal of Korean Society for Applied Biology and Chemistry*, 54(3), 325-331.

Lu W., Li D., Wu Y. (2003) Influence of water activity and temperature on xylanase biosynthesis in pilot scale solid-state fermentation by *Aspergillus sulphuros*. *Enzyme Microbial Technology*, 32, 305-311.

Mabrouk M.E.M., El Ahwany A.M.D. (2008) Production of - mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. *African Journal of Biotechnology*, 7(8), 1123-1128.

Malik S.K., Mukhtar H., Farooqi A.A., Haq I. (2010) Optimization of process parameters for the biosynthesis of cellulases by *Trichoderma viride*. *Pakistan Journal of Botany*, 42(6), 4243-4251.

McCoy M. (2001) Soaps and detergents: An update on the latest developments within the detergent industry also introducing the latest new enzyme, a mannanase. *Chemical Engineering News*, 20, 19-32.

Meenakshi M., Singh G., Bhalla A., Hoondal G. S. (2010) Solid state fermentation and characterization of partially purified thermostable mannanase from *Bacillus* sp. MG-33. *Bioresource*, 5(3), 1689-1701.

Mekala N.K., Singhania R.R., Sukumaran R.K., Pandey A. (2008). Cellulase production under solid-state fermentation by *Trichoderma reesei* RUT C30: Statistical optimization of process parameters. *Applied Biochemistry and Biotechnology*, 151(2-3), 122-131.

Mendoza N.S., Arai M., Sugimoto K., Kawaguchi T., Joson L.M. (1995) Cloning and sequencing of β-mannanase gene from *Bacillus subtilis* NM-39. *Biochemistry Biophys Acta*, 1243, 552-554.

Miller L., (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31, 208-218.

Mrudula S., Gopal R., Seenayya G. (2011). Effect of substrate and culture conditions on the production of amylase and pullanase by thermophilic *Clostridium thermosulfurogenes* SVM17 in solid state fermentation. *Malaysian Journal of Microbiology*, 7(1), 15-21.

Nagendra P.G., Chandrasekharan M. (1996) L-Glutaminase production by marine *Vibrio costicola* under solid state fermentation using different substrates. *Journal of Marine Biotechnology*, 4, 176-179.

Narasimh G., (2006) Nutrient effects on production of cellulytic enzymes by *Aspergillus niger*. *African Journal of Biotechnology*, 5, 472-476.

Phothichitto K., Nitisinprasert S., Keawsompong S. (2006) Isolation, screening and identification of mannanase producing microorganisms. *Kasetsart Journal* (Nat. Sci.), 40, 26-38.

Sachslehner A., Nidetzky B., Kulbe K.D., Haltrich D. (1998) Induction of mannanase, xylanase and endoglucanase activities in *Sclerotium rolfsii*. *Applied Environmental Microbiology*, 64, 594-600.

Sachslehner A., Foildl G., Foidl N., Gübitz G., Haltrich D. (2000) Hydrolysis of isolated coffee mannan and coffee extract by mannanases of *Sclerotium rolfsii*. *Journal of Biotechnology*, 80, 127-134.

Takahashi R., Kusakabe I., Kobayashi H., Murakami K., Maekawa A., Suzuki T. (1984) Purification and some properties of mannanase from *Streptomyces* sp. *Agricultural Biology and Chemistry,* 48, 2189-2195.

Wu G., Bryant M.M., Voitle R.A., Roland D.A. (2005) Effects of mannanase in corn-soy diets on commercial leghorns in second cycle hens. *Poultry Science*, 84, 894-897.

Zhang J., He Z.M., Hu K. (2000) Purification and characterization of β-mannanase from *Bacillus licheniformis* for industrial use. *Biotechnology Letters*, 22, 1375-1378.