

Full Length Research Paper

Exploration of different species of orange peels for mannanase production

Oladipo O. OLANIYI^{1*}, Charles A. OSUNLA² and Olawale O. Olaleye³

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

²Department of Microbiology, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria.

³Nigerian Institute for Trypanosomiasis Research, P.M.B 2077, Kaduna, Nigeria.

Accepted 12 February, 2014

Different species of orange peels were evaluated as carbon source in comparison to Locust Bean Gum for mannanase production by *Penicillium italicum*, *Trichosporonoides oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis*. Mannanase activity was determined by dinitrosalicylic acid method. The protein concentration was however determined by the Lowry method. Of all the substrates screened, the highest mannanase activity (573.89 U/ml) was achieved with *P. italicum* cultured on sweet orange peels. The highest mannanase activity for mixed substrate fermentation in ratio 1:1:1:1 and 1:1:1:2 (Lime, Grape, Tangerine and Sweet orange peels) was obtained in a medium inoculated with *P. italicum* while mixed culture of *P. italicum* and *T. oedocephalis* gave maximum mannanase activity in mixed substrate ratio 2:1:1:1. The mixed substrate fermentation gave appreciable enzyme activity and thus, they can be used as low-cost substrate for mannanase production by these organisms.

Key words: Locust Bean Gum, mixed substrate fermentation, carbon sources.

INTRODUCTION

Agro-wastes are the most abundant and renewable material produced on earth. Large quantities of agro-wastes are obtained from forests, agricultural practices, and industrial processes, particularly from agro-allied based industries such as breweries, paper and pulp, textile and timber industries. These wastes generally accumulate in the environment as pollutants (Abu et al., 2000). About 2.9×10^3 million tons of lignocellulosic residues are produced from cereal crops and 3×10^3 million tons from pulse and oil seed crops. In addition, 5.4×10^2 million tons is produced annually from crops worldwide (FAO, 2006) and these materials accumulate in enormous amounts (GOP, 2009). Agricultural and industrial wastes are among the causes of environmental pollution. Their conversion into useful products may ameliorate the problems they cause. Enzyme production from lignocellulosic biomass through the biological route seems to be very attractive and sustainable due to several reasons, the major being the

renewable and ubiquitous nature of biomass and its non-competitiveness with food crops (Singhania et al., 2010).

Mannanase (β -mannanase; EC 3.2.1.78) participates in the degradation of hemicellulose and similar polysaccharides by hydrolyzing the β -1, 4-glycosidic linkages within the main chain such as galactoglucomannan, the major hemicellulose of softwood (McCleary et al., 1988; Lundqvist et al., 2002). The hemicelluloses are the second richest renewable energy substances on earth (Wyman et al., 2005). Mannan, glucomannan, galactomannan, and galactoglucomannan are the major polysaccharides that constitute hemicellulose (Yan et al., 2008). Mannanase is useful in many fields including the feed, food as well as paper and pulps industries (Gubitz et al., 1997; Sachslehner et al., 2000; Daskiran et al., 2004; Kansoh and Nagie, 2004). Furthermore, it is employed for the preparation of mannooligosaccharides used as non-nutritional food additives for selective growth of human beneficial intestinal microflora (bifidobacteria and lactobacilli) (Alvarez-Mancedo et al., 2008). Despite of the high practical potentialities, the use of mannanase is still limited due to low yield and high-production cost. This has necessitated a renewed search for mannolytic

*Corresponding author. E-mail: microladit@gmail.com. Tel: 08068054636.

organisms with novel mannanase properties and strategies for low-cost enzyme production.

In search of viable mannolytic organisms, we isolated different mannolytic microfungi from agricultural wastes in Akure, Nigeria, in which *Penicillium italicum* and *Trichosporonoides oedocephalis* gave considerable mannanase activity (Arotupin and Olaniyi, 2013). In another study conducted by Akinyele et al. (2013), various agro-wastes were screened for quantitative mannanase production as substitute to Locust Bean Gum (LBG) in solid state fermentation and sweet orange peels were observed to give the highest mannanase activity. Based on this information, different species of orange peels (sweet, lime, grape and tangerine) were evaluated as alternative carbon source for mannanase production.

MATERIALS AND METHODS

Chemicals and Substrates

Different species of orange peels (sweet, lime, grape and tangerine) 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°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. Locust Bean Gum was purchased from Sigma Chemicals (St. Louis, MO). All other chemicals were of analytical grade.

Fungi Isolates

Penicillium italicum and *Trichosporonoides oedocephalis* isolated from agro-wastes previously confirmed positive for mannanase activity were used in this study (Arotupin and Olaniyi, 2013). The fungal isolates were identified in Microbiology Research Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria according to the method designed by Pitt and Hocking (1997) 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 organisms were maintained at 4°C on Malt Extract Agar (MEA).

Mannanase production

For the production of mannanase in solid state fermentation, the isolates were cultured at 30°C in 250 ml Erlenmeyer flasks containing 10 grams of the carbon source (sweet, lime, grape and tangerine peels). The substrate was suspended in 33 ml Mandels and Weber's medium modified by Olaniyi et al. (2013). This medium

(moistening agent) contained the following ingredients (g/L): Peptone 2, yeast extract 2, NaNO₃ 2, K₂HPO₄ 1, MgSO₄.7H₂O 0.5, KCl 0.5 and FeSO₄.7H₂O traces. After sterilization at 121°C for 15 min, it was cooled and inoculated with 2 discs of 8 mm diameter of the organism from MEA culture plates using sterile cup borer. The flask was incubated at 30°C for 5 days at static condition.

Mixed substrate fermentation

The effect of different substrate combinations was evaluated on mannanase production and protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis*.

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°C for 60 min. The solid materials and fungal biomass were separated by centrifugation (Centurion Scientific Limited) (6000 rpm, 15 min at 4°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.

Enzyme assays

Mannanase activity was assayed in the reaction mixture composing of 0.5 ml of 50 mM potassium phosphate buffer pH 7.0 and 1% LBG with 0.5 ml of supernatant at 45°C for 60 min (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 determination

The amount of protein liberated in the fermentation media was evaluated according to the method designed by Lowry et al. (1951) using Bovine Serum Albumin (BSA) as the standard.

RESULTS

The mannanase activities of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis* on different carbon sources is shown in Figure 1. The highest mannanase activity on grape peels was achieved

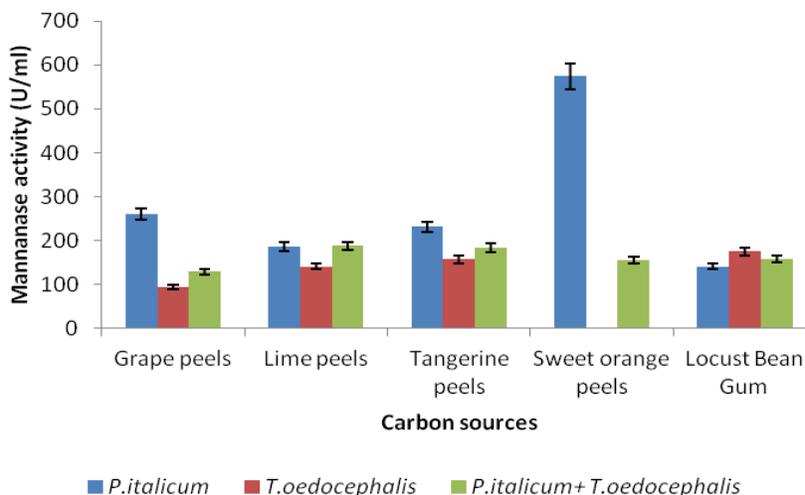


Figure 1. Effect of different orange peels on mannanase production potential of *P. italicum*, *T. oedocephalis* and co-culture of *P.italicum* and *T. oedocephalis*.

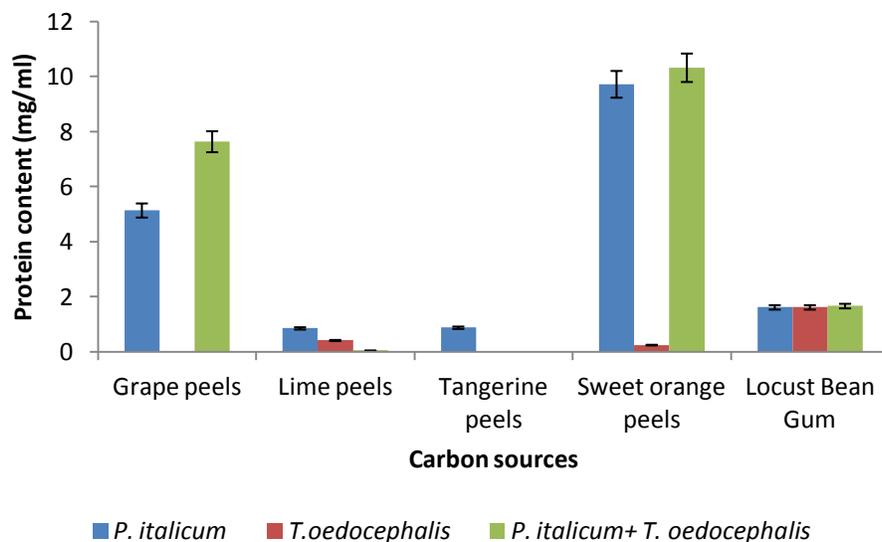


Figure 2. Effect of different orange peels on protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P.italicum* and *T. oedocephalis*.

with *P. italicum* with an activity of 260.14 U/ml while the lowest of value of 94.72 U/ml was obtained for *T. oedocephalis*. In lime peels containing medium, the highest mannanase activity was achieved with co-culture of *P. italicum* and *T. oedocephalis* while the lowest was obtained in a medium inoculated with *T. oedocephalis*. On tangerine and sweet orange peels, *P. italicum* gave maximum mannanase activities of 231.53 and 573.89 U/ml respectively while *T. oedocephalis* recorded the highest mannanase activity of 175 U/ml in a medium

supplemented with LBG (control). However, the overall highest mannanase activity was achieved with *P. italicum* cultured on sweet orange peels. The mannanase activity displayed by *P. italicum* on sweet orange peels was 4-fold higher than what was displayed by *T. oedocephalis* on LBG (control).

The protein yield of the organisms cultured in the media containing sweet, lime, grape and tangerine peels respectively is shown in Figure 2. *Penicillium italicum* and co-culture of *P. italicum* and *T. oedocephalis* produced

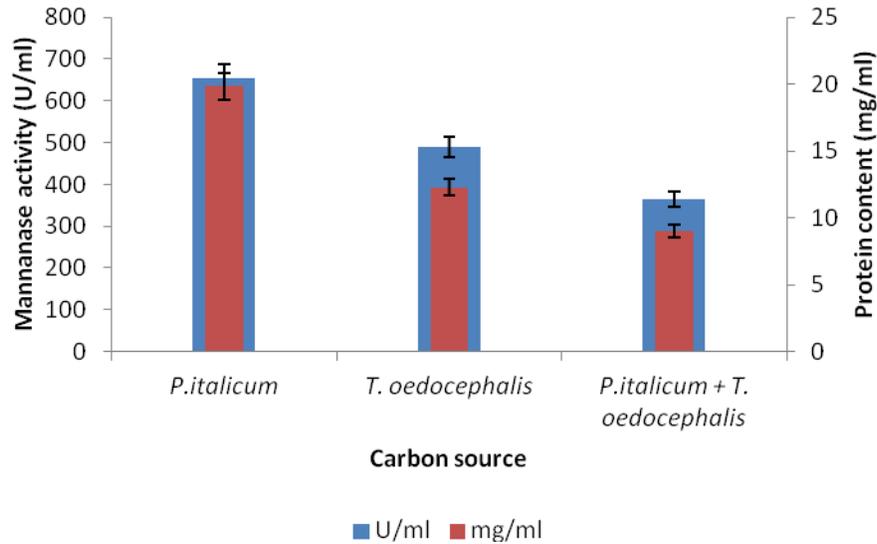


Figure 3. Effect of mixed substrate fermentation (1:1:1:1) of different orange peels (Lime, Grape, Tangerine and sweet orange peels) on mannanase activity and protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis*.

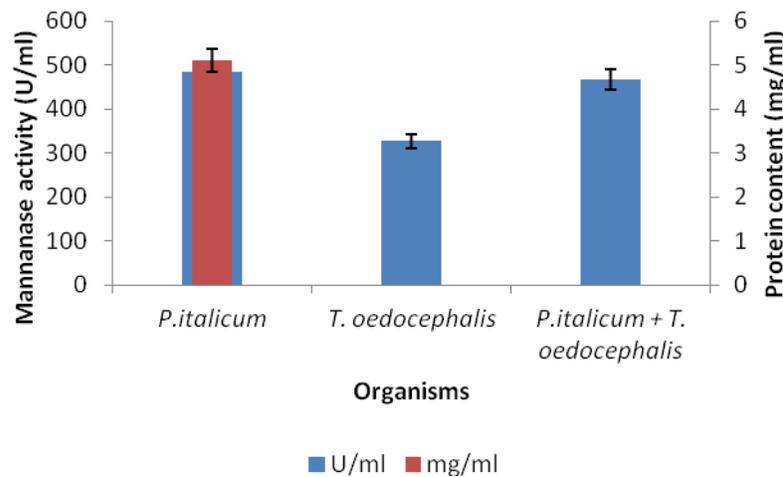


Figure 4. Effect of the mixed substrates (1:1:1:2) of different orange peels (Lime, Grape, Tangerine and sweet orange peels) on mannanase activity and protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis*.

much more protein in media containing sweet orange peels compared to other substrates. The protein content of culture filtrate in grape peels containing medium was highest in mixed culture (*P. italicum* and *T. oedocephalis*) inoculated medium while *P. italicum* gave maximum protein content on lime and tangerine peels. However, the protein content produced by mixed culture of *P. italicum* and *T. oedocephalis* was 10-fold higher than what was produced by any of the organism on LBG.

The effect of mixed substrate fermentation in ratio 1:1:1:1 of different orange peels (Lime, Grape, Tangerine

and sweet orange peels) on mannanase activity and protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis* is shown in Figure 3. The mannanase activities of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis* were 655.56, 489.17 and 363.33 U/ml respectively. Therefore, the highest mannanase activity and protein content were obtained in a medium inoculated with *P. italicum* while the lowest was achieved with mixed culture of *P. italicum* and *T. oedocephalis* inoculated medium. Similarly, in Figure 4, *P. italicum*

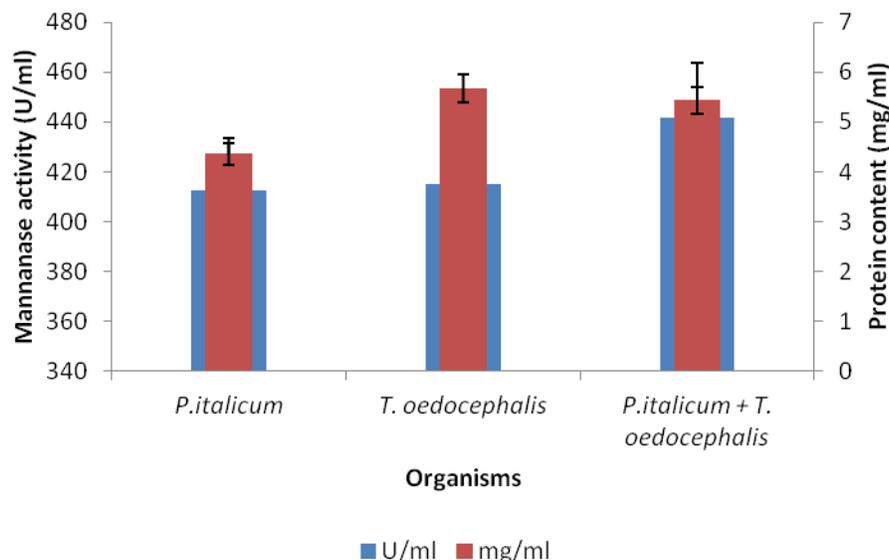


Figure 5. Effect of mixed substrate (2:1:1:1) of different orange peels (Lime, Grape, Tangerine and sweet orange peels) on mannanase activity and protein content of *P. italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis*.

gave the highest mannanase activity and protein content in mixed substrate fermentation in ratio 1:1:1:2 of different orange peels (Lime, Grape, Tangerine and sweet orange peels). In Figure 5, mannanase activity was highest in a medium inoculated with mixed culture of *P. italicum* and *T. oedocephalis* while the highest protein content was achieved with *T. oedocephalis* in mixed substrate fermentation in ratio 2:1:1:1 of different orange peels (Lime, Grape, Tangerine and sweet orange peels).

DISCUSSION

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 (Ray *et al.*, 2007). *Penicillium italicum*, *T. oedocephalis* and co-culture of *P. italicum* and *T. oedocephalis* were able to produce extracellular mannanase on different species of orange peels although with differences in the rate of enzyme production. The differences in mannanase activity on different species of orange peels could 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 (Mabrouk and El Ahwany, 2008; Akinyele *et al.*, 2013). The overall highest mannanase production was achieved with *P. italicum* cultured on sweet orange peels. It might be that sweet orange peels were readily assimilated and metabolized by the cells of *P. italicum* for enzyme biosynthesis.

Penicillium italicum and co-culture of *P. italicum* and *T.*

oedocephalis gave much more protein in media containing sweet orange peels compared to other substrates. The high protein released on sweet orange peels suggests the presence of other proteins (beside the mannanase enzyme) which may include other cell-wall hydrolyzing enzymes (de Vries and Visser, 2001). Hemicellulases particularly xylanases are also required for the hydrolysis of natural mannan (Khan, 1980). These organisms seem to secrete the hydrolytic enzymes for the breakdown of the sweet orange peels (polymer) into the growth media which largely accounts for the high protein contents.

The mixed substrate fermentation of different species of orange peels gave considerable mannanase activity. The remarkable differences in the enzyme production by the use of mixed substrate fermentation of different species of orange peels was not understood, it may be due to different requirements by the isolates.

Conclusion

Different species of orange peels are available in abundance and can be used as low-cost carbon source for the production of commercial mannanase. Such use could help reduce the pollution due to orange peels wastes. The present work indicated that mannanase production potential of these fungal isolates was improved when compared with LBG that is known to be expensive in enzyme production. An appreciable yield of mannanase was achieved when mixed substrate fermentation of different species of orange peels was employed.

REFERENCES

- Abu EA, Onyenekwe PC, Ameh DA, Agbaji AS, Ado SA (2000). Cellulase production from sorghum bran by *Aspergillus niger* SL 1: An assessment of pretreatment methods. Proceedings of the International Conference on Biotechnology: Commercialization and Food security, Abuja, Nigeria, pp. 153-159.
- Akinyele BJ, Olaniyi OO, Adetunji CO (2013). Screening and optimization of nutritional conditions for mannanase production by *Penicillium italicum* LAD-A5 in solid state cultivation. E3 J. Biotechnol. Pharm. Res., 4(2): 35-41.
- Alvarez-Macedo F, Landin M, Ram MP (2008). Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. European J. Pharm. Biopharma., 69(2), 573-581.
- Arotupin DJ, Olaniyi OO (2013). Screening and Identification of Mannanase-Producing Fungi Isolated from Selected Agricultural Wastes. Brit. Microbiol. Res. J., 3(4): 635-644.
- Daskiran M, Teeter R, Fodge D, Hsiao H (2004). An evaluation of endo- β -mannanase (Hemicell) effects on broiler performance and energy use in diets varying in mannan content., Poult. Sci., 83: 662-668.
- de Vries RP, Visser J (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol. Mol. Biol. Rev., 65: 497-552.
- EI-Naggar MY, Youssef SA, El-Assar SA, Beltagy EA (2006). Optimization of cultural conditions for β -mannanase production by a local *Aspergillus niger* isolate. Inter. J. Agric. Biol., 8(4): 539-545.
- Food and Agriculture Organization (FAO) (2006). FAO statistical databases, <http://faostat.fao.org/>.
- Gross Operating Profit (GOP) (2009). Food composition table for Pakistan. A collaborative report of NWFP University, UNICEF and Ministry of Planning and Development; Islamabad, Pakistan.
- Gubitz GM, Lisching T, Stebbing D, Saddler JN (1997). Enzymatic removal of hemicellulose from dissolving pulps. Biotechnol. Lett., 19(5): 491-495.
- Kansoh A, Nagie Z (2004). Xylanase and mannanase enzymes from *Streptomyces galbus* NR and their use in biobleaching of softwood kraft pulp. Antonie Van Leeuwenhoek. 85(2): 103-114.
- Khan AW (1980). Cellulolytic enzyme system of *Activibro cellulolyticus*, a newly isolated anaerobe. J. Gen. Microbiol., 121: 499-502.
- Lowry OH, Rosebrough NJ, Farv AL, Randall RJ (1951). Protein measurement with Folin Phenol reagent. J. Biol. Chem., 193: 265-275.
- Lundqvist J, Teleman A, Junel L, Zacchi G, Dahlman O, Tjerneld F, Stabrand H (2002). Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). Carbohydr. Polym., 48(1): 29-39.
- Mabrouk EM, Ahwany MD (2008). Production of mannanase by *Bacillus amylolequifaciens*10A1 cultured on potato peels. Afr. J. Biotechnol., 7(8):11-28.
- McCleary BV, Willis AW, Scott TK (1988). Synthesis of beta-mannopyranosides for the assay of beta-mannosidase and exo-beta-mannanase. Methods Enzymol., 160: 515-518.
- Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31: 426-428.
- Olaniyi OO, Igbe FO, Ekundayo TC (2013). Optimization studies on mannanase production by *Trichosporonoides oedocephalis* in submerged state fermentation. E3 J. Biotechnol. Pharm. Res., 4(7):110-116.
- Pitt JI, Hocking AD (1997). Fungi and food spoilage, 2th Edition, Blacki Academic, London, U.K. p. 414.
- Ray AK, Bairagi A, Ghosh KS, Sen SK (2007). Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 isolated from fish gut. Acta Ichthyologica Et Piscatoria., 37(1):47-53.
- Sachslehner A, Foidl G, Foidi N, Gubitz G, Haltrich D (2000). Hydrolysis of isolated coffee mannan and coffee extract by mannanases of *Scrotium rolfsii*. J. Biotechnol., 80:127-134.
- Singhania RR, Sukumarana RK, Patelb AK, Larrocheb C, Pandeya A (2010). Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzy. Microb. Technol., 46: 541-549.
- Wyman CE, Decker SR, Himmel ME, Brady JW, Skopec CE, Viikari L (2005). Polysaccharides: Structural Diversity and Functional Versatility, CRC Press Boca Raton, FL., pp. 953-1033.
- Yan X, An X, Gui L, Liang D (2008). From structure to function: Insights into the catalytic substrate specificity and thermostability displayed by *Bacillus subtilis* mannanase BCman. J. Mol. Biol., 379(3): 535-544.