



Full Length Research Paper

# Screening and optimization of nutritional conditions for mannanase production by *Penicillium italicum* LAD-A5 in solid state cultivation

Juliet B. AKINYELE<sup>1</sup>, Oladipo O. OLANIYI<sup>1\*</sup> and Charles O. ADETUNJI<sup>2</sup>

<sup>1</sup>Department of Microbiology, Federal University of Technology, Akure, Ondo State.

<sup>2</sup>Nigerian Stored Product Research Institute, Km 3 Asa Dam, P.M.B 1489, Ilorin, Kwara State.

Accepted 26 February, 2013

Different fungal isolates were screened for the production of mannanase under solid state cultivation at static condition. *Penicillium italicum* LAD-A5 was selected as the most potent in producing enzyme of high activity. It was therefore selected for further studies. The nutritional requirements of *Penicillium italicum* LAD-A5 for the production of mannanase in solid state fermentation were investigated. The process parameters optimized include; carbon and nitrogen sources, inoculum concentrations, percentage moisture content and sugar supplementation. Utilization of different agrowastes on mannanase production was evaluated. Orange peels were found to be the most effective carbon source with highest mannanase activity of 129.130 U/ml. Yeast extract meal was obtained to be the best nitrogen source with highest activity of 128.380 U/ml out of all the nitrogen sources screened in this study. The best percentage moisture content for maximum enzyme activity was obtained at 50%. Inoculum concentration of  $1 \times 10^6$  yielded highest mannanase activity of 37.562 U/ml. Addition of simple carbon sources to medium containing locust bean gum caused a slight catabolic repression of mannanase synthesis.

**Key words:** Screening, optimization, solid state cultivation, mannanase activity, *Penicillium italicum* LAD-A5.

## INTRODUCTION

“Mannan exists in nature in two forms, galactomannan and acetylated galacto-mannan. Galactomannan is present in the seeds of leguminous plants and composed of a homogenous backbone of  $\beta$ -1-4 linked mannose residues. Acetylated galactomannan, a principal component of hemicellulose, has a heterogenous backbone of  $\beta$ -1-4 linked mannose and glucose units. Mannanases (EC 3.2.1.78; 1,4- $\beta$ - D- mannan mannanohydrolases) occur widely in microorganisms in fungi, yeasts, and bacteria as well as from germinating seeds of terrestrial plants (Ferreira and Filho, 2004; Heck *et al.*, 2005; Jiang *et al.*, 2006; Lin *et al.*, 2007; Meenakshi *et al.*, 2010) ”.

“In recent years, mannanases have gained increasing attention because of their various biotechnological

applications in the food, feed, coffee extraction, oil drilling, detergent, as well as pulp and paper industries (Lin and Chen, 2004; Dhawan and Kaur, 2007; Titapoka *et al.*, 2008; Naganagouda *et al.*, 2009). They can also be used in the production of manno oligosaccharides, which were reported to be excellent prebiotics stimulating growth of beneficial intestinal microorganisms (Gibson *et al.*, 2000)”. Mannanases are useful in many fields including biobleaching of pulp and detergent industry (Gubitza *et al.*, 1997), bioconversion of biomass wastes to fermentable sugars (Chandrakant and Bisaria, 1998), and upgrading of animal feed stuff (Ray *et al.*, 1982). It can be used to reduce the viscosity of coffee extracts (Hashimoto and Fukumoto, 1969). The coffee preparation using  $\beta$ -mannanases showed better volatile aroma, taste properties and visual appearance of the final drink (Nunes and Coimbra, 1998).

Mannanases could be used as valuable food sweetener or additives (Tomotari, 1990) and also have

\*Corresponding author. Email: microladit@yahoo.com.

potential application for mannoooligosaccharide preparation to be used as prebiotic, which is expected to improve the growth performance of animal. Manno-oligosaccharides are generated when mannan is hydrolyzed by endo-1,4- $\beta$ -D-mannanase (EC 3.2.1.78) which catalyzes the random cleavage of  $\beta$ -D-1, 4-mannopyranosyl linkages within the main chain of galactomannan, glucomannan, galactoglucomannan and mannan (McCearry and Matheson, 1974). It has been reported that mannoooligosaccharides are special nutrient or growth promoter for probiotics, such as *Bifidobacterium* sp. and *Lactobacillus* sp. In this study, the nutritional parameters for solid state fermentation were optimized to enhance mannanase production by *Penicillium italicum* LAD-A5.

## MATERIALS AND METHODS

### Microorganisms

Eleven fungal strains from agro-wastes previously confirmed positive for mannanase activity by plate assay were used in this study. The fungal isolates were identified in the Microbiology Research Laboratory, Federal University of Technology, Akure, Ondo State, Nigeria. The isolates were: *Penicillium italicum* LAD-A5, *Aspergillus flavus* LAD-2A, *A. flavus* LAD-10A, *A. glaucus* LAD-9A11, *Rhizopus japonicus* PAP-1C, *R. japonicus* YP-5B, *R. japonicus* LBW-8A, *R. japonicus* BN-7A, *R. japonicus* LBW-7B, *R. japonicus* LAD-9A12 and *Trichosporonoides oedocephalis* FCN-12C.

### Screening for mannanase production

For the production of mannanase in solid state fermentation, the isolates were grown at 28°C in 250 ml Erlenmeyer flasks containing 10 grams of the coarsely ground copra meal. Mandels and Weber's medium modified by El-Naggar *et al.* (2006) was used to adjust the moisture content from 50% to 80%.

### Optimization of nutritional conditions

To ascertain the effect of nutritional conditions on mannanase production by *Penicillium italicum* LAD-A5, the present study was carried out using different carbon sources (yam peels, wheat bran, groundnut shell, palm kernel cake, cassava peels, pineapple peels, potato peels, rice bran, orange peels and locust bean gum (control)), inorganic and organic nitrogen sources (NH<sub>4</sub>Cl, NaNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>, yeast extract, whey, peptone, soybeans, urea and locust beans), inoculum concentrations (1×10<sup>3</sup>, 1×10<sup>4</sup>, 1×10<sup>5</sup>, 1×10<sup>6</sup>, 1×10<sup>7</sup>) and percentage moisture contents (20%, 40%, 50%, 60% and 80%). Different sugars were supplemented to evaluate its induction or repression effect on mannanase production.

### 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 (6000 rpm, 15 min at 4°C). The clear supernatant was used for enzyme assays and soluble protein determination.

### Enzyme assays

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

### Statistical analysis

Experiment data was subjected to ANOVA of SPSS programming. Duncan's multiple range tests was used to identify significant differences between means of treatments.

## RESULTS AND DISCUSSION

### Screening of mannan-degrading fungal strains

All the tested fungal strains were able to produce extracellular mannanase in solid state fermentation although with differences in the rate of enzyme production. The highest mannanase activity was reached by *P. italicum* LAD-A5 followed by *Trichosporonoides oedocephalis* FCN-12C and *R. japonicus* YP-5B, while the lowest was recorded in *R. japonicus* LBW-8A (Table 1). Therefore, *P. italicum* LAD-A5 was selected for further studies. The highest yield and protein content was recorded for *P. italicum* LAD-A5 which shows that there was direct relationship between the yield, protein content of the tested cultures and the production of mannanase. It was also observed that the final pH of all the tested cultures was in acidic range (4.49-6.59). Mannanase activity had been reported in a variety of molds under solid state cultivation (Ademark *et al.*, 1998; Regalado *et al.*, 2000; El-Naggar *et al.*, 2006; Sae-Lee, 2007; Dhawan and Kaur, 2007; Hashem *et al.*, 2009) but few data are available on mannanase activity of *P. italicum*.

### The effect of carbon sources

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). Table 2 shows that several types of agro-industrial by-products were evaluated as

**Table 1.** Mannanase production by different fungal isolates in solid state cultivation.

Isolates	Final pH	Mannanase activity (U/ml)	Protein (mg/ml)	Yield (U/g)
1C	5.46 <sup>g</sup> ±0.03	11.667 <sup>d</sup> ±0.31	1.111 <sup>g</sup> ±0.00	1.667 <sup>d</sup> ±0.02
2A	5.93 <sup>h</sup> ±0.03	16.482 <sup>g</sup> ±0.06	0.694 <sup>c</sup> ±0.00	2.355 <sup>g</sup> ±0.01
5A	5.30 <sup>f</sup> ±0.02	18.056 <sup>j</sup> ±0.02	1.667 <sup>j</sup> ±0.01	2.579 <sup>j</sup> ±0.06
5B	4.49 <sup>a</sup> ±0.06	17.500 <sup>i</sup> ±0.15	1.250 <sup>h</sup> ±0.01	2.500 <sup>i</sup> ±0.01
7A	5.48 <sup>g</sup> ±0.03	15.370 <sup>f</sup> ±0.00	0.139 <sup>a</sup> ±0.00	2.186 <sup>f</sup> ±0.01
7B	6.38 <sup>i</sup> ±0.03	6.204 <sup>b</sup> ±0.21	0.972 <sup>f</sup> ±0.01	0.886 <sup>b</sup> ±0.01
8A	6.59 <sup>j</sup> ±0.00	5.000 <sup>a</sup> ±0.04	0.556 <sup>b</sup> ±0.01	0.714 <sup>a</sup> ±0.01
9A11	4.73 <sup>b</sup> ±0.06	10.000 <sup>c</sup> ±0.13	0.833 <sup>e</sup> ±0.03	1.429 <sup>c</sup> ±0.01
9A12	4.81 <sup>c</sup> ±0.03	13.056 <sup>e</sup> ±0.05	0.833 <sup>e</sup> ±0.01	1.865 <sup>e</sup> ±0.01
10A	5.06 <sup>d</sup> ±0.01	17.222 <sup>h</sup> ±0.11	1.250 <sup>h</sup> ±0.05	2.460 <sup>h</sup> ±0.03
12C	5.23 <sup>e</sup> ±0.03	17.685 <sup>f</sup> ±0.07	1.486 <sup>i</sup> ±0.00	2.527 <sup>i</sup> ±0.01

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

**1C**= *Rhizopus japonicus* PAP-1C, **2A**= *Aspergillus flavus* LAD-2A, **5A**= *Penicillium italicum* LAD-A5, **5B**= *R. japonicus* YP-5B, **7A**= *R. japonicus* BN-7A, **7B**= *R. japonicus* LBW-7B, **8A**=*R. japonicus* LBW-8A, **9A11**=*A. glaucus* LAD-9A11, **9A12**=*R. japonicus* LAD-9A12, **10A**= *A. flavus* LAD-10A, **12C**= *Trichosporonoides oedocephalis* FCN-12C.

**Table 2.** Effect of different carbon sources.

Carbon sources	Final pH	Mannanase activity (U/ml)	Protein (mg/ml)	Yield (U/g)
YP	5.09 <sup>d</sup> ±0.04	55.09 <sup>f</sup> ±0.07	1.806 <sup>g</sup> ±0.03	5.509 <sup>f</sup> ±0.01
WB	3.29 <sup>a</sup> ±0.05	102.78 <sup>g</sup> ±0.05	0.694 <sup>c</sup> ±0.02	10.278 <sup>g</sup> ±0.00
GNS	7.15 <sup>h</sup> ±0.06	1.94 <sup>a</sup> ±0.00	0.139 <sup>a</sup> ±0.00	0.194 <sup>a</sup> ±0.00
PKC	5.16 <sup>de</sup> ±0.09	25.46 <sup>c</sup> ±0.05	0.694 <sup>c</sup> ±0.03	2.546 <sup>c</sup> ±0.03
CP	4.03 <sup>b</sup> ±0.10	26.85 <sup>d</sup> ±0.05	0.278 <sup>b</sup> ±0.01	2.685 <sup>d</sup> ±0.01
PAP	4.71 <sup>c</sup> ±0.19	48.15 <sup>e</sup> ±0.01	4.953 <sup>h</sup> ±0.03	4.815 <sup>e</sup> ±0.01
RB	6.55 <sup>g</sup> ±0.07	3.24 <sup>b</sup> ±0.00	1.482 <sup>d</sup> ±0.01	0.324 <sup>b</sup> ±0.00
PPP	6.02 <sup>f</sup> ±0.31	105.56 <sup>h</sup> ±0.03	0.694 <sup>c</sup> ±0.01	10.556 <sup>h</sup> ±0.03
ORP	5.12 <sup>d</sup> ±0.02	129.13 <sup>i</sup> ±0.01	1.528 <sup>e</sup> ±0.04	13.611 <sup>i</sup> ±0.01
LBG (control)	5.34 <sup>e</sup> ±0.10	141.67 <sup>j</sup> ±0.01	1.620 <sup>f</sup> ±0.03	14.167 <sup>j</sup> ±0.01

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

**YP**= Yam peels  
**WB**= Wheat bran  
**GNS**= Groundnut shell  
**PKC**= Palmkernel cake  
**CP**= Cassava peels  
**PAP**= Pineapple peels  
**RB**=Rice bran  
**PPP**=Potato peels  
**ORP**=Orange peels  
**LBG**= Locust bean gum

substrates for mannanase production by *P. italicum* LAD-A5 in comparison to locust beans gum (control). *P. italicum* LAD-A5 grew well on various raw materials of commercial potential with significant differences in the rate of 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 (Mabrouk and El Ahwany, 2008).

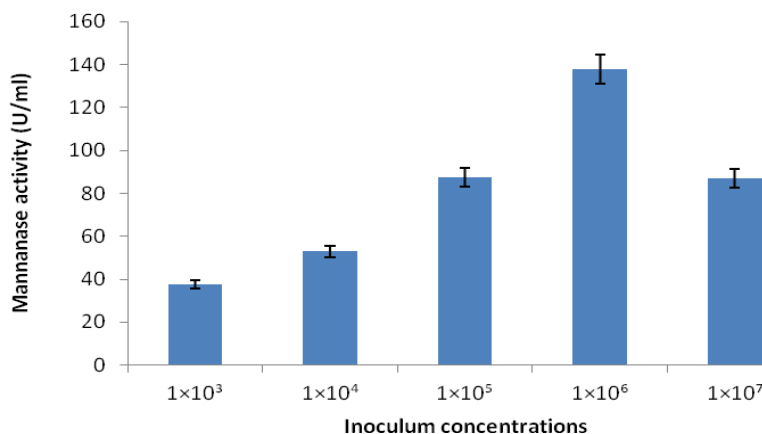
Of all the substrates tested, orange peels were found to be the best substrate for the production of mannanase, which gave maximum mannanase activity of 129.130 U/ml. However, the value obtained for orange peels was significantly lower than that of locust bean gum (control) (141.667 U/ml). It might be due to the fact that orange peels provided adequate amount of nutrients like proteins, carbohydrates, fats, fibers, ash, calcium, magnesium, phosphorous, potassium, sulphur, various

**Table 3.** Effect of different nitrogen sources.

Nitrogen sources	Mannanase activity (U/ml)	Protein (mg/ml)	Yield (U/g)
YP	128.38 <sup>h</sup> ±0.31	25.482 <sup>e</sup> ±0.39	18.34 <sup>f</sup> ±1.00
WH	43.472 <sup>b</sup> ±0.15	24.537 <sup>d</sup> ±0.28	6.21 <sup>b</sup> ±0.98
PP	75.278 <sup>d</sup> ±0.38	26.056 <sup>f</sup> ±0.34	10.754 <sup>c</sup> ±0.02
NH <sub>4</sub> Cl	100.88 <sup>e</sup> ±0.43	0.976 <sup>b</sup> ±0.25	14.411 <sup>d</sup> ±0.33
SB	117.407 <sup>g</sup> ±0.17	24.963 <sup>e</sup> ±0.24	16.773 <sup>e</sup> ±0.35
UR	112.13 <sup>f</sup> ±7.51	22.157 <sup>c</sup> ±0.27	16.019 <sup>e</sup> ±0.16
LB	48.704 <sup>c</sup> ±0.46	25.417 <sup>e</sup> ±0.38	6.958 <sup>b</sup> ±0.40
NaNO <sub>3</sub> (control)	9.111 <sup>a</sup> ±0.47	0.528 <sup>a</sup> ±0.02	1.302 <sup>a</sup> ±0.26

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

YP: Yeast extract, WH: Whey, PP: Peptone, SB: Soybeans: UR: Urea, LB: Locust beans  
NH<sub>4</sub>Cl: Ammonium chloride, NaNO<sub>3</sub>: Sodium nitrate

**Figure 1.** Effect of different inoculum concentrations (spores/ml).

amino acids and porosity for oxygen supply (Bakri *et al.*, 2003; Javed *et al.*, 2006).

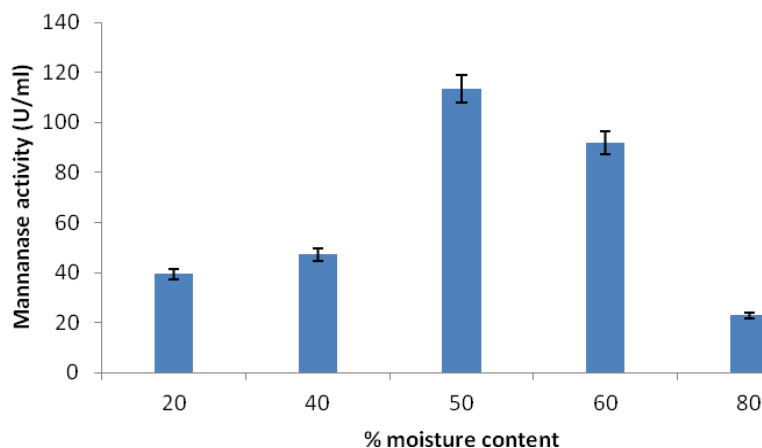
### The effect of inorganic and organic nitrogen sources

In this present work, different nitrogen sources were separately added to the fermentation medium at 0.2% concentration replacing all nitrogen sources from minimal salt medium used as moistening agent. Among all the nitrogen sources tested; soybean meal gave maximum production of mannanase (117.407 U/ml) when compared with the NaNO<sub>3</sub> (control) (Table 3). Similar result was reported by Javed *et al.* (2006) when soybeans meal was observed to be best nitrogen source for β-glucosidase production and this was attributed to the ease of mycelium to get nitrogen from it. But Okeke and Obi (1993) reported the more enzyme production using inorganic nitrogen sources as compared to the organic nitrogen sources from *Arthrographis* sp. They

mentioned that the use of the organic nitrogen sources could also be advantageous than the inorganic ones, as the organic sources are cheaper in cost and also not involved in the competition with active site of the enzyme. The study is in agreement with the work done by Kansoh and Gammal (2001). They used organic nitrogen sources and reported more activity than inorganic nitrogen sources.

### The effect of inoculum concentrations (spores/ml)

Inoculum concentration has profound effect on the production of mannanase and protein synthesis. The best inoculum size on mannanase production by *P. italicum* LAD-A5 was recorded at 1×10<sup>6</sup> spores/ml which gave highest activity of 37.562 U/ml, and as the inoculum size increased the production decreased as indicated in Figure 1. At an increased inoculum size, the fungus might over grow and led to the phenomena where the fungus



**Figure 2.** Effect of different percentage moisture content.

**Table 4.** Effect of sugar supplementation.

Sugars	Mannanase activity (U/ml)	Protein (mg/ml)	Yield (U/g)
Maltose	98.15 <sup>g</sup> ±0.41	1.852 <sup>bc</sup> ±0.02	19.63 <sup>g</sup> ±0.10
Glucose	88.43 <sup>f</sup> ±0.26	3.103 <sup>f</sup> ±0.13	17.686 <sup>f</sup> ±0.01
Mannose	51.39 <sup>b</sup> ±1.82	1.852 <sup>ab</sup> ±0.37	10.278 <sup>b</sup> ±0.12
Arabinose	62.96 <sup>d</sup> ±0.39	1.944 <sup>c</sup> ±0.04	12.592 <sup>d</sup> ±0.04
Mannitol	34.26 <sup>a</sup> ±0.57	1.574 <sup>a</sup> ±0.05	6.852 <sup>a</sup> ±0.03
Lactose	80.09 <sup>e</sup> ±0.54	2.685 <sup>e</sup> ±0.09	16.018 <sup>e</sup> ±0.08
Galactose	56.94 <sup>c</sup> ±0.29	3.102 <sup>f</sup> ±0.12	11.388 <sup>c</sup> ±0.01
LBG (control)	115.278 <sup>h</sup> ±0.32	2.315 <sup>d</sup> ±0.02	28.056 <sup>h</sup> ±0.05

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

lacked nutrient for the production of enzyme (Abd-Aziz *et al.*, 2008). It has also been reported that the inoculum size influenced the mycelia growth and exo-biopolymer production (Park *et al.*, 2001; Lee *et al.*, 2004). It was observed that small inoculum size controls and shortens the initial lag phase whereas larger inoculums size increased the moisture content to significant extent. The free excess liquid present an additional diffusion barrier together with that imposed by solid nature of the substrate and leads to a decreased in growth and enzyme production (Jatinder *et al.*, 2006a,b; Abo-State *et al.*, 2010).

### The effect of moisture

“The moisture content is an important factor that influences the growth and product yield in SSF (Alam *et al.*, 2005; Mrudula *et al.*, 2011)”. “Moisture is reported to cause swelling of the substrates, thereby facilitating better utilization of the substrate by microorganisms” (Kim *et al.*, 1985; Nagendra and Chandrasekaran, 1996). The

data presented in the Figure 2 clearly indicates that the yield of mannanase by *P. italicum* LAD-A5 increased with an increase in percentage moisture content from 20% to 50% with an optimum activity of 56.123 U/ml obtained at 50%. Any further increase in the percentage moisture content beyond 50% resulted in the decrease of enzyme yields may be due to clumping of solid particles which results in the decrease of inter-particle space leading to decreased diffusion of nutrients (Babu and Satyanarayana, 1996; Alam *et al.*, 2005; Mrudula *et al.*, 2011). In contrast, the low moisture content leads to the decrease solubility of nutrients present in the substrate thereby decreases enzyme yields.

### The effect of sugar supplementation

The effect of various sugars (0.2 g/l) supplemented to the enzyme production medium containing locust bean gum in order to evaluate its induction or repression effect on mannanase production were tested (Table 4). The highest activity was exhibited by 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 (Moussa and Thawat, 2007; Mabrouk and El-Ahwany, 2008).

## Conclusion

In conclusion, *P. italicum* LAD-A5 is capable of producing copious titre value of mannanase activity under solid state fermentation when commercial and agro-wastes were utilized as carbon sources. The optimal nutritional factors for the production of mannanase were proposed at  $1 \times 10^6$  inoculum size and 50% moisture content.

## REFERENCES

- Abd-Aziz S, Ong LGA, Hassan MA, Karim MIA (2008). Process parameters optimization of mannanase production from *Aspergillus niger* FTCC 5003 using palm kernel cake as carbon source. *Asian J. Biotechnol.*, 3(5): 297-307.
- Abo-State MAM, Swelim M, Hammad AI, Gannam RB (2010). Some critical factors affecting cellulases production by *Aspergillus terreus* Mam-F23 and *Aspergillus flavus* Mam-F35 under solid state fermentation of wheat straw. *World Appl. Sci. J.*, 9(10): 1171-1179.
- Ademark P, Varga A, Medve J, Harjunpa AV, Drakenberg T, Tjerneld F (1998). Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: Purification and properties of a  $\beta$ -mannanase. *J. Biotechnol.*, 63: 199-210.
- Alam MZ, Muhammad N, Mahmat ME (2005). Production of cellulase from oil palm biomass as substrate by solid state bioconversion. *Am. J. Appl. Sci.*, 2(2): 569-572.
- Babu KR, Satyanarayana T (1996).  $\alpha$ -Amylase production by thermophilic *Bacillus coagulans* in solid state fermentation. *Process Biochem.*, 30: 305-309.
- Bakri Y, Jacques P, Thonart P (2003). Xylanases production by *Penicillium canescens* 10-10c in solid state fermentation. *Appl. Biochem. Biotechnol.*, 108: 737-748.
- Chandrakant P, Bisaria VS (1998). Simultaneous bioconversion of cellulose and hemicellulose to ethanol. *Crit. Rev. Biotechnol.* 18: 295-331.
- Dhawan S, Kaur J (2007). Microbial mannanases: an overview of production and applications, *Crit. Rev. Biotechnol.*, 27(4):197-216.
- El-Naggar MY, Youssef SA, El-Assar SA, Beltagy EA (2006). Optimization of cultural conditions for  $\beta$ -mannanase production by a local *Aspergillus niger* isolate. *Int. J. Agric. Biol.*, 8(4): 539-545.
- Ferreira HM, Filho EXF (2004). Purification and characterization of a mannanase from *Trichoderma harzianum* strain T4, *Carbohydr. Polym.*, 57: 23-29.
- Gibson GR, Ottaway PB, Rastall RA (2000). *Prebiotics: New Developments in Functional Foods*, Chandos Publishing, Oxford, UK.
- Gubitz GM, Hayn M, Urbanz G, Steiner W (1997). Purification and properties of an acid  $\beta$ -mannanase from *Sclerotium rolfsii*. *J. Biotechnol.*, 45: 165-172.
- Hashem AM, Ismail AMS, El-Refai MA, Abdel-Fattah AF (2009). Purification and some properties of  $\beta$ -mannanase from *Aspergillus oryzae* NRRL 3448. *J. Appl. Sci. Res.*, 5(12): 2067-2073.
- Hashimoto Y, Fukumoto J (1969). Studies on the enzyme treatment of coffee beans. *Nippon Nogeikagaku Kaishi*, 43: 317-322.
- Heck JX, Soares HB, Ayub MAZ (2005). Optimization of xylanase and mannanase production by *Bacillus circulans* strain BL53 on solid state cultivation, *Enzyme Microb. Technol.*, 37: 417-423.
- Jatinder K, Chadha BS, Saini HS (2006a). Optimization of medium components for production of cellulases by *Melanocarpus* sp. MTCC 3922 under solid-state fermentation. *World J. Microbiol. Biotechnol.*, 22: 15-22.
- Jatinder K, Chadha BS, Saini HS (2006b). Optimization of culture conditions for production of cellulases and xylanases by *Scytalidium thermophilum* using Response Surface Methodology. *World J. Microbiol. Biotechnol.*, 22: 169-176.
- Javed MM, Ikram UH, Siddiq Z, Saleem T (2006). Triggering of  $\beta$ -glucosidase production in *Trichoderma viride* with nutritional and environmental control. *J. Appl. Sci. Res.*, 2(11): 884-889.
- Jiang ZQ, Wei Y, Li D, Li L, Chai P, Kusakabe I (2006). High level production, purification and characterization of a thermostable mannanase from the newly isolated *Bacillus subtilis* WY34, *Carbohydr. Polym.*, 66: 88-96.
- Kansoh AL, Gammal A (2001). Xylanolytic activities of *Streptomyces* sp. taxonomy production, partial purification and utilization of agricultural wastes. *Acta Microbiol. Immunol. Hung.*, 48: 39-52.
- Kim JH, Hosobuchi M, Kishimoto M, Seki T, Ryu DDY (1985). Cellulase production by a solid state culture system. *Biotechnol. Bioeng.*, 27: 1445-1450.
- Lee BC, Bae JT, Pyo HB, Choe TB, Kim SW, Hwang HJ, Yun JW (2004). Submerged culture conditions for the production of mycelia biomass and exopolysaccharides by the edible Basidiomycete *Grifola frondosa*. *Enzyme Microb. Technol.*, 35(5): 369-376.
- Lin SS, Dou WF, Xu HY, Li HZ, Xu ZH, Ma YH (2007). Optimization of medium composition for the production of alkaline beta-mannanase by alkaliphilic *Bacillus* sp. N16-5 using response surface methodology, *Appl. Microbiol. Biotechnol.*, 75: 1015-1022.
- Lin TC, Chen C (2004). Enhanced mannanase production by submerged culture of *Aspergillus niger* NCH-189 using defatted copra based media, *Process Biochem.*, 39(9): 1103-1109.
- Mabrouk MEM, El Ahwany AMD (2008). Production of  $\beta$ -mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. *Afr. J. Biotechnol.*, 7(8): 1123-1128.
- McCearry VB, Matheson NK (1974). Galactomannan structure and  $\beta$ -mannanase and  $\beta$ -mannosidase activity in germinating legume seeds. *Phytochem.*, 14: 1187-1194.
- Meenakshi M, Singh G, Bhalla A, Hoondal GS (2010). Solid state fermentation and characterization of partially purified thermostable mannanase from *Bacillus* sp. MG-33. *Bioresour* 5(3): 1689-1701.
- Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.*, 31: 426-428.
- Moussa TAA, Tharwat NA (2007). Optimization of cellulase and  $\beta$ -glucosidase induction by sugarbeet pathogen *Sclerotium rolfsii*. *Afr. J. Biotechnol.*, 6: 1048-1054.
- Mrudula S, Gopal R, Seenayya G (2011). Effect of substrate and culture conditions on the production of amylase and

- pullulanase by thermophilic *Clostridium thermosulfurogenes* SVM17 in solid state fermentation. *Malaysian J. Microbiol.*, 7(1): 15-21.
- Naganagouda K, Salimath PV, Mulimani VH (2009). Purification and characterization of endo- $\beta$ -1,4 mannanase from *Aspergillus niger* gr for application in food processing industry. *J. Microbiol. Biotechnol.*, 19(10): 1184-1190.
- Nagendra PG, Chandrasekharan M (1996). L-Glutaminase production by marine *Vibrio costicola* under solid state fermentation using different substrates. *J. Mar. Biotechnol.*, 4: 176-179. *Malaysian J. Microbiol.*, 7(1): 15-21.
- Nunes FM, Coimbra MA (1998). Influence of polysaccharide composition in foam stability of espresso coffee. *Carbohydr. Polym.*, 37: 283-285.
- Okeke BC, Obi CKS (1993). Production of cellulolytic and xylanolytic enzymes by an *Arthrographis* species. *World J. Microbiol. Biotechnol.*, 9: 345-349.
- Park JP, Kim SW, Hwang HJ, Yun JW (2001). Optimization of submerged culture conditions for the mycelia growth and exopolymer production by *Cordyceps militaris*. *Lett. Appl. Microbiol.*, 33(2): 76-81.
- 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.
- Ray S, Pubols MH, Mgginnis J (1982). The effect of a purified guar degrading enzyme on chicken growth. *Poult. Sci.*, 61: 488-494.
- Regalado CB, Garcia-Almendarez E, Venegas-Barrera LM (2000). Production, partial purification and properties of  $\beta$ -mannanases obtained by solid substrate fermentation of spent soluble coffee wastes and copra paste using *Aspergillus oryzae* and *Aspergillus niger*. *J. Sci. Food Agric.*, 80(9): 1343-1350.
- Sae-Lee N (2007). The production of fungal mannanase, cellulase and xylanase using palm kernel meal as a substrate. *Walailak J. Sci. Technol.* 4(1): 67-82.
- Titapoka S, Keawsompong S, Haltrich D, Nitisinprasert S (2008). Selection and characterization of mannanase-producing bacteria useful for the formation of prebiotic manno-oligosaccharides from copra meal. *World J. Microbiol. Biotechnol.*, 24(8): 1425-1433.
- Tomotari M (1990). Bifidobacteria and their role in human health. *J. Industrial Microbiol.*, 6: 263-268.