

EVALUATION OF ENZYMATIC ACTIVITIES AND DEGRADATION ABILITIES OF ANTAGONISTIC MICROORGANISMS ASSOCIATED WITH COMPOST

Nadia Ramzan*, Nayara Noreen, Zahida Perveen and Saleem Shahzad

Pest & Disease Research Lab., Department of Agriculture & Agribusiness Management, University of Karachi, Karachi-75270, Pakistan

*Corresponding author's email: nrbotany2006@yahoo.com

ABSTRACT

During the present studies, thirteen antagonistic compost inhabiting bacteria and fungi selected from *in vitro* studies against soil borne pathogens were evaluated for their amylase, cellulase and pectinase activities. Most of the antagonists showed the enzymatic activities. The antagonists were also inoculated in to the grass clippings to see their degradation abilities. All the antagonists showed the ability to degrade the grass clippings but with varied rate of degradation. The highest rate of degradation shown by *Bacillus licheniformis* and *Aspergillus fumigatus* followed by *Bacillus subtilis*, *Trichoderma harzianum*, *Bacillus cereus*, *Bacillus megaterium*, *Penicillium citrinum*, *Bacillus pumilus*, *Micrococcus varians*, *Trichoderma virens*, *Acrophialophora fusispora*, *Stachybotrys charatum* and *Pseudomonas fluorescens*.

Key words: Amylase, cellulase, pectinase, compost inhabiting bacteria and fungi, degradation abilities.

INTRODUCTION

Compost is a solid mature product obtained from composting process. Composting is the recycling of solid waste. By the action of bacteria and fungi, organic waste change in to humus (Adediran *et al.*, 2003). The composting material contains complex compounds and degradation of these compounds requires the use of extracellular enzymes produced by microorganisms (Miyatake and Iwabuchi, 2005). Many of the extracellular enzymes common in both bacteria and fungi convert complex compounds in to simpler. Beneficial microbes *viz.*, *Bacillus*, *Pseudomonas*, *Aspergillus*, *Penicillium* and *Trichoderma* species associated with compost (Ryckeboer *et al.*, 2003). Many of them produce lytic enzymes that can hydrolyze a variety of compounds, including chitin, proteins, cellulose and hemicelluloses (Tripathi *et al.*, 2008).

Amylase enzymes that play a major role in the utilization of polysaccharides (Zoltowska, 2001) occur in various bacteria, fungi, plants and animals (Da Lagea *et al.*, 2007). α -amylase and glucoamylase are the two major classes of amylase enzymes (Gomes and Steiner, 2004). Commercially about 25-35% of the world enzymes consist of α -amylase produced by different microorganisms (Nguyen *et al.*, 2002). The utilization of agriculture waste materials reduced the pollution and upgraded the materials. Agriculture waste used for both liquid and solid fermentation consists of carbon and nitrogen sources necessary for the growth and metabolism of organisms (Tang-Yao, 2002). These nutrients sources include corn, orange waste, wheat and rice as flours used for amylase production (Djekrif-Dakhmouche *et al.*, 2006). It is also used in the fermentation industries for the conversion of starch in to simplest sugars (Farid *et al.*, 2002).

Cellulose is one of the most important sources of carbon (Nowak *et al.*, 2005). Cellulolytic enzymes include endoglucanases, exoglucanases and β -glucosidases that play an important role in the hydrolysis of cellulose and biomass utilization (Bhat and Bhat, 1997; Subramaniyan and Prema, 2000). Cellulolytic microorganisms degrade the cellulose into simplest sugar by producing cellulase enzyme (Kasana *et al.*, 2008). During organic degradation, several microorganisms such as species of *Aspergillus*, *Penicillium* and *Trichoderma* have been reported to generate cellulolytic enzymes (Gautam *et al.*, 2010b; Wilson, 2011). Many bacteria including species of *Bacillus*, *Pseudomonas*, *Streptomyces* and *Staphylococcus* are also capable to degrading cellulose (Gautam *et al.*, 2010c). At industrial scale the cost of enzyme production can be reduced by obtaining the enzymes from microorganisms.

Pectin is a polysaccharide present in the cell wall of plants (Alphons *et al.*, 2009). Various bacteria, fungi and higher plants produced pectinolytic enzymes that catalyze the degradation of pectic substances (Chinedu *et al.*, 2008). The most important commercial pectinase is polygalacturonase. Several bacterial and fungal strains have potential to produce pectinolytic enzymes (Pericin *et al.*, 2007; Nitinkumar and Bhushan, 2010). Pectinases are also produced from several agriculture pectin containing wastes (Reda *et al.*, 2008), such as citrus peel (Mamma *et al.*, 2008). The composting process can be speed up by microbial inoculations (Sarkar *et al.*, 2010). The microbes generate enzymes and increase the degradation of organic materials (Ghaffari *et al.*, 2011). The microbial

inoculation efficiency is usually affected by competition with indigenous microorganisms (Xi *et al.*, 2005). Selection of suitable microbes is, therefore, an important factor for effectiveness of inoculation. The aim of the present study is to find out the beneficial microbes which can accelerate the composting process by producing extra cellular enzymes.

MATERIAL AND METHODS

Antagonistic microorganisms

Antagonistic microorganisms isolated from compost and check their antagonistic activity against soil borne pathogens by dual culture plate assay.

Amylase activity

A 5mm inoculum disc from pure culture of each antagonistic fungus was inoculated in the centre of Petri plates containing Starch Peptone Agar (SPA) medium (Starch 5g, peptone 10g, agar 20g, distilled water, 1000ml) amended with penicillin and streptomycin. The antagonistic bacteria were streaked on SPA medium without antibiotics. There were three replicates of each bacterium and fungus. The plates were incubated at their respective temperatures for 2-3 days before exposure to iodine vapours. Formation of a clear zone around the colonies showed amylolytic activity and indicated a positive reaction. Medium where starch was not utilized turned blue when exposed to iodine vapours.

Cellulase activity

A 5mm inoculum disc from pure culture of each antagonistic fungus was inoculated in the center of Petri plates containing Cellulose agar (CA) medium (Cellulose 10g, agar 20g, distilled water, 1000ml) amended with penicillin and streptomycin. The antagonistic bacteria were streaked on CA medium without antibiotics. There were three replicates of each bacterium and fungus. The plates were incubated at their respective temperatures for 2-3 days. The plates were then exposed to iodine vapours. Formation of clear zone around the colonies showed the cellulolytic activity and indicated a positive reaction. Medium where cellulose was not utilized turned pinkish when exposed to iodine vapours.

Pectinase activity

A 5mm inoculum disc from pure culture of each antagonistic fungus was inoculated in the center of Petri plates containing Hanskin's medium. To prepare the medium, mineral salts *viz.*, $(\text{NH}_4)_2\text{SO}_4$ 2g, K_2HPO_4 4g, Na_2HPO_4 6g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2g, CaCl_2 0.001g, H_3BO_3 0.00001g, ZnSO_4 0.00007g, MoO_3 0.00001g, all dissolved in 100ml distilled water and volume makeup to 500ml; Yeast extract 1g, pectin 5g, agar 20g, distilled water 500ml and then both the 500ml solutions mixed to get 1000ml medium. Penicillin and streptomycin added to the medium for inhibition of bacteria. The antagonistic bacteria were streaked on Hanskin's medium without antibiotics. There were three replicates of each bacterium and fungus. The plates incubated at their respective temperatures for 2-3 days and then flooded with 1% aqueous solution of hexa-decyle-tri-methyl ammonium bromide. Formation of a clear zone around the colonies showed the pectinase activity and indicated a positive reaction. Medium where pectin was not utilized showed no change in colour.

Degradation abilities of antagonists

Four ml water was added to 10g of grass (*Cynodon dactylon* L.) clippings in 100ml flasks. The flasks were plugged with cotton and autoclaved. After autoclaving, the flasks were allowed to cool down and then inoculated with antagonist. For each antagonist there were three replicates. Flasks without antagonist served as control. Time that each antagonist took to degrade grass clippings was recorded.

RESULTS

Antagonistic microorganisms

Thirteen microorganisms (six fungi and seven bacteria) were selected by dual culture plate assay.

Amylase activity

Five fungi *viz.*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Stachybotrys charatum*, *Trichoderma harzianum*, *T. virens* and five bacteria *viz.*, *Bacillus cereus*, *B. licheniformis*, *B. megaterium*, *B. subtilis* and *Micrococcus varians* showed amylolytic activity (Table 1; Fig. 1).

Cellulase activity

All fungi viz., *Acrophialophora fusispora*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Stachybotrys charatum*, *Trichoderma harzianum*, *T. virens* and six bacteria viz., *Bacillus cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis* and *Micrococcus varians* showed cellulolytic activity (Table 1; Fig. 2).

Pectinase activity

Four fungi viz., *Acrophialophora fusispora*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Trichoderma harzianum* and seven bacteria viz., *Bacillus cereus*, *Bacillus licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *Micrococcus varians* and *Pseudomonas fluorescens* showed pectinolytic activity (Table 1; Fig. 3).

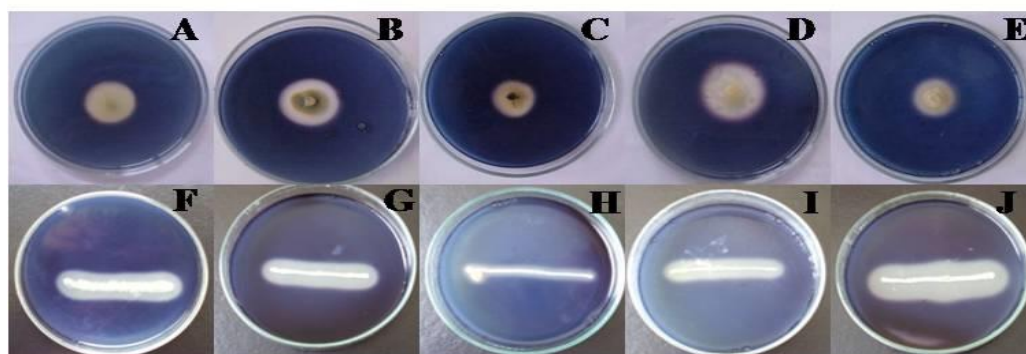


Fig. 1. Amylase activities of antagonists

A= *Aspergillus fumigatus*, B= *Penicillium citrinum*, C= *Stachybotrys charatum*, D= *Trichoderma harzianum*, E= *Trichoderma virens*, F= *Bacillus cereus*, G= *Bacillus licheniformis*, H= *Bacillus megaterium*, I= *Bacillus subtilis*, J= *Micrococcus varians*.

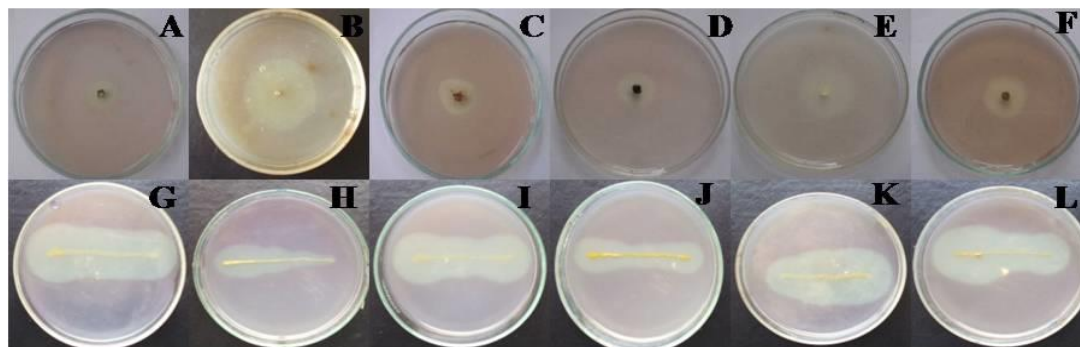


Fig. 2. Cellulase activities of antagonists

A= *Acrophialophora fusispora*, B= *Aspergillus fumigatus*, C= *Penicillium citrinum*, D= *Stachybotrys charatum*, E= *Trichoderma harzianum*, F= *Trichoderma virens*, G= *Bacillus cereus*, H= *Bacillus licheniformis*, I= *Bacillus megaterium*, J= *Bacillus pumilus*, K= *Bacillus subtilis*, L= *Micrococcus varians*.

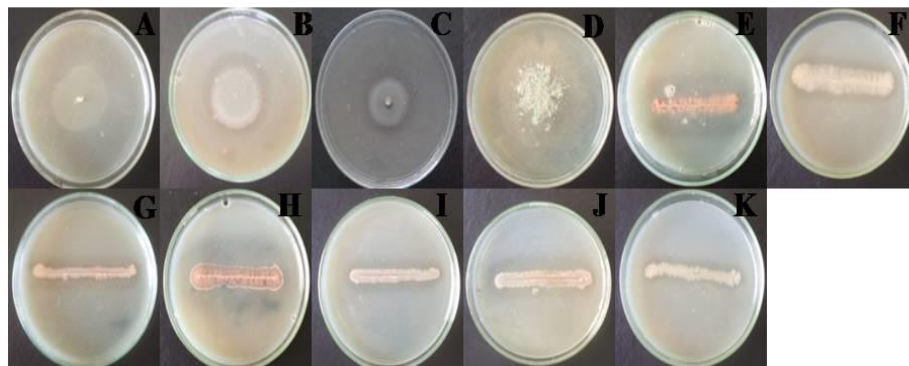


Fig. 3. Pectinase activities of antagonists

A= *Acrophialophora fusispora*, B= *Aspergillus fumigatus*, C= *Penicillium citrinum*, D= *Trichoderma harzianum*, E= *Bacillus cereus*, F= *Bacillus licheniformis*, G= *Bacillus megaterium*, H= *Bacillus pumilus*, I= *Bacillus subtilis*, J= *Micrococcus varians*, K= *Pseudomonas fluorescens*.

Degradation abilities of antagonists

All the antagonists showed ability to degrade the grass clippings. However, the microorganisms showed varied rate of degradation. The highest rate of degradation was showed by *Bacillus licheniformis* and *Aspergillus fumigatus* followed by *Bacillus subtilis*, *Trichoderma harzianum*, *Bacillus cereus*, *Bacillus megaterium*, *Penicillium citrinum*, *Bacillus pumilus*, *Micrococcus varians*, *Trichoderma virens*, *Acrophialophora fusispora*, *Stachybotrys charatum* and *Pseudomonas fluorescens* (Table 2).

Table 1. Enzymatic activities of antagonists.

S.No.	Test organisms	Amylase	Cellulase	Pectinase
	Fungi			
1	<i>Acrophialophora fusispora</i>	-	+	+
2	<i>Aspergillus fumigatus</i>	+	+	+
3	<i>Penicillium citrinum</i>	+	+	+
4	<i>Stachybotrys charatum</i>	+	+	-
5	<i>Trichoderma harzianum</i>	+	+	+
6	<i>Trichoderma virens</i>	+	+	-
	Bacteria			
7	<i>Bacillus cereus</i>	+	+	+
	<i>Bacillus licheniformis</i>	+	+	+
9	<i>Bacillus megaterium</i>	+	+	+
10	<i>Bacillus pumilus</i>	-	+	+
11	<i>Bacillus subtilis</i>	+	+	+
12	<i>Micrococcus varians</i>	+	+	+
13	<i>Pseudomonas fluorescens</i>	-	-	+

Table 2. Degradation of grass clippings by biocontrol agents.

S.No.	Name of microorganisms	Days of incubation	Degradation
1	<i>Acrophialophora fusispora</i>	11	++
2	<i>Aspergillus fumigatus</i>	5	++ +
3	<i>Bacillus cereus</i>	6	++
4	<i>Bacillus licheniformis</i>	5	++ +
5	<i>Bacillus megaterium</i>	6	++
6	<i>Bacillus pumilus</i>	7	++
7	<i>Bacillus subtilis</i>	6	++ +
8	<i>Micrococcus varians</i>	8	++
9	<i>Penicillium citrinum</i>	6	++
10	<i>Pseudomonas fluorescens</i>	12	+
11	<i>Stachybotrys charatum</i>	14	++
12	<i>Trichoderma harzianum</i>	6	++ +
13	<i>Trichoderma virens</i>	8	++

+ = Good Degradation
 ++ = Better Degradation
 +++ = Best Degradation

DISCUSSION

Fungi and bacteria are involved in the cycling of nutrients since they have ability to produce enzymes that degrade the complex organic molecules into simplest, that are unavailable to many organisms (Orth *et al.*, 1993). Bacteria secrete many extracellular enzymes that are involved in assortment recognition and important for symbiosis (Van Workum Wat *et al.*, 1998). Enzymes such as chitinase, B-1,3 glucanases and cellulases produce by *Trichoderma harzianum* (Lorito *et al.*, 1994; Di Pietro, 1995) help in degradation of the cell wall of pathogens and therefore, biological activity increases.

During the fermentation of starch many bacteria and fungi produce extracellular amylases (Adeniran and Abiose, 2009). The microbes produced amylase enzyme convert the starch into the oligosaccharides with quick reduction in blue colour and appearance of clear zone (Najafi *et al.*, 2005). In starch industries, α - amylase play a major role (Pandey *et al.*, 2000). The antifungal activity of *Trichoderma* species is due to the greater variety of lytic enzymes. These lytic enzymes play a great role in biocontrol (Kubicek *et al.*, 2001). During the present studies, amylase enzymes produced by antagonists viz., *T. harzianum*, *T. virens*, *A. fumigatus*, *P. citrinum*, *S. charatum*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. subtilis* and *Micrococcus varians*. Similarly report made by (Das *et al.*, (2004), Mohamed *et al.* (2011), and Hamuel (2011). Some species of *Bacillus* produced enzymes in stationary or some in exponential phase. Stability of enzymes is sensitive to pH and temperature (Declerk *et al.*, 2003).

The cellulase producing fungi and bacteria were selected on the formation of clear zone around their colonies on coboxymethyl cellulose agar plates (Immanuel et al., 2006). During the present studies *T. harzianum*, *T. virens*, *A. fumigatus*, *A. fusispora*, *P. citrinum*, *S. charatum*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis* and *M. varians* showed cellulase activity. Mishra et al. (1982) isolated different cellulolytic fungi viz., *Chaetomium globosum*, *Fusarium solani*, *Paecilomyces variotii* and *Penicillium chrysogenum* from soil and compost. Cellulolytic fungi such as *Aspergillus*, *Chaetomium*, *Penicillium* and *Trichoderma* hasten composting and reduce the composting period for one month (Dubey and Maheshwari, 2005). *B. cereus* (Lah et al., 2012), *B. pumilus* (Ariffin et al., 2006), *B. licheniformis* (Fujimoto et al., 2011), *B. subtilis* (Kim et al., 2012), *A. fumigatus*, (Kumari et al., 2011) have been reported earlier to produced cellulases.

Pectic substances are the main component of cell wall. These substances are degraded by the enzyme pectinase. The major degrader of fruit wastes are the pectinolytic bacteria (Rolz et al., 2011). Many workers observed pectin production using agricultural wastes as substrate (Castilho et al., 2000). *T. harzianum*, *P. citrinum*, *A. fumigatus*, *A. fusispora*, *M. varians*, *P. fluorescens* and thermotolerant species of *Bacillus* showed pectinase activity during present studies. Bhardwaj and Garg, (2010) observed similar results. Different workers isolated pectinase from bacteria and fungi viz., *A. fumigatus* (Phutela et al., 2005), *T. harzianum* (Nabi et al., 2003), *Bacillus* species (Marcia et al., 1999). Through bacteria and fungi important enzymes could be produced cheaply and it will reduce the cost of final products.

All the species that produced enzymes are important from the biotechnological point of view and in the decomposition of agricultural residues (Singh and Hayashi, 1995). The abundant growth in the fermentation substrate indicated the ability of fungi and bacteria to produce enzymes, which degraded the substrate. Enzymes can be produced on both solid and liquid substrates. However, solid-state fermentation has many advantages as compared to liquid state fermentation (Aguilar and Huitron, 1990). To reduce the cost of fermentation, agricultural waste is used for fermentation. Mostly bacteria and fungi are involved in the waste bioconversion. These wastes mostly consist of carbon and nitrogen sources. In the present studies, grass was used as a substrate. For decomposition of grass clippings, spore suspensions of selected biocontrol agents were added to grass clippings. The degradation of grass clippings was much faster in inoculated grass as compared to non-inoculated grass clippings. *A. fumigatus* and *B. licheniformis* showed the fastest degradation of grass clippings within five days as compared to other biocontrol agents, when inoculated singly. There are many microbiological agents involved in the biodegradation. *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., produced cellulase enzyme during the degradation of organic waste (Gautam et al., 2010b). The microbes present in compost produced a variety of enzymes, which help in the degradation. Vargas-Garcia et al. (2005) reported that waste from the agriculture sources could be used as substrates for the growing and preservation of lingo-cellulolytic fungi.

Normally natural composting process takes five to six months (Jilani, 2007), but this period can be shortened by inoculating the beneficial microbes with high enzymatic activities in to the composting material. Biswas and Narayanasamy (2002) inoculated *Trichoderma* and *Aspergillus* species into the composting material and they observed that composting period was shortened and maturity of compost occurs within one month. Composts represent an optimal substrate for biocontrol agents, thus encouraging their establishment into the soil environment (Leandro et al., 2007).

REFERENCES

- Adediran, J.A., L.B. Taiwo and R.A. Solubo (2003). Effect of organic wastes and method of composting on compost maturity. Nutrient composition of compost and yields of two vegetable crops. *J Sustainable Agriculture*, 22(4): 95-101.
- Adeniran, A.H. and S.H. Abiose (2009). Amylolytic potentially of fungi isolated from some Nigerian agriculture waste. *Afr J Biotechnol.*, 8: 667-672.
- Aguilar, G. and C. Huitron (1990). Constitutive exo pectinases produced by *Aspergillus niger* sp. CH-Y-1043 on different carbon sources. *Biotechnol Lett*, 12(9): 655- 660.
- Alphons, G., J. Voragen, J.C. Gerd, P.V.E. René and A.S. Henk (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Struct Chem.*, 20: 263-275.
- Ariffin, H., N. Abdullah, M.S. Umi Kalsom, Y. Shirai and M.A. Hassan (2006). Production and characterization of cellulose by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology*, 3(1): 47-53.
- Bhardwaj, V. and N. Garg (2010). Exploitation of microorganisms for isolation and screening of pectinase from environment. Proceeding in 8th International Conference-Globelics. Kuala Lumpur, Malaysia.
- Bhat, M. K. and S. Bhat (1997). Cellulose degrading enzymes and their potential industrial applications. *Biotechnology Advances*, 15(3-4): 583-620.
- Biswas, D.R. and G. Narayanasamy (2002). Mobilization of phosphorus from rock phosphate through composting using crop residue fertilizer. *News B.* pp. 53-56.

- Castilho, L.R., T.L.M. Alves and R.A. Medronho (2000). Production and extraction of pectinases obtained by solid-state fermentation of agro-industrial residues with *Aspergillus niger*. *Bioresour Technol*, 71: 45-50.
- Chinedu, S.N., U.A. Okafor, T.N. Emezue and Okochi (2008). Xylanase production of *Aspergillus niger* and *Penicillium chrysogenum* from ammonia pretreated cellulosic waste. *Res J Microbiol*, 3(4): 246- 253.
- Da Lagea, J.L., G.J. Etienne, E. G.J. Danchinc and D. Casane (2007). Where do animal α -amylases come from? An inter kingdom trip. *Feder Eur Biochem Soc.*, 581: 3927-3935.
- Das, K., R. Doley and A.K. Mukherjee (2004.) Purification and characterization of a thermostable alkaliphilic, extracellular alpha-amylase from *Bacillus subtilis* DM-03, a strain isolated from the traditional fermented food of India. *Biotechnol Appl Biochem*, 40(3): 291-298.
- Declerck, N., M. Machius, P. Joyet, G. Wiegand, R. Huber and C. Gaillardin (2003). Hyperthermostabilization of *Bacillus licheniformis* α -amylase and modulation of its stability over a 50°C temperature range. *Protein Eng.*, 16(4): 287-293.
- Di Pietro, A. (1995). Fungal antibiosis in biocontrol of plant diseases. Chapter 20 In: Allelopathy: Organisms, Processes and Applications. Inderjit, Dakshini, K. M.M. and Einhellig, F.A. (Eds). pp. 271-279.
- Djekrif-Dakhmouche, S., Z. Gheribi-Aoulmi, Z. Meraihi and L. Bennamoun (2006). Application of a statistical design to the optimization of culture medium for α -amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder. *J Food Eng.*, 73: 190-197.
- Dubey, R.C. and D.K. Maheshwari (2005). A textbook of Microbiology. S. Chan and Company Ltd. Ram Nagar, New Dehli 110055.
- Farid, M.A., H.A. El-Enshasy and A.M. Noor El-Deen (2002). Alcohol production from starch by mixed cultures of *Aspergillus awamori* and immobilized *Saccharomyces cerevisiae* at different agitation speeds. *J Basic Microbiol.*, 42: 162-171.
- Fujimoto, N., T. Kosaka, T. Nakao and M. Yamada (2011). *Bacillus licheniformis* bearing a high cellulose-degrading activity, which was isolated as a heat-resistant and micro-aerophilic microorganism from Bovine Rumen. *The Open Biotechnology Journal*, 5: 7-13.
- Gautam, S.P., P.S. Bundela, A.K. Pandey, M.K. Awasthi and S. Sarsaiya (2010b). Effect of different carbon sources on production of cellulases by *Aspergillus niger*. *Journal of Applied Science Environmental Sanitation*, 5(3): 277-281.
- Gautam, S.P., P.S. Bundela, A.K. Pandey, Jamaluddin, M.K. Awasthi and S. Sarsaiya, (2010c). Cellulase production by *Pseudomonas* sp. isolated from municipal solid waste compost. *International Journal of Academic Research*, 2(6): 330-333.
- Ghaffari, S., A.A. Sepahi, M.R. Razavi, F. Malekzadeh and H. Haydarian (2011). Effectiveness of inoculation with isolated *Anoxybacillus* sp. MGA110 on municipal solid waste composting process. *Afr J Microbiol Res.*, 5: 5373-5378.
- Gomes, J. and W. Steiner (2004). The biocatalytic potential of extremophiles and Extremozymes. *Food Technol.*, 42(4): 223-239.
- Hamuel, D.J. (2011). Production of β -glucanase enzyme from *Penicillium oxalicum* and *Penicillium citrinum*. *African Journal of Biotechnology*, 10(47): 9657-9660.
- Immanuel, G., R. Dhanusha, P. Prema and A. Palavesam (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *Int J Environ Sci Tech.*, 3: 25-34.
- Jilani, S. (2007). Municipal solid waste composting and its assessment for re-use in plant production. *Pakistan Journal of Botany*, 39(1): 271-277.
- Kasana, R.C., R. Salwan, H. Dhar, S. Dutt and A. Gulati (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Curr Microbiol*, 57: 503-507.
- Kim, Y.K., S.C. Lee, Y.Y. Cho, H.J. Oh and Y.H. Ko (2012). Isolation of cellulolytic *Bacillus subtilis* strains from agricultural environments. *ISRN Microbiology*, pp. 1-9.
- Kubicek, C.P., R.L. Mach, C.K. Peterbauer and M. Lorito (2001). *Trichoderma*: From genes to biocontrol. *J Plant Pathol.*, 83: 11-23.
- Kumari, B.L., M. Hanumasri and P. Sudhakar (2011). Isolation of cellulose producing fungi from soil, optimization and molecular characterization of the isolate for maximizing the enzyme yield. *World Journal of Science and Technology*, 1(5): 1-9.
- Lah, T.N.T., N.N.N. Ab- Rahman and M.M. Ben Nama (2012). Cellulase activity and glucose production by *Bacillus cereus* monoculture and co-culture utilizing palm kernel cake (PKC) under solid-state fermentation. *International Conference on Environment, Energy and Biotechnology*, 33: 172-177.
- Leandro, L.F.S., T. Guzman, L.M. Ferguson, G.E. Fernandez and F.J. Louws (2007). Population dynamics of *Trichoderma* in fumigated and compost amended soil and on strawberry roots. *Applied Soil Ecology*, 35: 237-246.
- Lorito, M., C.K. Hayes, A. Di Pietro, S.L. Woo and G.E. Harman (1994). Purification, characterization and synergistic activity of a glucan 1,3 β -glucosidase and N-acetyl- β glucosaminidase from *Trichoderma harzianum*. *Phytopathology*, 84: 394-405.
- Mamma, D., E. Kourtoglou and P. Christakopoulos (2008). Fungal multienzyme production on industrial by products of citrus-processing industry. *Bioresour Technol.*, 99: 2373-2383.

- Marcia, M.C.N., R.D. Silva and E. Gomes (1999). Screening of bacterial strains for pectinolytic activity: characterization of the polygalacturonase produced by *Bacillus* sp. *Revista de Microbiologia*, 30: 299-303.
- Mishra, M.M., K.K. Kapoor and K.S. Yadav (1982). Effects of compost enriched with Mussoorie rock phosphate on crop yield. *Indian J Agric Sci.*, 52: 674-678.
- Miyatake, F. and K. Iwabuchi (2005). Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost. *Bioresour Technol.*, 10: 1016.
- Mohamed, S.A., E.I. Azhar, M.M. Ba-Akdah, N.R. Tashkandy and T.A. Kumosani (2011). Production, purification and characterization of α -amylase from *Trichoderma harzianum* grown on mandarin peel. *African Journal of Microbiology Research*, 5(9): 1018-1028.
- Nabi, N.Z., M. Asgher, A.H. Shah, M.A. Sheikh and M.J. Asad (2003). Production of pectinase by *Trichoderma harzianum* in solid fermentation of citrus peel. *Pak J Agri Sci.*, 40: (3-4).
- Najafi, M.F., D. Deobagkar and D. Deobagkar (2005). Purification and characterization of an extracellular α -amylase from *Bacillus subtilis* AX20. *Protein Expr Puri.*, 41: 349-354.
- Nguyen, Q.D., J.M. Rezessy-Szabo, M. Claeysens, I. Stals and A. Hoschke (2002). Purification and characterization of amylolytic enzymes from thermophilic fungus *Thermomyces lanuginosus* strain ATCC 34626. *Enzyme Microb Technol.*, 31: 345-352.
- Nitinkumar, P.P. and L.C. Bhushan (2010). Production and purification of Pectinase by soil isolate *Penicillium* sp. and search for better agro-residue for its SSF. *Recent Res Sc Tech.*, 2(7): 36-42.
- Nowak, J., M. Florek, W. Kwiatak, J. Lekki, P. Chevallier and E. Zieba (2005). Composite structure of wood cells in petrified wood. *Mater Sci Eng.*, 25:119-30.
- Orth, A.B., D.J. Royse and M. Tien (1993). Ubiquity of lignin-degrading peroxidases among various wood-degrading fungi. *Appl. Environ. Microbiol.*, 59: 4017-4023.
- Pandey, A., P. Nigam, C.R. Soccol, V.T. Soccol, D. Singh and R. Mohan (2000). Advances in microbial amylases. *Biotechnol Appl Biochem*, 31:135-152.
- Pericin, D.M., S.Z. Madarev, L.M. Radulovic and M. Skrinjar (2007). Production of exo-pectinase by *Penicillium roqueforti* using pumpkin oil cake. *Nat Sci.*, 113: 313-320.
- Phutela, U., V. Dhuna, S. Sandhu and B.S. Chadha (2005). Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposing orange peels. *Braz J Microbiol*, 36: 63- 69.
- Reda, A.B., M. Hesham and M. Yassin (2008). Production of Bacterial Pectinase from Agro-industrial Wastes Under solid-state fermentation conditions. *Journal of Applied Science Research*, 4(12): 1708-1721.
- Rolz, C., R. De Leon and M.C. De Arricola (2011). Biotechnology in washed coffee processing. *Process Biochemistry*, 16: 8-11.
- Ryckeboer, J., J. Megaert, J. Coosemans, K. Deprins and J. Swings (2003). Microbiological aspects of biowaste during composting in a monitored compost bin. *J Appl Microbiol*, 94 (1): 127-137.
- Sarkar, S., R. Banerjee, C. Chanda, P. Das, D. Ganguly and S. Pal (2010). Effectiveness of inoculation with isolated *Geobacillus* strains in the thermophilic stage of vegetable waste composting. *Bioresour Technol.*, 101: 2892-2895.
- Singh, A. and K. Hayashi (1995). Microbial cellulases, protein architecture, molecular properties and biosynthesis. *Adv Appl Microbiol.*, 40: 1-44.
- Subramaniam, S. and P. Prema (2000). Cellulase-free xylanases from *Bacillus* and other microorganisms. *FEMS Microbiol Lett*, 183-187.
- Tang-Yao, H. (2002). Isolation and biochemical characterization of an endo-1, 3- β -glucanase from *Streptomyces sioyaensis* containing a C-terminal family 6 carbohydrate binding 1,3- β -glucan. *Microbiol.*, 148: 1151-1159.
- Tripathi, S., S. Kamal, I. Sheramati, R. Oelmuller and A. Varma (2008). Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. *Mycorrhiza* 3rd edition Springer pp. 281-306.
- Van Workum, W.A.T., S. Van Slageren, A.A.N. Van Brussel and J.W. Kijne (1998). Role of exopolysaccharides of *Rhizobium leguminosarum* bv. *viciae* as host plant-specific molecules required for infection thread formation during nodulation of *Vicia sativa*. *Mol Plant Microbe Interact.*, 11: 1233-1241.
- Vargas- Garcia, M.C., M.J. Lopez, F.F. Suarez and J. Moreno (2005). Laboratory study of inocula production for composting processes. *Bioresour Technol*, 96(7): 797-803.
- Wilson, D.B. (2011) Microbial diversity of cellulose hydrolysis. *Current Opinion in Microbiology*, 14, 259-263.
- Xi, B.D., G.J. Zhang and H.L. Liu (2005). Process kinetics of inoculation composting of municipal solid waste. *J Hazard Mater, B.*, 124: 165-172.
- Zoltowska, K. (2001). Purification and characterization of alpha-amylases from the intestine and muscle of *Ascaris suum* (Nematoda). *Acta Biochem Pol.*, 48: 763-774.

(Accepted for publication December 2015)

