



Original Article

## Suitable conditions for xylanases activities from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) and their properties for agricultural residues hydrolysis

Sudathip Chantorn<sup>1\*</sup>, Chanitchote Piyapittayanun<sup>1</sup>, Phataraporn Khumphai<sup>1</sup>, Srisuda Pannanusorn<sup>1</sup>,  
Kulwadee Phannachet<sup>2</sup>, and Jirawan Apiraksakorn<sup>3</sup>

<sup>1</sup> Department of Biotechnology, Faculty of Science and Technology,  
Thammasat University, Rangsit Campus, Khlong Luang, Pathumthani, 12120 Thailand.

<sup>2</sup> Department of Microbiology, Faculty of Medicine,  
Chiang Mai University, Mueang, Chiang Mai, 50200 Thailand.

<sup>3</sup> Department of Biotechnology, Faculty of Technology,  
Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand.

Received: 28 November 2014; Accepted: 6 October 2015

---

### Abstract

*Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) were isolated from soybean field in Khon Kaen province, Thailand. Crude enzymes from both isolates showed the activities of cellulase, xylanase, and mannanase at 37°C for 24 h. The highest xylanase activities of *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) were 1.58±0.25 and 0.82±0.16 U/ml, respectively. The relative xylanase activities from both strains were more than 60% at pH 5.0 to 8.0. The optimum temperature of xylanases was 50°C in both strains. The residual xylanase activities from both strains were more than 70% at 60°C for 60 min. Five agricultural wastes (AWs), namely coffee residue, soybean meal, potato peel, sugarcane bagasse, and corn cobs, were used as substrates for hydrolysis properties. The highest reducing sugar content of 101±1.32 µg/ml was obtained from soybean meal hydrolysate produced by *Bacillus* sp. GA2(1) xylanase.

**Keywords:** *Bacillus* sp., xylanase, mannanase, cellulase, agricultural wastes

---

### 1. Introduction

Lignocellulosic materials such as agricultural hardwood and softwood residues are the most abundant and cost effective biomass available to the world (Hsu *et al.*, 2010). The composition of lignocellulosic material is different depending on species. It consists of mainly three different types of polymers: cellulose, hemicelluloses, and lignin (Hendriks and Zeeman, 2009). Hemicellulose is the second most abundant

renewable biomass product. Xylan is recognized as the major hemicelluloses component of agricultural residue, and is composed of a linear backbone of β-1,4-linked-D-xylopyranosyl units, which are differently substituted branch chains (Bajaj *et al.*, 2012). The complete hydrolysis of xylan is achieved by the combination of several enzyme activities due to its complex heterogeneous structure. The major enzymes involved in xylan hydrolysis are endo-1,4-β-xylanase (1,4-β-D-xylanohydrolase; EC 3.2.1.8) that cleaves glycosidic bonds to produce xylo-oligosaccharides and β-D-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37) which is responsible for the final breakdown of xylo-oligosaccharide into xylose. The xylanolytic enzymes have attracted a great deal of attention

---

\* Corresponding author.  
Email address: sudathip@tu.ac.th

because their applications in various industrial processes such as paper and pulp, food and feed and biofuel industries (Susan van Dyk *et al.*, 2010). Furthermore, five agricultural wastes (AWs): coffee residue, soybean meal, potato peel, bagasse, and corn cobs, are found in large quantities in Thailand. All AWs contain cellulose and hemicelluloses that could be hydrolyzed by cellulase and hemicellulase. Xylanases, the major hemicellulase, are produced by many microorganisms including bacteria, yeast, actinomycetes, and filamentous fungi (Fang *et al.*, 2007; Ko *et al.*, 2010). Many studies have been conducted to identify the new xylanase with high activity and stability which can be used in extreme conditions in various industries. The use of low cost agricultural by-products as substrates for xylanase production is attractive in terms of economic production (Gupta *et al.*, 2001; Sanghi *et al.*, 2008). In this study, xylanases from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) were characterized. In addition, we compared the oligosaccharide production by xylanase digestibility of various agricultural materials.

## 2. Materials and Methods

### 2.1 Materials

All chemicals, media and media components were of analytical grade obtained from Sigma-Aldrich Chemical Ltd., USA; Carlo Erba reagent, France; and HiMedia Laboratories Ltd.

### 2.2 Microorganisms and cultivation

*Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) were isolated from soil in a soybean field in Khon Kaen province using xylan agar medium (pH 7.0) at 37°C. Stock cultures were maintained and stored at -20°C on nutrient broth at culture collection of the Department of Biotechnology, Khon Kaen University, Thailand.

### 2.3 Enzyme production

The enzyme was produced in an 250 ml Erlenmeyer flask containing 50 ml of basal medium [(% w/v): 0.20 NaNO<sub>3</sub>, 0.05 K<sub>2</sub>HPO<sub>4</sub>, 0.02 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 MnSO<sub>4</sub>·2H<sub>2</sub>O, 0.02 CaCl<sub>2</sub>·H<sub>2</sub>O, 0.02 FeSO<sub>4</sub>·7H<sub>2</sub>O, and 0.50 corn cob]. The overnight grown *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) with the OD 600 of 0.5 was inoculated with 1%(v/v) and shaken at 150 rpm for 18 h at 37°C. The cell suspension was centrifuged at 10,000Xg for 20 min at 4°C, and the crude enzymes were collected and stored at -20°C for further study.

### 2.4 Enzyme assay

The activities of extracellular enzymes; cellulase, xylanase, and mannanase were determined at 37°C for 15 min. To determine the respective cellulase, xylanase, and mannanase activities, the reaction mixtures contained 0.5 ml

of crude enzyme and 0.5 ml of 10 mM phosphate buffer pH 7.0 with 1%(w/v) substrate in which carboxymethylcellulose (CMC), oat spelt xylan, and locust bean gum were used. The amounts of reducing sugar were measured by the dinitrosalicylic acid (DNS) method (Miller, 1959). All experiments were conducted in triplicate, and results represented as mean values of the activities.

One unit of cellulase, xylanase or mannanase activity was defined as the amount of enzyme producing 1 micromole of glucose, xylose or mannose per minute respectively, under experimental conditions.

### 2.5 Effect of pH on enzyme activity

The optimum pH of xylanase activity was evaluated of pH of 3.0-10.0 by 10 mM of citrate (pH 3.0-6.0), phosphate (pH 6.0-8.0), and glycine-NaOH (pH 8.0-10.0). The pH stability was determined by incubating xylanase preparation for 24 h at 4°C in different buffers. At regular intervals of 6 h samples were withdrawn and residual enzyme activity was determined.

### 2.6 Effect of temperature on enzyme activity

The optimum temperature was determined by incubating the enzyme in 10 mM of appropriate buffers at different temperatures between 30°C and 80°C. The thermostability was evaluated by incubation at different temperatures for 60 min. The remaining enzyme activities were measured.

### 2.7 Hydrolysis experiment

#### 2.7.1 Agricultural wastes preparation

Five AWs: coffee residue, soybean meal, potato peel, sugarcane bagasse, and corn cobs, were used as substrates for this study. All AWs were dried at 60°C for 48 h, blended, milled by a hammer mill (IKA Labortechnik; Janke & Kunkel, Germany) and then sieved to obtain products with an average particle size of 30 mesh. All samples were kept dessicated until used.

#### 2.7.2 Enzymatic hydrolysis

One unit of crude xylanase from *Bacillus* sp. GA2(1) and from *Bacillus* sp. GA1(6) were incubated with each type of AW in 10 mM of suitable buffers at 50°C, 150 rpm for 5 h. The reduced sugar content was analyzed using DNS method.

## 3. Results and Discussion

### 3.1 Enzyme production

The production of extracellular enzyme by *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) was studied in a shaken flask using corn cobs as an inducer. Table 1 shows the activi-

Table 1. Extracellular enzyme activities from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6)

Culture / Enzyme	Cellulase (U/ml)	Xylanase (U/ml)	Mannanase (U/ml)
<i>Bacillus</i> sp. GA2(1)	0.33 ± 0.10	1.58 ± 0.25	0.53 ± 0.08
<i>Bacillus</i> sp. GA1(6)	0.15 ± 0.04	0.82 ± 0.16	0.25 ± 0.07

ties of cellulases, mannanases, and xylanases. It was obvious that xylanase produced the highest activity in both strains, with the xylanase activity of *Bacillus* sp. GA2(1) (1.58±0.25 U/ml) being higher than that of *Bacillus* sp. GA1(6) (0.82±0.16U/ml).

Many microorganisms such as *Bacillus circulans*, *B. stearothermophilus* strain T6 (Khasin *et al.*, 1993), *Streptomyces cuspidosporus* (Maheswari and Chandra, 2000), *Rhodothermus marinus* (Hreggvidsson *et al.*, 1996), *Cellulomonas fimi* (Shallom and Shoham, 2003), *Cellvibrio japonicas* (Hogg *et al.*, 2001), *Fusarium merismoides* (Fernandez-Martin *et al.*, 2000), *Aspergillus niger* (Park *et al.*, 2002), *Trichoderma reesei* and *Penicillium* sp (Mikan and Castellanos, 2004) have been shown to possess cellulase and hemicellulase (xylanase and mannanase) activities. In this study, *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6), gram-positive bacteria, also exhibited activities of cellulase, mannanase, and xylanases but the highest enzyme activity was obtained from xylanases in both strains of bacteria.

### 3.2 Effect of pH on xylanase activity

The effect of pH on xylanase activities is shown in Figure 1. Crude xylanases from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) presented highest activities of 1.50±0.15 U/ml at pH 6.0 and 0.76±0.11 U/ml at pH 5.0, respectively.

The optimum pH of 6.0 of *Bacillus* sp. GA2(1) crude xylanase was not only consistent with that of *Bacillus* sp. strain SPS-0, *Bacillus* sp. W1 (JCM2888), and *Bacillus* sp. strain TAR-1 (Subramaniyan and Prema, 2002), but comparable to the optimum pH of some fungal xylanases such as those of *Aspergillus fischeri*, *A. nidulans*, and *Fusarium oxysporum* F3 (Raj and Chandra, 1996; Fernandez-Espinar *et*

*al.*, 1994; Christakopoulos *et al.*, 1996). The optimum pH of 5.0 of crude xylanase from *Bacillus* sp. GA1(6) was the same as many other xylanases from *Streptomyces* sp. T-7, *Streptomyces* sp. No 3137, and from some fungi such as *Aspergillus* sp., *A. awamori*, *A. sojae*, *A. oryzae*, *Trichoderma longibrachiatum*, *T. viride*, and *T. harzianum* (Khanna *et al.*, 1995). However, the slightly acidic pH of the enzymes matched the value characteristic of the family 11 endoxylanase (Subramaniyan and Prema, 2002).

The pH stability of *Bacillus* sp. GA2(1) xylanase was such that it retained more than ~60% residual activity after 12 h of incubation in pH range from 3.0 to 10.0. Moreover, xylanase activity from *Bacillus* sp. GA2(1) revealed stability in pH range from 5.0 to 8.0 and showed ~80% residual activity after 24 h of incubation (Figure 2A). Thus, *Bacillus* sp. GA2(1) xylanase possessed broad pH stability and this could be an important tool for application in industrial processes.

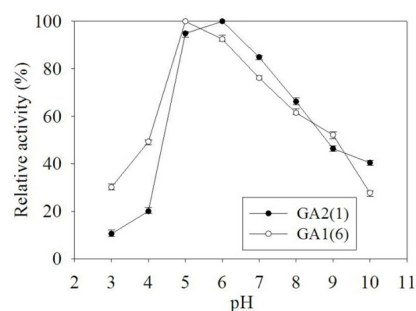


Figure 1 Optimum pH of xylanase from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6). The influence of pH on xylanase activity was determined at 50°C using 10 mM of different buffers.

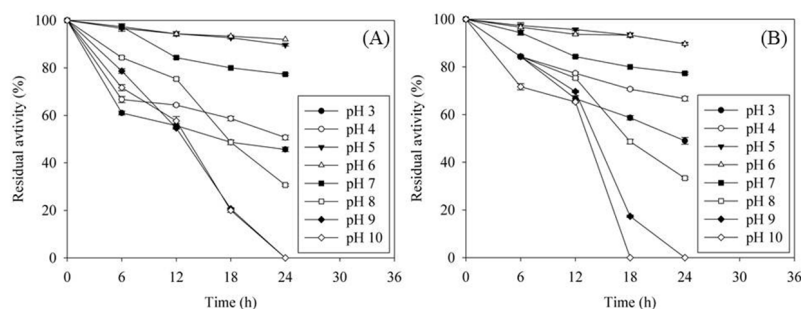


Figure 2. Effect of pH on the stability of xylanase from *Bacillus* sp. GA2(1) (A) and *Bacillus* sp. GA1(6) (B). The enzyme solutions were incubated at 4°C for 24 h in different buffers (pH 3 to 10).

Furthermore, the stability of *Bacillus* sp. GA1(6) xylanase exhibited a broad pH range of 4.0-7.0 with ~70% residual activity after 24 h of incubation while the activities rapidly decreased at pH 8.0-10.0 after 12 h of incubation (Figure 2B). The enzyme was relatively less stable at high pH than at low pH. These results suggested that the *Bacillus* sp. GA1(6) xylanase was rather stable in the acidic region. The difference in pH for extracellular xylanases might be due to the post transcriptional modifications in xylanase excretion process, such as glycosylation, that improved stability in more extreme pH conditions (Adhyaru *et al.*, 2014). However, to confirm this result, further study should be performed employing purified xylanase.

**3.3 Effect of temperature on xylanase activity**

An optimum temperature of xylanases was 50°C for both *Bacillus* sp. GA2(1) (1.62±0.13 U/ml) and *Bacillus* sp. GA1(6) (0.75±0.11 U/ml) at standard assay as shown in Figure 3A. Temperature stabilities were evaluated by pre-incubating in the 30-80°C range for 60 min (Figure 3B). They retained more than 70% of their activities at 30-60°C, and above the range, the activities rapidly decreased.

An optimum temperature of 50°C xylanases for both *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) was similar to that of *B. pumilus* (Prema and Asha Poorna, 2006), *Bacillus* sp. KS09, *Bacillus* sp. Strain 41-1, *Streptomyces* sp. CD3, *S. exfoliates* (Mittal *et al.*, 2012), and *Jonesia denitrificans* (Nawel *et al.*, 2011). Most of the bacterial xylanases exhibit optimum activity at 50-60°C (Bajaj and Manhas, 2012). However, thermostability of different *Bacillus* sp. xylanases varies between 55°C and 80°C (Kumar and Satyanarayana, 2011). The thermostability of enzyme depends on molecular interactions such as hydrogen bond, hydrophobic interactions, and disulfide bond which can improve conformation structure for enzyme stability (Bajaj and Sharma, 2011).

**3.4 AWs hydrolysis**

Five AWs including coffee residue, soybean meal, potato peel, sugarcane bagasse and corn cobs contain carbohydrate structures. The maximum reducing sugar content of 101±1.32 µg/ml was obtained from soybean meal hydrolysate produced by *Bacillus* sp. GA2(1) xylanase, while the maximum reducing sugar content of 54.32±0.55 µg/ml was observed from coffee residue hydrolysate produced by *Bacillus* sp. GA1(6) xylanase as shown in Figure 4.

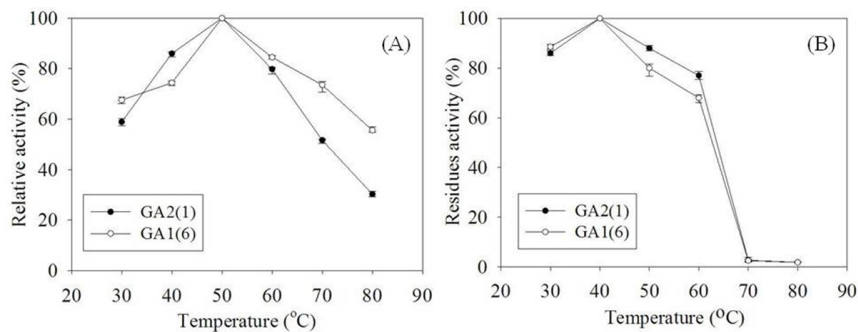


Figure 3. Optimum temperature (A) and thermostability (B) of xylanase from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6). The optimum temperature was measured at different temperatures. For determination of thermostability, the residual activity of xylanase was measured after 60 min pre-incubation at different temperatures at pH 6.0 for *Bacillus* sp. GA2(1) and pH 5.0 for *Bacillus* sp. GA1(6), respectively.

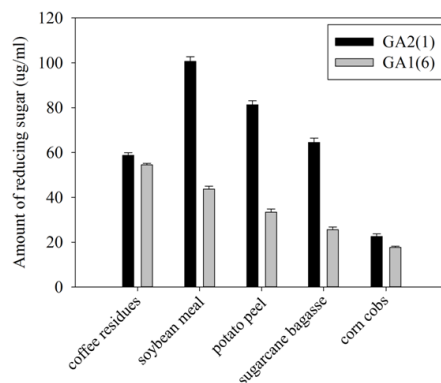


Figure 4. Reducing sugar content of various agricultural waste hydrolysates produced by crude xylanase from *Bacillus* sp. GA 2(1) (■) and *Bacillus* sp. GA 1(6) (■)

Carbohydrates of all AWs in this study were mainly cellulose and hemicellulose. Hemicelluloses are heteropolymers of pentoses (xyloses and arabinose), hexoses (mannose, galactose and glucose), and sugar acid. Bioconversion of hemicellulose has received more attention because of its practical application in various industrial processes, such as for production of fuels and chemicals (Saha, 2003). Although soybean meal is used primarily as a source of protein, it contains significant quantities of carbohydrates. These are mainly arabinogalactans, which are soluble, and therefore easily fermentable by beneficial microflora in animal intestinal tracts, especially in poultry and swine (Huisman *et al.*, 1999). Moreover, soybean meal hydrolysate may also result in promoting antimicrobial activity. Thus, the xylanase from *Bacillus* sp. GA2(1) could be used to produce soybean meal hydrolysate as a feed additive. When compared to *Bacillus* sp. GA1(6) xylanase, *Bacillus* sp. GA2(1) showed higher potential in hydrolysing various kinds of AWs, suggesting that xylanase *Bacillus* sp. GA2(1) could be applied in various industries.

#### 4. Conclusions

*Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) were isolated from a soybean field in Khon Kaen Province in Thailand. Crude xylanases from *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) showed the highest activity of  $1.50 \pm 0.15$  U/ml at pH 6.0 and  $0.76 \pm 0.11$  U/ml at pH 5.0, respectively. The pH stability xylanases from *Bacillus* sp. GA2(1) presented a wide pH range of 3-10 with ~60% residual activity and more than 80% residual activity after 12 h and 24 h of incubation at 4°C, respectively. In addition, xylanases from *Bacillus* sp. GA1(6) were stable in acid region with ~70% residual activity after 24 h of incubation at 4°C. Both isolates possess the optimum temperature of 50°C. The enzymes retained residual activity up to 70% after preincubating at 60°C for 1 h, and then rapidly decreased. With their action at acidic pH and stability at high temperature, both enzymes are good candidates for several industrial processes. Because *Bacillus* sp. GA2(1) and *Bacillus* sp. GA1(6) also produced cellulase and mannanase, and AWs hydrolysate produced sugars, we will characterize these enzymes and types of hydrolysate sugar in the near future.

#### Acknowledgements

This work was supported by Faculty of Science and Technology, Thammasat University research Fund.

#### References

- Adhyaru, D.N., Bhatt, N.S. and Modi, H.A. 2014. Enhanced Production of Cellulase-free, Thermo-alkali-solvent-stable Xylanase from *Bacillus altitudinis* DHN8, its Characterization and Application in Sorghum Straw Saccharification. *Biocatalysis and Agricultural Biotechnology*. 3(2), 182-190.
- Bajaj, B.K., Khajuria, Y.P. and Singh, V.P. 2012. Agricultural Residues as Potential Substrates for Production of Xylanase from Alkali-thermotolerant Bacterial Isolate. *Biocatalysis and Agricultural Biotechnology*. 1(4), 314-320.
- Bajaj, B.K. and Manhas, K. 2012. Production and Characterization of Xylanase from *Bacillus licheniformis* P11 (C) with Potential for Fruit Juice and Bakery Industry. *Biocatalysis and Agricultural Biotechnology*. 1(4), 330-337.
- Bajaj, B.K. and Sharma, P. 2011. An Alkali-thermotolerant Extracellular Protease from a Newly Isolated *Streptomyces* sp. DP2. *New Biotechnology*. 28(6), 725-732.
- Christakopoulos, P., Nerinckx, W., Kekos, D., Macris, B. and Claeysens, 1996. M. Purification and Characterization of Two Low Molecular Mass Alkaline Xylanases from *Fusarium oxysporum* F3. *Journal of Biotechnology*. 51(2), 181-189.
- Fang, H.Y., Chang, S.M., Hsieh, M.C. and Fang, T.J. 2007. Production, Optimization Growth Conditions and Properties of the Xylanase from *Aspergillus carneus* M34. *Journal of Molecular Catalysis B: Enzymatic*. 49(1-4), 36-42.
- Fernandez-Espinar, M.T., Pinaga, F., de Graaff, L., Visser, J., Ramon, D. and Valles, S. 1994. Purification, Characterization and Regulation of the Synthesis of an *Aspergillus nidulans* Acidic Xylanase. *Applied Microbiology Biotechnology*. 42(4), 555-562.
- Fernandez-Martin, R., Domenech, C., Cerda-Olmedo, E. and Avalos, J. 2000. ent-Kaurene and Squalene Synthesis in *Fusarium fujikuroi* Cell-free Extracts. *Phytochemistry*. 54(7), 723-728.
- Gupta, S., Kuhad, R.C., Bhushan, B. and Hoondal, G.S. 2001. Improved Xylanase Production from a Haloalkalophilic *Staphylococcus* sp. SG-13 Using Inexpensive Agricultural Residues. *World Journal of Microbiology and Biotechnology*. 17(1), 5-8.
- Hendriks, A.T.W.M. and Zeeman, G. 2009. Pretreatments to Enhance the Digestibility of Lignocellulosic Biomass. *Bioresource Technology*. 100(1), 10-18.
- Hogg, D., Woo, E.J., Bolam, D.N., MaKie, V.A., Gillbert, H.J. and Pickersgill, R.W. 2001. Crystal Structure of Mannanase 26A from *Pseudomonas cellulosa* and Analysis of Residues Involved in Substrate Binding. *The Journal of Biological Chemistry*. 276(33), 31186-31192.
- Hreggvidsson, G.O., Kaiste, E., Holst, O., Eggertsson, G., Palsdottir, A. and Kristjansson, J.K. 1996. An Extremely Thermostable Cellulase from the Thermophilic Eubacterium *Rhodothermus marinus*. *Applied Environmental Microbiology*. 62(8), 3047-3049.
- Hsu, T., Guo, G., Chen, W. and Hwang, W. 2010. Effect of Dilute Acid Pretreatment of Rice Straw on Structural Properties and Enzymatic Hydrolysis. *Bioresource Technology*. 101(13), 4907-4913.

- Huisman, M.M.H., Schols, H.A. and Voragen, A.G.J. 1999. Enzymatic Degradation of Cell Wall Polysaccharides from Soybean Meal. *Carbohydrate Polymers*. 38(4), 299-307.
- Khanna, P., Sundari, S.S. and Kumar, N.J. 1995. Production, Isolation and Partial Purification of Xylanase from an *Aspergillus* sp. *World Journal of Microbiology and Biotechnology*. 11(2), 242-243.
- Khasin, A., Alchanati, I. and Shoham, Y. 1993. Purification and Characterization of a Thermostable Xylanase from *Bacillus stearothermophilus* T-6. *Applied Environmental Microbiology*. 59(6), 1725-1730.
- Ko, C., Lin, Z., Tu, J., Tsai, C., Liu, C., Chen, H. and Wang, T. 2010. Xylanase Production by *Paenibacillus campinasensis* BL11 and Its Pretreatment of Hardwood Kraft Pulp Bleaching. *International Biodeterioration and Biodegradation*. 64(1), 13-19.
- Kumar, V. and Satyanarayana, T. 2011. Applicability of Thermo-alkali-stable and Cellulase-free Xylanase from a Novel Thermo-halo-alkaliphilic *Bacillus halodurans* in Producing Xylooligosaccharides. *Biotechnology Letters*. 33(11), 2279-2285.
- Maheswari, M.U. and Chandra, T.S. 2000. Production and Potential Applications of a Xylanase from a New Strain of *Streptomyces cuspidosporous*. *World Journal of Microbiology and Biotechnology*. 16(3), 257-263.
- Mikan, V.J.F. and Castellanos, S.D.E. 2004. Screening for Isolation and Characterization of Microorganisms and Enzymes with Useful Potential for Degradation of Cellulose and Hemicellulose. *Revista Colombiana de Biotecnología*. 6(1), 58-67.
- Miller, G.L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars. *Analytical Chemistry*. 31(3), 426-428.
- Mittal, A., Nagar, S., Kirti, K.S.J. and Gupta, V.K. 2012. Isolation, Purification and Characterization of Alkali and Thermostable Xylanase from *Bacillus* sp KS09. *International Journal Research and Development Pharmacy and Life Sciences*. 1(2), 63-68.
- Nawel, B., Said, B., Estelle, C., Hakin, H. and Duchiron, F. 2011. Production and Partial Characterization of Xylanase Produced by *Jonesia denitrificans* Isolated in Algerian Soil. *Process Biochemistry*. 46(2), 519-525.
- Park, Y.S., Kang, S.W., Lee, J.S., Hong, S.I. and Kim, S.W. 2002. Xylanase Production in Solid State Fermentation by *Aspergillus niger* Mutant Using Statistical Experimental Design. *Applied Microbiology Biotechnology*. 58(5-6), 762-766.
- Prema, P. and Asha Poorna, C. 2006. Production and Partial Characterization of Endoxylanase by *Bacillus pumilus* Using Agro Industrial Residues. *Biochemical Engineering Journal*. 32(2), 106-112.
- Raj, K.C. and Chandra, T.S. 1996. Purification and Characterization of Xylanase from Alkali-tolerant *Aspergillus fischeri* Fxn1. *FEMS Microbiology Letters*. 145(3), 457-461.
- Saha, B.C. 2003. Hemicellulose Bioconversion. *Journal of Industrial Microbiology and Biotechnology*. 30, 279-291.
- Sanghi, A., Garg, N., Sharma, J., Kuhar, K., Kuhad, R.C. and Gupta, V.K. 2008. Optimization of Xylanase Production Using Inexpensive Agro-residues by Alkalophilic *Bacillus subtilis* ASH in Solid-state Fermentation. *World Journal of Microbiology and Biotechnology*. 24(5), 633-640.
- Shallom, D. and Shoham, Y. 2003. Microbial hemicellulases. *Current Opinion in Microbiology*. 6(3), 219-228.
- Subramaniyan, S. and Prema, P. 2002. Biotechnology of Microbial Xylanases: Enzymology, Molecular Biology and Application. *Critical Reviews in Biotechnology*. 22(1), 33-46.
- Susan van Dyk, J., Sakka, M., Sakka, K. and Pletschke, B.I. 2010. Characterization of the Multi-enzyme Complex Xylanase Activity from *Bacillus licheniformis* SVD1. *Enzyme and Microbial Technology*. 47(4), 174-177.