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Some Properties of a New Thermostable Xylanase from Alkaliphilic and Thermophilic *Bacillus* sp. Isolate DM-15

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Abstract: Alkaliphilic and thermophilic *Bacillus* sp. DM-15 was isolated from ciftehan thermal spring, Turkey, produced thermostable xylanase at 55°C at pH 9. The molecular weight of the enzyme was estimated to be 95.6 kDa by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The enzyme showed broad working pH range of 4.5-10.0 with an optimum pH of 6.5. The temperature optimum of the enzyme was found to be 60-70°C. Xylanase production by thermophilic *Bacillus* sp. strain cultivated in liquid media reached a maximum at 36 h, with levels of 192.8 μmol mg^{-1} protein/min. The enzyme was stable for 15 min at 60°C while 13, 86, 95 and 96% of the original activities were lost at 70, 80, 90 and 100°C, respectively. Enzyme activity was increased in the presence of 5 mM $CaCl_2$ (137%), $CoCl_2$ (133%), $CuSO_4$ (112%), $MgCl_2$ (106%) and KCl (105%) and inhibited in the presence of 5 mM EDTA, $HgCl_2$, $FeSO_4$, SDS (1%), $MnCl_2$, Triton X-100 (1%) up to 93, 89, 73, 41, 29 and 8%, respectively. The DM-15 thermostable xylanase may be suitable for several industrial applications, such as food, agricultural and biofuel.

Key words: Thermostable xylanase, *Bacillus* sp., characterization, optimum, molecular weight, thermal spring

INTRODUCTION

The major structure of hemicellulose is xylan which is the second most abundant polysaccharide after cellulose in nature (Whistler and Richards, 1970; Goheen, 1982; Coral *et al*., 2002; Collin *et al*., 2005; Faulet *et al*., 2006). It is found in large amounts in wood and plant material (Luthi *et al*., 1990). Xylan is a polymer β-1,4-linked xyloses with arabinosyl and/or 4-O-methylglucurosyl side chains (Faulet *et al*., 2006; Whistler and Richards, 1970).

Xylan can be degraded to xylose units by xylanolytic enzymes. These degredation requires the catalysis of both endo-xylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37) (Faulet *et al*., 2006). In natural environment, xylanases are produced mainly by microorganisms, marine algae, protozoans, crustaceans, insects and snails (Ratanachomsri *et al*., 2006). Among the microorganisms, many reports on xylanases from *Bacillus* sp., *Clostridium* sp., *Streptomyces* sp., *Aspergillus* sp., *Thermomyces lanuginosus*, *Thermoascus aurantiacus* and other microorganisms are available (Wong *et al*., 1988; Bastawde, 1992; Alam *et al*., 1994; Faulet *et al*., 2006). Xylanases show potential applications in biotechnology include baking, fruit juice, paper, textile, pulp, ethanol production, aroma and animal feed (Bhat, 2000). In industrial applications, xylanases have to be alkalophilic and thermostable. However, most of the xylanases known are optimally active at temperatures below 50°C and acidic or neutral pH (Gessesse, 1998; Ryan *et al*., 2003). Only a few xylanases are reported to be active and stable at high temperature and alkaline pH (Dhillon *et al*., 2000; Rani and Nand, 2000; Khandeparkar and Bhosle, 2006a).

In this research, isolated thermostable microorganism, *Bacillus* sp. DM-15, from the thermal spring in Turkey, producing thermostable xylanase which active at broad range pH values and described partial characterization of the enzyme.

MATERIALS AND METHODS

Organisms and cultivation conditions: *Bacillus* sp. DM-15 was isolated from the thermal spring in Ciftehan, Nigde,

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Turkey. The samples were incubated at 80°C for 10 min for selection of gram-positive spore forming bacteria, *Bacillus* sp. The isolates screened for xylanase production on LB-xylan-agar plates containing (g L^{-1}) peptone 10, yeast extract 5, NaCl 5, xylan 1, agar 15 at 55°C. The pH was adjusted to 7.5 with 1N NaOH. Xylanase positive colonies were selected by Congo-red staining procedure (Teather and Wood, 1982).

Enzyme production: Strain DM-15 was grown up in LB medium at 55°C with shaking at 200 rpm for 24 h. After removal of cells by centrifugation (Hettich Universal EBA12) (5,000 rpm, 10 min), the supernatant was used for all enzyme assays.

Enzyme assay: Xylanase activity was assayed by adding 1 mL enzyme to 0.02 g xylan (2%, w/v) in 0.1M phosphate buffer, pH 6.5 and incubating at 55°C for 30 min. The reaction was stopped by the addition of 3 mL of 3,5-dinitrosalicylic acid reagent and $A_{540\ nm}$ was measured in a Pharmacia spectrophotometer (Miller, 1959). An enzyme unit is defined as the amount of enzyme releasing 1 mmol of glucose from the substrate in 1 min at 55°C.

Effect of pH and temperature on activity and stability: Temperature and pH effects on enzyme activity were assayed at various temperatures ranging from 40-100°C and at pH values ranging from 4-10 for 30 min. Following buffers were used in the reactions: 100 mM Na-acetate (pH 4-6), 100 mM Na-phosphate (pH 6-7) and 100 mM Tris (pH 7-10) (Ozcan *et al*., 1996).

For the measurement of thermal stability, the enzyme was pre-incubated at temperatures between 40-100°C for 15 min at optimum pH. The enzyme activity was then determined under standard enzyme assay condition (60°C, 30 min and pH 6.5).

Effect of various metals ions, surfactants, chelating agents and inhibitors on activity: The effect of metal ions, chelating agents, surfactants and inhibitors on the activity of xylanase were determined by pre-incubating the enzyme in the presence of EDTA (5 mM), SDS (1%), $CaCl_2$ (5 mM), $MgCl_2$ (5 mM), KCl (5 mM), $FeSO_4$ (5 mM), $CuSO_4$ (5 mM), $CoCl_2$ (5 mM), $MnCl_2$ (5 mM), $HgCl_2$ (5 mM) and TritonX-100 (1%) for 30 min at 55°C before adding the substrate. Subsequently relative xylanase activities were measured at standard enzyme assay conditions (Faulet *et al*., 2006; Egas *et al*., 1998; Lo *et al*., 2001). The control (without any additive) was taken as having 100% activity.

SDS-PAGE and zymogram analysis: SDS-PAGE and SDS-Xylan-PAGE (0.2% xylan) were done as described by Laemmli (1970) with slab gels (12% w/v acrylamide). For visualizing of total proteins, SDS-PAGE was stained for 1 h with the solution of 0.1% Coomassie blue R 250-40% methanol-10% glacial acetic acid and then destained overnight in the same solution without dye. For activity staining (zymogram) of xylanase by SDS-Xylan-PAGE, SDS was removed by washing the gel at room temperature in solution-A (50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2), isopropanol) for 1 h and solution-B (50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2)) for 1 h, respectively. The gel was kept overnight in solution-C (50 mM Na_2HPO_4, 50 mM NaH_2PO_4 (pH 7.2), 5 mM β-mercaptoethanol, 1 mM EDTA) at 4°C for renaturation of the enzyme. It was then sealed with film and incubated at 55°C for 4 h. After incubation, the gel was stained in 0.1% (w/v) Congo-red dye for 1 h and washed with 1% (w/v) NaCl for 30 min to visualize the clear band of xylanase activity (Saul *et al*., 1990; Lee *et al*., 1994; Coral *et al*., 2002).

RESULTS AND DISCUSSION

Bacillus sp. DM-15 was gram positive, rod shaped, spore forming and aerobic bacterium. It was identified as *Bacillus* sp. depending on various morphological and biochemical characteristics. The growth was observed between pH 6 and 10 in LB medium at 55°C. The maximum enzyme synthesis occurred 12 h later from inoculation in LB medium at 55°C.

Determination of molecular mass: Molecular mass determined by SDS-PAGE electrophoresis revealed single band showing xylanase activity in gel using BioCapt MW software with a molecular weight of 95.6 kDa (Fig. 1).

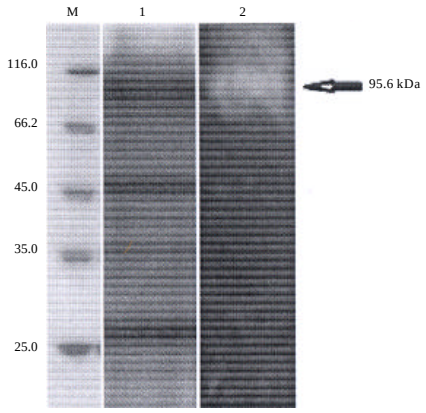

Fig. 1: Zymogram analysis of xylanase on SDS-PAGE. The gel was cut into two pieces, the total protein and mekar was visualized with Coomassie Brillant Blue (Lane: 1) staining and the activity of anzyme relealed by Congo-red (Lane: 2)

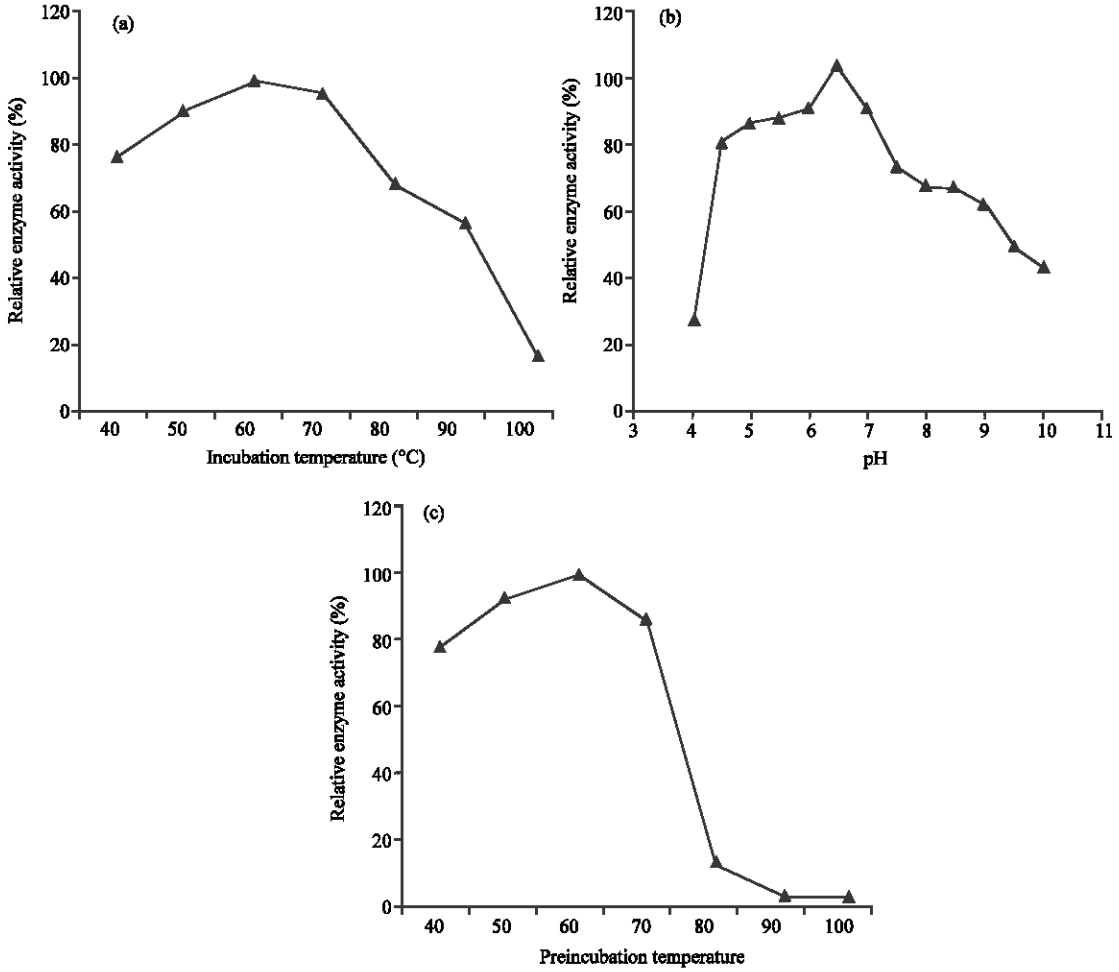

Fig. 2: Enzyme properties of *Bacillus* sp. DM-15 xylanase. a) Effect of temperature, b) Effect of pH and c) Thermal stability

Enzyme properties: The enzyme had a broad temperature range between 40-90°C and the optimum activity was observed at 60°C. The relative enzyme activities were 77, 91, 96, 68 and 57% at 40, 50, 70, 80 and 90°C, respectively, whereas only 17% activity was retained at 100°C for 30 min (Fig. 2a). The enzyme also showed a significant relative activity (73%) between pH 4.5 and 10 with an optimum pH of 6.5 (100%) (Fig. 2b). For thermal stability estimation, the retaining activity was determined at optimum pH and temperature (Fig. 2c). The enzyme was relatively thermostable at 70, 80, 90 and 100°C with, 13, 86, 95 and 96% lost of the original activities, respectively.

Table 1: Effect of different metal ions surfactans, chalating agents and inhibitors on xylanase from *Bacillus* sp. strain DM-15

Chemical	Concentration	Relative enzyme activity (%)
Control	None	100
$CaCl_2$	5 mM	137
EDTA	5 mM	7
SDS	1%	59
Triton X-100	1%	92
$MnCl_2$	5 mM	71
KCl	5 mM	105
$HgCl_2$	5 mM	11
$MgCl_2$	5 mM	106
$FeSO_4$	5 mM	27
Co	5 mM	133
$CuSO_4$	5 mM	112

Effect on some metal ions, surfactants, chelating agents and inhibitors on enzyme activity: The enzyme was pre-incubated at 60°C for 30 min at different concentration of the metal ions and various chemicals prior to standard enzyme activity assay. The results for residual enzyme activity have shown in Table 1. The relative activity of enzyme was significantly increased to 137 and 133% by adding $CaCl_2$ and $CoCl_2$ (5 mM each), respectively. Slightly stimulation was also observed by addition of $CuSO_4$ (112%), $MgCl_2$ (106%) or KCl (105%). On the other hand, partial inhibition of original enzyme activity was observed with use of SDS (1%), $MnCl_2$ (5 mM) and Triton

X-100 (1%) up to 59, 71 and 92%, respectively. DM-15 xylanase enzyme activity was strongly inhibited up to 7, 11 and 27% by the use of EDTA (5 mM), $HgCl_2$ (5 mM) or $FeSO_4$ (5 mM), respectively (Table 1). It has been reported that there are many microorganisms producing xylanolytic enzymes. They include species such as *Aspergillus niger*, *Marasmius* sp., *Termitomyces* sp., *Enterobacter* sp., *Bacillus* sp. (Coral *et al*., 2002; Ratanachomsri *et al*., 2006; Faulet *et al*., 2006; Gessesse, 1998; Saleem *et al*., 2002; Anuradha *et al*., 2007). The xylanases from thermophilic bacteria such as *Thermonospora fusca* and thermophilic *Bacillus* species show an optimum temperature in the range of 65-85°C (Kulkarni *et al*., 1999).

We report here the partial characterization of thermostable xylanase from alkaliphilic and thermophilic bacterium *Bacillus* sp. DM-15. *Bacillus* sp. DM-15 showed growth at a wide range of pH from 6-10 with an optimum pH of 9. The bacterium is called typically alkaliphilic, as it grows optimally at pH values above 8 and grows poorly under neutral pH value of 7 (Horikoshi, 1999). The optimum temperature for enzyme production and growth was 55°C. Vieille and Zeikus (2001) reported that thermophilic organisms grow optimally between 50 and 80°C. This suggests that strain *Bacillus* sp. DM-15 could be thermophilic bacterium.

Xylanase from the isolate *Bacillus* sp. DM-15 exhibited a broad range temperature profile with a peak of maximal activity at 60°C (Fig. 2a). Enzyme activity at 60°C was 192.8 μmol mg^{-1} protein/min and it showed good working temperature at 40-70°C at optimum pH value. It has been reported that fungal and bacterial xylanases are generally show temperature optimum between at 40 and 60°C (Butt *et al*., 2008). Enzyme activity was reduced to half at the temperature of 90°C with broad temperature interval. Also, enzyme thermostability was maintained until 70°C and then sharply reduced.

The pH activity of the DM-15 xylanase was in the range 4.5-10 with an optimum pH of 6.5 (Fig. 2b). This pH values are similar to thermostable xylanase from *Bacillus stearothermophilus* T-6 in the range 5-11 with an optimum pH of 6.5-7 (Khasin *et al*., 1993). The bacterial growth was found to be the best at pH 9. However, the optimum pH activity of xylanase was lower then growth pH optimum of bacteria. D-Xylanases from different organisms show broad pH optima and are usually stable over a wide pH range (3-10) and show optimum pH in the range of 4-7 (Kulkarni *et al*., 1999). Xylanases from *Aspergillus kawachii* and *Penicillium herquei* exhibit an optimum pH of 2-6 (Ito *et al*., 1992; Funaguma *et al*., 1991). However, the optimal pH of the xylanases isolated from *Bacillus* sp. C-59-2 (Horikoshi and Atsukawa, 1973) and *Bacillus* sp NCL 87-6-10 (Srinivasan and Rele, 1999) was 9. Although, DM-15 xylanase has slightly acidic optimal pH value, it show some activity at alkaline pH values with optimum growth at pH 9.0 with almost 50% activity. Similarly, the xylanase from *Bacillus* sp. with optimum growth at pH 10.0 showed remarkable stability at pH 9-10 but was not highly active above pH 8.0 (Okazaki *et al*., 1984).

Enzymes with high thermostability and an ability to function at wide pH range are desirable for many industrial applications as many industrial processes take place at very high or low pH and high temperature (Ratanachomsri *et al*., 2006). With this respect, the strain could be a good source for biotechnological applications.

Molecular weight of DM-15 thermostable xylanase was 95.6 kDa. Molecular weights of xylanases vary from 8-145 kDa (Sunna and Antranikian, 1997; Kulkarni *et al*., 1999). However, it has been reported that a high molecular weight of 340 kDa xylanase was produced by *Bacillus subtilis* (Sa-Pereira *et al*., 2002).

The effect of metal ions, surfactants, chelating agents and inhibitors on enzyme activity have been investigated. EDTA, $HgCl_2$, $FeSO_4$ and detergent agents inhibited the activity of the DM-15 xylanase. So that these chemicals must be eliminated after treatment of the oligosaccharide, polysaccharide or glycoprotein substrate when these needs to be denatured before its hydrolysis by the enzyme (Faulet *et al*., 2006). Similar results have been also reported (Bastawde, 1992; Tatjana and Vladimir, 1996; Chivero *et al*., 2001; Khandeparkar and Bhosle, 2006b). It is reported that the inhibition of xylanase enzyme by Hg^{+2} ions may be due to its interaction with sulphydryl groups of cysteine residue in or close to the active site of the enzyme (Khandeparkar and Bhosle, 2006b). The strong inhibition of DM-15 xylanase activity in the presence of EDTA suggests that metals are needed for the enzymatic reaction (Khandeparkar and Bhosle, 2006b). This was confirmed by the observed increase in xylanase activity in the presence of $CaCl_2$, $CoCl_2$, $CuSO_4$, $MgCl_2$ and KCl (Table 1). The stimulation of xylanases by $CaCl_2$ and $CuSO_4$ (*Bacillus* sp. strain AR-009, Gessesse, 1998), $MgCl_2$ (Khasin *et al*., 1993; Gessesse, 1998) and K^+ (Khasin *et al*., 1993; Gessesse, 1998; Faulet *et al*., 2006) have previously been reported and is in accordance with the present study. On the other hand, the inhibition of xylanases by Ca^{2+} (Faulet *et al*., 2006), Cu^{2+} (Khasin *et al*., 1993; Faulet *et al*., 2006) and $MgCl_2$ (Gessesse, 1998) have also been reported.

However, to reveal the mode of action of xylanase, further research using more inhibitors and combinations would be necessary. The DM-15 xylanase is sensitive to SDS and triton X-100. It was reported that denaturant detergent agents must be eliminated after treatment of the oligosaccharide, polysaccharide or glycoprotein substrate when this needs to be denatured before its hydrolysis by the enzyme (Faulet *et al*., 2006).

CONCLUSION

The *Bacillus* sp. DM-15 strain produced high levels of thermostable xylanase. The DM-15 xylanase is a slightly acidophilic with wide range of pH (4.5-10), thermostable and resistant to most of chelators and metal ions. Hence, it is qualified for use in biotechnological applications and all its properties make it a useful tool for biobleaching in pulp and paper industry. The DM-15 xylanase production process can be commercialized after further optimization for enhanced enzyme production.

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REFERENCES

Alam, M., I. Gomes, G. Mohiuddin and M.M. Hoq, 1994. Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* grown on lignocelluloses. Enzyme Microbiol. Technol., 16: 298-302.

Anuradha, P., K. Vijayalakshmi, N.D. Prasanna and K. Sridevi, 2007. Production and properties of alkaline xylanase from *Bacillus* sp. isolated from sugarcane fields. Curr. Sci. India, 92: 1283-1286.

Bastawde, K.B., 1992. Xylan structure, microbial xylanases and their mode of action. World J. Microbiol. Biotechnol., 8: 353-368.

Bhat, M.K., 2000. Cellulases and related enzymes in biotechnology. Biotechnol. Adv., 18: 355-383.

Butt, M.S., M. Tahir-Nadeem, Z. Ahmad and M.T. Sultan, 2008. Xylanases and their applications in baking industry. Food Technol. Biotechnol., 46: 22-31.

Chivero, E.T., A.N. Mutukumira and R. Zvauya, 2001. Partial purification and characterization of a xylanase enzyme produced by a micro-organism isolated from selected indigenous fruits of Zimbabwe. Food Chem., 72: 179-185.

Collin, T., C. Gerday and G. Feller, 2005. Xylanases, xylanases families and extremophilic xylanases. FEMS Microbiol. Rev., 29: 3-23.

Coral, G., B. Arikan, M.N. Unaldi and H.K. Guvenmez, 2002. Some properties of thermostable xylanase from an *Aspergillus niger* strain. Ann. Microbiol., 52: 299-306.

Dhillon, A., J.K. Gupta and S. Khanna, 2000. Enhanced production, purification and characterization of a novel cellulase poor thermostable, alkali tolerant xylanase from *Bacillus circulans* AB 16. Process. Biochem., 35: 849-856.

Egas, M.C., M.S. Da Costa, D.A. Cowan and E.M. Pires, 1998. Extracellular α-amylase from *Thermus filiformis* Ork A2: Purification and biochemical characterization. Extremophiles, 2: 23-32.

Faulet, B.M., S. Niamke, J.T. Gonnety and L.P. Kouame, 2006. Purification and biochemical properties of a new thermostable xylanase from symbiotic fungus, *Termitomyces* sp. Afr. J. Biotechnol., 5: 273-282.

Funaguma, T., S. Naito, M. Morita, O.M. Kumara and M. Sugiura *et al*., 1991. Purification and some properties of xylanase from *Penicillium herquei* Banier and Sartory. Agric. Biol. Chem., 55: 1163-1165.

Gessesse, A., 1998. Purification and properties of two thermostable alkaline xylanases from an alkaliphilic *Bacillus* sp. Applied Environ. Microbiol., 64: 3533-3535.

Goheen, D.W., 1982. Chemicals from wood and other biomass. Part I: Future supply of organic chemicals. J. Chem. Educ., 58: 465-468.

Horikoshi, K. and Y. Atsukawa, 1973. Xylanase produced by alkalophilic *Bacillus* No. C-59-2. Agric. Biol. Chem., 37: 2097-2103.

Horikoshi, K., 1999. Alkaliphiles: Some applications of their products for Biotechnology. Microb. Mol. Biol. Rev., 63: 735-750.

Ito, K., J. Ogassawara, T. Sugimoto and T. Ishikawa, 1992. Purification and properties of acid stable xylanases from *Aspergillus kawachii*. Biosci. Biotechnol. Biochem., 56: 547-550.

Khandeparkar, R. and N.B. Bhosle, 2006a. Purification and characterization of thermoalkalophilic xylanase isolated from the *Enterobacter* sp. MTCC 5112. Res. Microbiol., 157: 315-325.

Khandeparkar, R. and N.B. Bhosle, 2006b. Isolation, purification and characterization of the xylanase produced by *Arthrobacter* sp. MTCC 5214 when grown in solid-state fermentation. Enzyme Microb. Tech., 39: 732-742.

Khasin, A., I. Alchanati and Y. Shoham, 1993. Purification and characterization of a thermostable xylanase from *Bacillus stearothermophilus* T-6. Applied Environ. Microbiol., 59: 1725-1730.

Kulkarni, N., A. Shendye and M. Rao, 1999. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev., 23: 411-456.

Laemmli, U.K., 1970. Cleauage of structural proteins during the assembly of the head of the bacteriophage T4. Nature, 227: 680-685.

Lee, S.P., M. Morikawa, M. Takagi and T. Imanaka 1994. Cloning of the aapT gene and characterization of its product, α-amylase-pullulanase (AapT), from thermophilic and alkaliphilic *Bacillus* sp. strain XAL601. Applied Environ. Microbiol., 60: 3764-3773.

Lo, H.F., L.L. Lin, H.L. Chen, H.H. Hsu and C.T. Chang, 2001. Enzymatic properties of a SDS-resistant *Bacillus* sp. TS-23 α-amylase produced by recombinant *Escherichia coli*. Process Biochem., 36: 743-750.

Luthi, E., N.B. Jasmat and P.L. Berqouist, 1990. Xylanase from the extremely thermophilic bacterium *Caldocellum saccharolyticum*: Overexpression of the gene in *Escherichia coli* and characterization of the gene product. Applied Environ. Microbiol., 56: 2677-2683.

Miller, G.L., 1959. Use of dinitrosalicylic acid for determination of reducing sugar. Anal. Chem., 31: 426-428.

Okazaki, W., T. Akiba, K. Horikoshi and E. Akahoshi, 1984. Production and properties of two types of xylanases from alkalophilic thermophilic *Bacillus* sp. Applied Microbiol. Biotechnol., 19: 335-340.

Ozcan, N., C. Cunningham and W.J. Haris, 1996. Cloning of a cellulase gene from the rumen anaerobe *Fibrobacter succinogenes* SD35 and partial characterization of the gene product. Lett. Applied Microbiol., 22: 85-89.

Rani, D.S. and K. Nand, 2000. Production of thermostable cellulase-free xylanase by *Clostridium absonum* CFR-702. Process. Biochem., 36: 355-362.

Ratanachomsri, U., R. Sriprang, W. Sornlek, B. Buaban, V. Champreda, S. Tanapongpipat and L. Eurwilaichitr, 2006. Thermostable xylanase from *Marasmius* sp.: Purification and characterization. J. Biochem. Mol. Biol., 39: 105-110.

Ryan, S.E., K. Nolan, R. Thompson, G.M. Gubitz, A.V. Savage and M.G. Tuohy, 2003. Purification and characterization of a new low molecular weight endoxylanase from *Penicillium capsulate*. Enzyme Microbiol. Tech., 33: 775-785.

Sa-Pereira, P., M. Costa-Ferreira and M.R. Atres-Barros, 2002. Enzymatic properties of a neutral endo-1,3(4)-β-xylanase Xyl II from *Bacillus subtilis*. J. Biotechnol., 94: 256-272.

Saleem, M., M.S. Akhtar and S. Jamil, 2002. Production of xylanase on natural substrates by *Bacillus subtilis*. Int. J. Agric. Biol., 4: 211-213.

Saul, D.J., L.C. Williams, R.A. Grayling, L.W. Chamley, D.R. Love and P.L. Berquist, 1990. celB, a gene coding for a bifunctional cellulase from the extreme thermophile *Caldocellum saccharolyticum*. Applied Environ. Microbiol., 56: 3117-3124.

Srinivasan, M.C. and M.V. Rele, 1999. Microbial xylanases for paper industry. Curr. Sci., 77: 137-142.

Sunna, A. and G. Antranikian, 1997. Xylanolytic enzymes from fungi and bacteria. Critical Rev. Biotechnol., 17: 39-67.

Tatjana, C. and M. Vladimir, 1996. Purification and properties of the xylanase produced by *Thermomyces lanuginosus*. Enzyme Microbiol. Technol., 19: 289-296.

Teather, R.M. and P.J. Wood, 1982. Use of Congo-red polysaccaride interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Applied Environ. Microbiol., 43: 777-780.

Vieille, C. and G.J. Zeikus, 2001. Hyperthermophilic enzymes: Source, uses and molecular mechanisms for thermostability. Microbiol. Mol. Biol. R., 65: 1-43.

Whistler, R.L. and E.L. Richards, 1970. Hemicelluloses. In: The Carbohydrates, Pigman, W. and D. Horton (Eds.). Academic Press, New York, pp: 447-469.

Wong, K.K.Y., L.U. Tan and J.N. Saddler, 1988. Multiplicity of β-1,4-xylanase in microorganisms: Functions and applications. Microbiol. Rev., 52: 305-317.