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Purification and characterization of a thermoalkaline, cellulase free thermostable xylanase from a newly isolated *Anoxybacillus* sp. Ip-C from hot spring of Ladakh

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An alkaline, highly thermostable cellulase free xylanase was purified from a thermophilic *Anoxybacillus* sp. Ip-C, newly isolated from hot spring of Ladakh. The enzyme was purified using ammonia sulphate precipitation followed by Sephadex G-75. The molecular weight of the xylanase was about 45 kDa, as analyzed by SDS-PAGE. The enzyme had optimum activity at pH 9.0 and 70°C temperature; the enzyme retained 90% of its original activity for 96 hrs at 70 °C. V_{max} and K_m of the enzyme were found to be 13.5 µmol min^{-1} mg^{-1} protein and 4.59 mg ml^{-1}, respectively. Metal ions, Ca^{+2}, Fe^{+2} and Mg^{+2} highly enhance the enzyme activity to 122.45, 119.06 and 118.98% respectively; whereas SDS and Hg^{+2} completely inhibit the enzyme activity. TLC analysis of enzymatic hydrolysis products showed that this xylanase is an endoxylanase, and generates xylooligosaccharides. Thus, it provides a potential thermostable alkaline xylanase for industrial applications.

Keywords: Thermoalkaline xylanase, Purification, *Anoxybacillus* sp., Thermostability, Hydrolysis product.

Xylan, a major component of cell walls in plant tissues and the second most abundant renewable resource in nature. Biodegradation of xylan using xylanases [Endo-1,4-b-D- xylanohydrolase, EC 3.2.1.8] produces xylooligosaccharides (Bakri *et al.*, 2013), can be used as ingredients of functional food, cosmetics, biofuel, pharmaceuticals or agricultural products (Dhillon *et al*., 2008; Andre *et al*., 2009; Hauli *et al*., 2013), without environmental pollution (Sunna *et al*., 2000). In the last decade, xylanases have widespread potential applications in different industrial areas such as pulp bleaching, baking and brewing, animal feeding, waste-treating and bioenergy conversion (Li *et al.,* 2009; Hauli *et al*., 2013). As catalysts in pulp and paper industries, tolerance to alkaline pH and high temperature is the desirable property of xylanase (Techapun *et al.,* 2003). However, most of the xylanases known to date are optimally active at temperatures below 50°C and are active in acidic or neutral pH (Ryan *et al*., 2003; Wang *et al*., 2003). Conversely, only a few xylanases are reported to be active and stable at alkaline pH and high temperature (Dhillon *et al*., 2000; Bajaj *et al*., 2011). Thus it

has attracted considerable research interest to seek thermostable alkaline xylanases (Dhillon *et al*., 2000; Lama *et al*., 2004; Mamo *et al*., 2006). In this paper, we purified a thermotolerant alkaline xylanase, from the newly isolated thermophilic *Anoxybacillus* sp. Ip-C from hot spring of Ladakh. Its high thermostability and alkali stability enhances its potentiality in industrial applications.

Materials and methods

Isolation and identification of the strain capable of producing xylanase:

Water sample from a hot spring in Chumathung city, Ladakh was spread on nutrient agar plate with 0.1% yeast extract and 2% agar (w/v) and grown at 60^{o} C for 48 hrs. Among the three types of colonies appeared on the plates after incubation, xylanase producing strains was screened on plates consisting of 0.5% oat spelt xylan (w/v) and agar 2% (pH-7) using Congo red method (Wood *et al*., 1988) and designated as Ip-C. No cellulase activity was found in this strain.

The purity of the isolate was assessed by colony morphology and microscopy after 24 hours growth on nutrient agar plate. For further identification, genomic DNA of Ip-C was extracted from fresh culture following protocol of Ausubel *et al.* (1994). The 16S rRNA gene of the strain was amplified from the genomic DNA, using the universal primers (8F-5'AGA GTT TGA TCC TGG CTC AG3' and 1492R-5'TAC GGT TAC CTT GTT ACG ACT T3'). 16S rRNA gene is amplified from genomic DNA and sequenced by Chromus Biotech Pvt. Ltd., Bangalore. The 16S rRNA gene sequence of the isolate was aligned with reference 16S rRNA gene sequences of the European Microbiological Laboratory (EMBL), GenBank (gb, Germany) and the database of Japan (dbj) using the BLAST algorithm (Altuschul *et al*., 1997) available online in NCBI (National Centre for Biotechnology Information).

Enzyme assay:

Xylanase activity was assayed following the method of Bailey *et al.*(1992) using 1% soluble oat spelt xylan as the substrate at 70°C (pH 9.0), and the reducing sugars were determined according to DNS method (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme required produced 1 µmol of xylose per minute under standard assay condition. The cellulase activity was also assayed by DNS method, using 1% (w/v) carboxy-methylcellulose as the substrate and glucose as the standard (Li *et al*., 2008).

The effect of pH on xylanase activity:

The effect pH was determined by standard assay protocol after incubating 0.25 ml of enzyme and 0.75 ml of the following buffers at the indicated pH (between pH 4.0-10.0) containing 1% oat spelt xylan. [citrate phosphate buffer (pH 4.0-6.0) , phosphate buffer (pH 7.0-8.0), glycine-NaOH buffer (pH 9.0- 10.0)].

The effect of temperature on xylanase activity:

The effect of temperature on the enzyme activity was determined under standard assay procedure, mention earlier, at various temperatures ranging from 30°C to 90°C for 10 min at pH 9.0.

Thermostability of the xylanase:

In order to determine the thermal stability of the enzyme, aliquots of enzyme in plastic tubes were incubated from 0 to 96 hours at 70 and 80°C temperature. The tubes were removed at different times (at 0, 6, 24, 48 and 96 hr) and rapidly cooled in an ice bath. Then, these heat-treated enzymes were used to determine the residual enzyme activity through dinitrosalicylic acid method (Miller, 1959).

Time course growth and xylanase production:
The time course of growth and the production of extracellular xylanase were studied using minimal medium supplemented with xylan (1%). The mineral medium used for the production of xylanase contained gram per liter: KH_2PO_4 3.0; K_2HPO_4 2.0; $MgSO_4 \cdot 7H2O$ 0.5; $CaCl_2 \cdot 2H_2O$ 0.1; $FeSO_4 \cdot 7H_2O$ 0.05; $MnSO_4 \cdot 4H_2O$ 0.02; $ZnSO_4 \cdot 7H_2O$ 0.01; $CoCl_2 \cdot 6H_2O$ 0.02 and NH_4Cl 10.0. Culture samples ware collected at different time intervals over the period of cultivation, analyzed for xylanase activity using the procedure described above. Growth was measured at 600 nm.

Purification of xylanase:
The culture supernatant was collected by centrifugation at 10,000g for 20 min at 4°C. The enzyme was precipitated by slow addition of solid ammonium sulfate (40% saturation). The precipitate was left for overnight and recovered by centrifugation at 10,000g for 20 min at 4°C and then, dissolved in 50 mM glycine NaOH buffer (pH 9.0) and dialyzed against the same buffer for overnight. Enzyme activity of the dialyzed fraction was measured and further purified by gel chromatography (Sephadex G-75). Protein concentration was also measured by the method of Bradford (1976) using bovine serum albumin as a standard, at each purification steps.

Kinetic Parameters:
The effect of substrate concentration, ranging from 0.3125 to 32 mg ml^{-1}, on the xylanase activity was evaluated under standard assay conditions. The kinetic parameters Michaelis–Menten constant, K_m and maximal reaction velocity, V_{max}, were estimated by Michaelis–Menten plot. The apparent K_m and V_{max} values were calculated according to the Lineweaver Burk method (Lineweaver, 1934).

Effect of chemicals and metal ions on the xylanase activity:
Effects of several metal ions and chemical agents on the enzyme were determined. Enzyme activity was determined by pre incubating the enzyme for 1 hour at room temperature with different metal ions (1 mM and 10 mM solution) such as Mg^{+2}, Fe^{+2}, Pb^{+2}, Co^{+2}, Cr^{+2}, Ca^{+2}, Ni^{+2}, K^{+}, Na^{+}, Zn^{+2}, Cu^{+2}, Li^{+2}, Ag^{+2}, Hg^{+2}, Mn^{+2} and chemicals (the chelating agent EDTA, reducing agents dithiothreitol and *β*-mercaptoethanol, detergents SDS & Triton X100) under optimal assay condition mention above. The activity of the enzyme without any metal treatment was taken as control and the activity was considered as 100% (Annamalai *et al*., 2009).

SDS-PAGE analysis and zymogram assay:
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970)**.** Duplicate samples were analyzed in a 12% polyacrylamide gel containing 0.2% oat spelt xylan. After the electrophoresis, the gel was split into two parts. One of them was stained with Coomassie brilliant blue R-250, and the other was developed for zymogram analysis. Zymogram analysis for xylanase activity was detected by adding oat spelt xylan (0.2%) in the gel before polymerization. Samples were heated for 10 min at boiling water bath in sample buffer before being applied to gel. After electrophoresis, gel was soaked in 2.5% (w/v) Triton X-100 for 30 min, washed in 50 mM glycine-NaOH buffer pH 9.0 for 30 min, and incubated at 70°C for 30 min in the same buffer. Gel was stained with 0.1% Congo red for 15 min and washed with 1 M NaCl until xylanase bands became visible. The gel was then immersed in 5% (w/v) acetic acid, in which the background turned into dark blue and photographed (Kacagan *et al*., 2008).

Thin layer chromatography (TLC) analysis of hydrolysis products from xylan:
The hydrolytic pattern of xylanase on oat

spelt xylan was determined by the modified method of Apiraksakorn *et al.* (2008) for the thin layer chromatography (TLC) with xylooligosaccharides (xylose, xylobiose, xylotriose, xylotetrose, xylopentose). 1% xylan solution was treated with partially purified enzyme for 6 hour at 70°C. After incubation the unused xylan was precipitated with isopropanol and the precipitate was removed by centrifugation (10,000 rpm, 10 min) and the collected supernatant containing hydrolyzed products was concentrated using vacuum evaporator. 20µl of the hydrolyzed products were applied on TLC plate (Merck). Chromatography was performed by the ascending method on silica gel TLC plates (Merck) with a solvent system consisting of n-butanol, acetic acid and water (2:1:1). Sugars on the plates were detected by heating the plates at 120°C for about 10 min after they were sprayed with 5% (v/v) sulfuric acid in ethanol (Kacagan *et al*., 2008).

Results

Isolation and identification of strain Ip-C for producing xylanase:

The strain Ip-C, which was isolated from hot spring of Ladakh, exhibited evident clear zones around the colonies on xylan agar plates following staining with 1% Congo red solution, indicating that it secretes prominent amounts of xylanase. Microscopic observation showed that strain Ip-C belongs to *Bacillus.* The purity of the isolate was assessed by colony morphology and microscopy after 24 hours growth on nutrient agar plate. Colonies of strain Ip-C was small, creamish, irregular shaped with smooth edges (Fig. 1a). Phase contrast microscopy revealed that cells of the strain were Gram positive, rod-shaped (~2.5 µm long), motile, contain terminal spherical endospore (Fig. 1b).

Figure 1. Colony morphology of the pure culture and microscopic observation of the strain: (a) Quadrant streaking of the isolated *Anoxybacillus* sp. Ip-C after growth at 60°C for 2 days on nutrient agar (NA), from which the colony character was determined; (b) Gram positive character of *Anoxybacillus* sp. Ip-C with terminal endospore (using Leica DM1000 microscope).

To further identify this strain, the partial 16S rRNA gene of Ip-C was sequenced. The comparison with other 16S rRNA gene in GenBank revealed that the strain belongs to the genus *Anoxybacillus,* and named as *Anoxybacillus* sp. Ip-C (Fig. 2).

Accession number:

The 16S ribosomal RNA gene of *Anoxybacillus* sp. Ip-C, was submitted to GenBank and the following accession numbers was assigned for isolates Ip-C is JF968627**.**

Production of xylanase:
Production of xylanase by strain Ip-C reached maximum after 48 hours at pH 7.0 and 60° C temperature in medium supplement with 1% oat spelt xylan, through examining the effect of different temperature (40, 50, 60, 70 and 80°C) and pH (4.0-8.0) on the growth and xylanase production. The optimum temperature has been found to be at 60°C and pH 7.0 (Fig. 3). The xylanase production medium contained 1% Oat spelt xylan, 0.5% Yeast Extract, 0.5% NaCl(w/v), 0.2% NH_4Cl, 0.3% Na_2HPO_4 & 0.3% KH_2PO_4.

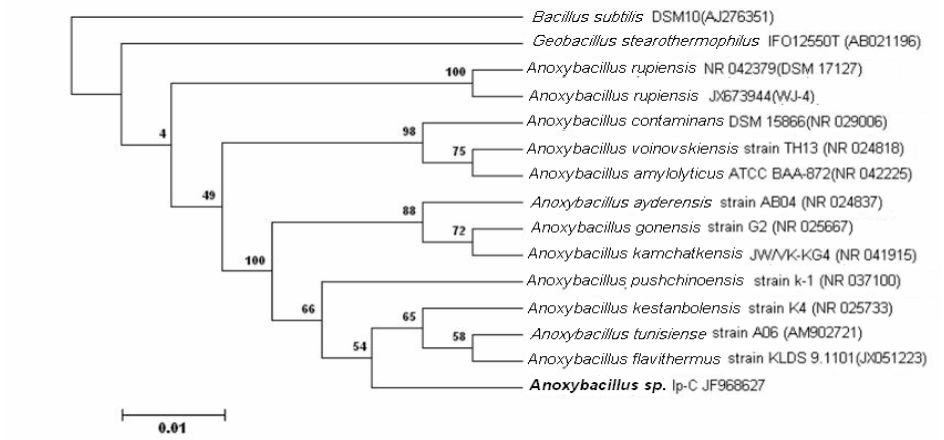

Figure 2. Phylogenetic tree of *Anoxybacillus* sp. Ip-C with the closely related species: 16s rDNA sequence based phylogenetic neighbour joining tree, using MEGA5, showing the phylogenetic relationship of strain Ip-C relative to the closely related species in the genera *Anoxybacillus*. 0.01 base substitutions per site.

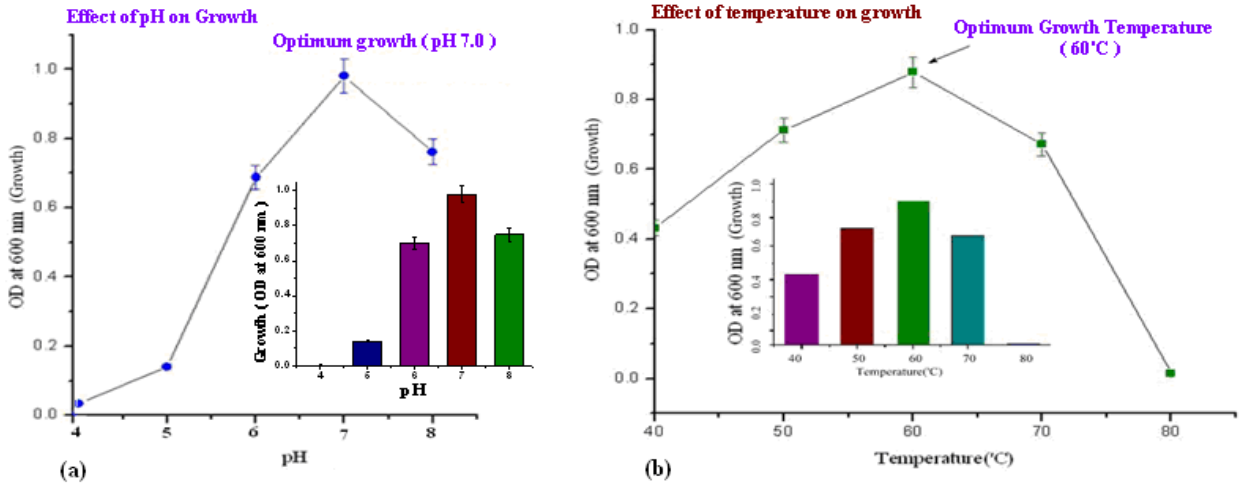

Figure 3. Optimum growth conditions of the strain: (a) The optimum temperature has been found to be at 60°C and (b) the optimum pH has been found to be pH 7.0.

Effect of pH and temperature on the xylanase and its thermostability:
Xylanase assays were performed under different conditions for enzyme activity. The optimum pH is 9.0 (Fig. 4) and the optimum temperature of the enzyme is 70°C (Fig. 5). Cellulase activity assay was performed and no cellulase activity could be detected at various pH values and at different enzyme dilutions. This indicated that this xylanase is a cellulase free xylanase (data not shown).

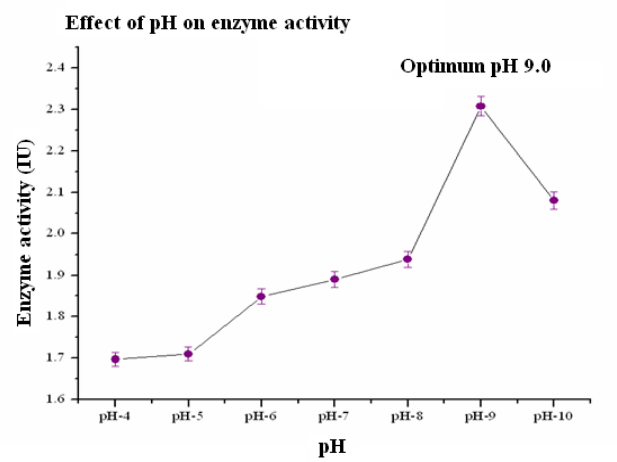

Figure 4. Optimum pH for xylanase activity of strain Ip-C is pH 9.0

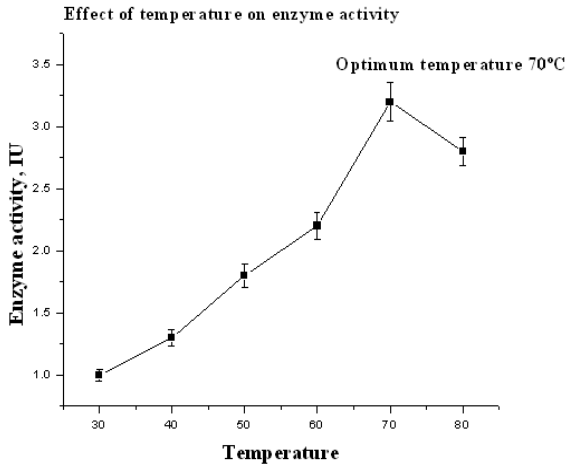

Figure 5. Optimum Temperature for xylanase activity of strain Ip-C is 70 °C.

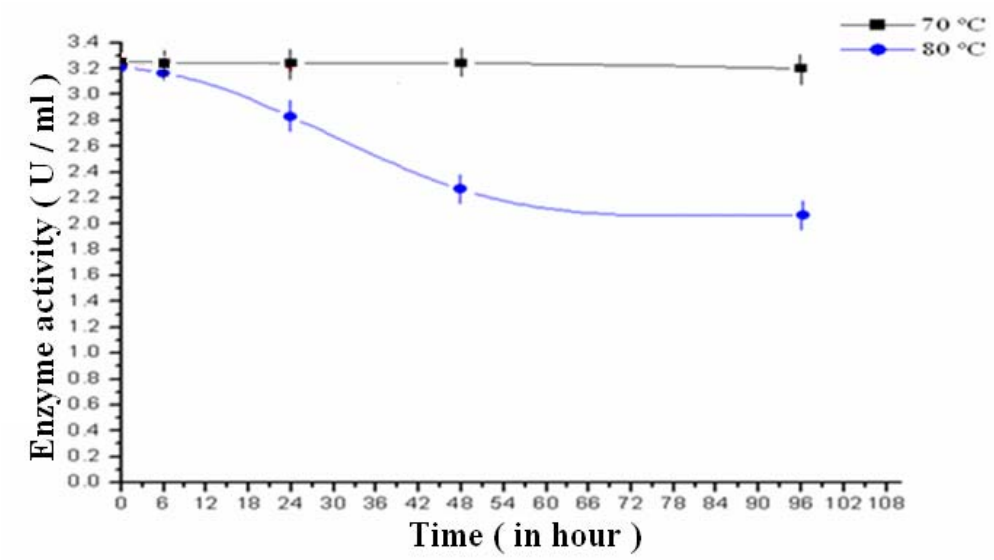

Figure 6. Effect of temperature on the stability of the xylanase of *Anoxybacillus* sp. Ip-C .The enzyme was incubated in buffer at pH 9.0 at different temperature and the enzyme activity was assayed at different time interval with oat spelt xylan using standard assay protocol.

The thermal stability of xylanase produced by this strain Ip-C was also determined. The thermostability of enzyme was investigated for a period of 96 hours at 70 and 80°C temperature. The enzyme was stable at 70 °C up to 96 hr. (Fig 6).

Time course growth and xylanase production:

The time course of growth and the production of extracellular xylanase were studied using minimal medium with xylan (1%). Soon after inoculation the culture started growing and reached a highest biomass at around 52 hour, remain constant up to 96 hours. After this the culture growth started decreasing. In comparison with growth, the xylanase production increased gradually and reached a highest value at around 96 hrs of the incubation and remained same thereafter (Fig 7).

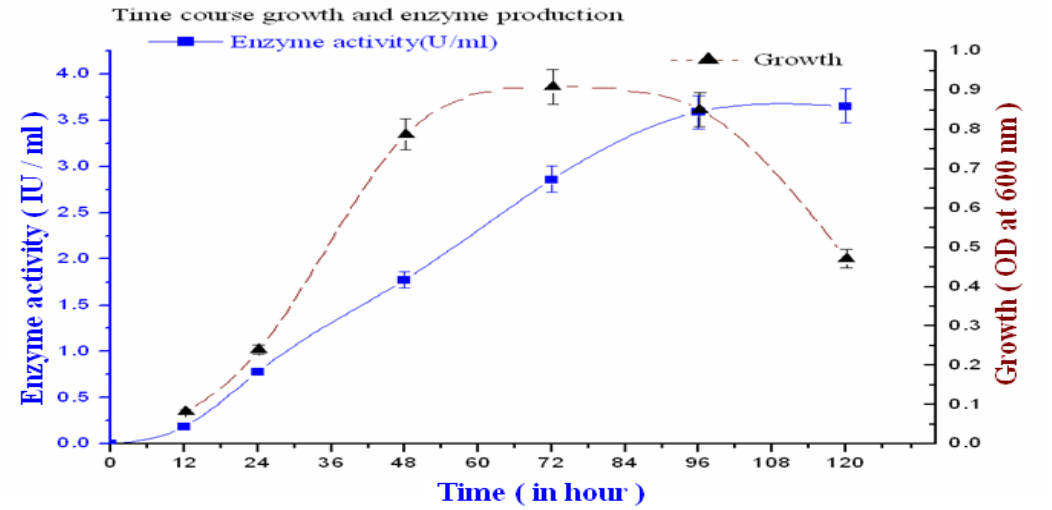

Figure 7. Time course of growth and enzyme production by *Anoxybacillus* sp. Ip-C : Cells were grown at 60°C in minimal medium supplemented with oat spelt xylan as a sole source of carbon (1 %). Growth was monitored by measuring optical density at 600 nm. Xylanase activity was assayed from the cell free supernatant as described above.

Table 1. Purification of xylanase from thermophilic *Anoxybacillus* sp. Ip-C

Purification step	Activity (U)	Protein (mg)	Specific activity (U/mg)	Purification (fold)
Culture supernatant	3.56	477.91	8.908	1
$(NH_4)_2SO_4$ fraction	4.54	150.54	30.265	1.28
Sephadex G 75	12.16	64.32	189.054	3.42

*All experiments are performed in triplicate and the data represented are the mean of three.

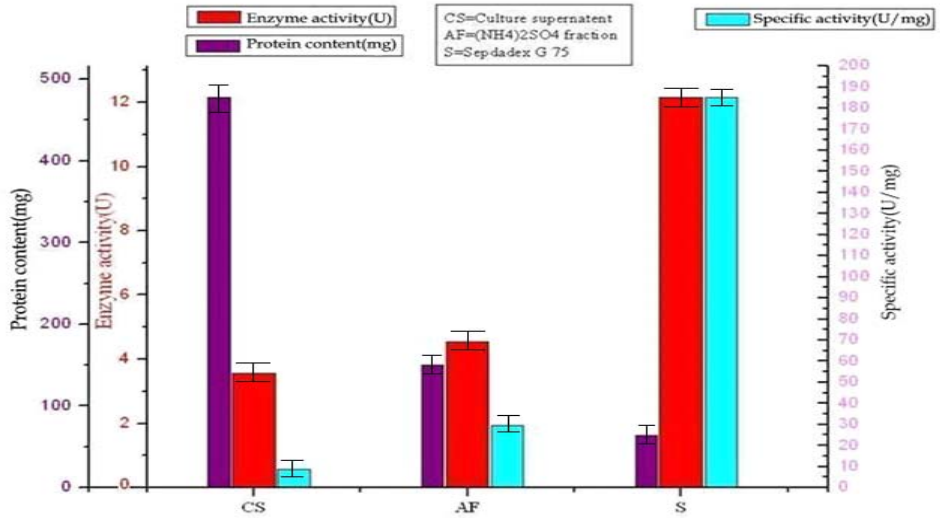

Figure 8. Activity of thermophilic *Anoxybacillus* sp. Ip-C xylanase at different purification stages. The enzyme activity, protein content and specific activity of CS (crude culture supernatant), AF (ammonia sulphate fractionated protein after dialysis) and S (enzyme after purified through Sephadex G75), were assayed after each of these purification steps.

Purification of xylanase from *Anoxybacillus* sp. Ip-C:

The xylanase activity assayed after each purification step is summarized in (Table 1).The ammonium sulfate (40%) precipitation yielded an activity of 4.54 IU (1.28 fold). Pooled fractions of xylanase activity when subjected to Sephadex G-75 showed increasing xylanase activity of 12.16 IU (3.42 fold) suggesting the purification of xylanase (Fig. 8).

Kinetic Parameters:

The kinetic constant K_m and maximal reaction velocity, V_{max} of xylanase were found to be 13.5µmol min^{-1} mg^{-1} protein and 4.59 mg ml^{-1}, respectively (Fig. 9).

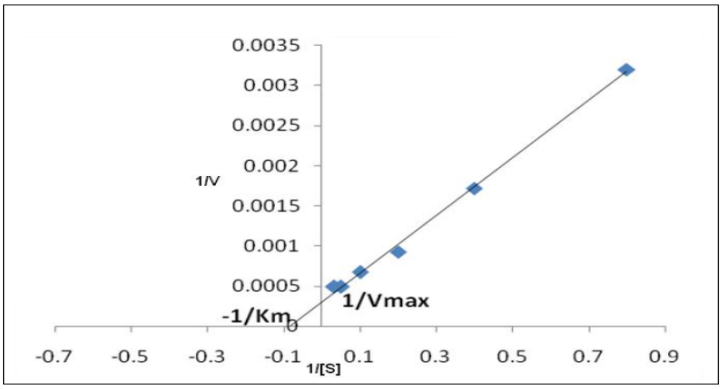

Figure 9. Lineweaver-Burke plot of initial velocity data of *Anoxybacillus* sp. Ip-C Xylanase on oat-spelt xylan

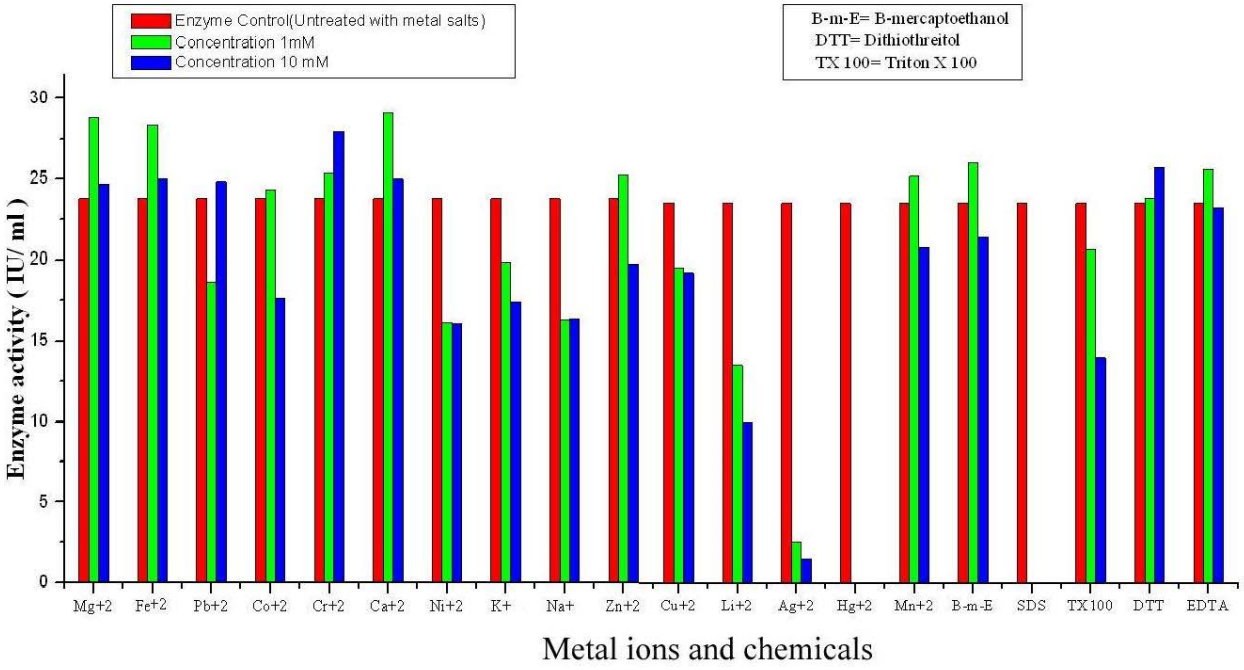

Figure 10. Effect of different chemical and metal ions on the activity of the partially purified xylanase

Table 2. Effect of metal ions and chemicals on the xylanase activity

Metal ions & Chemicals	Relative Enzyme Activity (%) *	
	1mM	10mM
Mg^{+2}	118.98 +/- 1.20	103.62 +/- 2.42
Fe^{+2}	119.06 +/- 1.45	104.98 +/- 1.65
Pb^{+2}	78.11 +/- 2.47	54.36 +/- 3.21
Co^{+2}	102.23 +/- 3.56	74.56 +/- 2.50
Cr^{+2}	106.63 +/- 0.50	117.42 +/- 4.26
Ca^{+2}	122.45 +/- 4.20	104.95 +/- 2.12
Ni^{+2}	67.62 +/- 1.80	67.25 +/- 3.18
K^{+}	83.41 +/- 2.65	73.07 +/- 2.88
Na^{+}	88.33 +/- 2.56	68.45 +/- 3.12
Zn^{+2}	105.97 +/- 1.66	82.94 +/- 1.12
Cu^{+2}	82.99 +/- 0.93	81.56 +/- 4.50
Li^{+2}	57.31 +/- 3.45	42.44 +/- 0.95
Ag^{+2}	10.85 +/- 2.60	6.51 +/- 1.45
Hg^{+2}	0	0
Mn^{+2}	107.10 +/- 2.30	88.45 +/- 1.70
Beta mercaptoethanol	110.61 +/- 1.86	91.21 +/- 2.52
SDS	0	0
Triton X100	87.86 +/- 1.85	71.21 +/- 2.52
Dithiothreitol (DTT)	109.30 +/- 1.50	100.45 +/- 3.80
EDTA	108.89 +/- 2.36	98.86 +/- 1.25

* Values represents the mean +/- SD (n = 3) relative to untreated control samples.

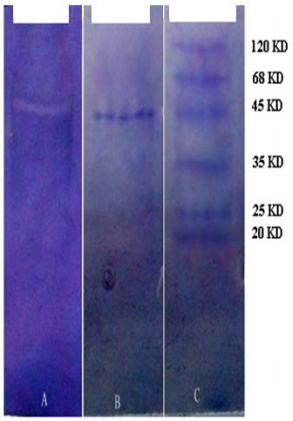

Figure 11. SDS PAGE of partially purified *Anoxybacillus* sp. Ip-C xylanase and zymogram. Lane A, zymogram visualizing activity; Lane B, purified xylanase; Lane C, molecular weight markers.

Effect of metal ions and chemicals on the xylanase activity:

Effects of different metal ions and chemicals on the activity of xylanase were determined (Fig. 10) by using oat spelt xylan as a substrate. It was seen that Ca^{+2}, Fe^{+2} and Mg^{+2} highly enhance the enzyme activity to 122.45%, 119.06 % and 118.98% respectively; whereas SDS and Hg^{+2} completely inhibit (0 U/ml) the enzyme activity (Table 2).

Figure 12. Thin-layer chromatography of hydrolysis products obtained from oat spelt xylan. Lane A, xylopentose; Lane B, xylotetrose; Lane C, xylotriose; Lane D, xylobiose; Lane E, xylose and sample refers to the xylanase treated hydrolysed products obtained from oat spelt xylan.

SDS-PAGE and Zymogram assay:
SDS-PAGE and Zymogram assay of the resultant xylanase band is about 45 kDa (Fig. 11)

TLC analysis of enzymatic hydrolysis products:
TLC analysis of enzymatic hydrolysis products showed that Ip-C xylanase catalyzed the hydrolysis of oat spelt xylan, producing xylose and xylooligosaccharides (Fig 12).

DISCUSSION
Depending on phylogenetic analysis based on 16S rDNA sequence homology, the strain Ip-C from hot spring of Ladakh, was accepted as a member of the genus *Anoxybacillus* and named as *Anoxybacillus* sp. Ip-C. The genus *Anoxybacillus* was firstly separated from *Bacillus* by Pikuta *et al.* (2000) based on the phenotypic properties and 16S rDNA sequence. Many microorganisms belonging to *Anoxybacillus* have been isolated, including *A. flavithermus* (Pikuta *et al*., 2000), *A. pushchinoensis* (Pikuta *et al*., 2003), *A. gonensis* (Belduz *et al*., 2003), *A. contaminans* (De Clerck *et al*., 2004) and others, most of them were thermoalkaliphilic. Though the isolated strain, *Anoxybacillus* sp. Ip-C showed optimal growth at pH 7.0, it produces a thermo-alkaline xylanase (70° C & pH 9.0) with high thermostability.

Thermostability of the xylanase shows greater than 96 hrs of stability, retaining more than 90% of its initial activity, at 70 ^{0}C. It is evident from similar report by other workers on thermostability of various types of microbial strains (Bajaj *et al*., 2011; Zhang *et al*., 2011) that our isolate is a much better candidate in this respect for industrial use. Some of the report (Dhillon *et al*., 2000) showed xylanase having increased temperature optima (80°C) than the test strain but stability of that xylanase was much less than our isolate as that reported xylanase retained only 70 % of its activity at 65 ^{0}C. In other reports (Annamalai *et al*., 2009; Kacagan *et al*., 2008) xylanases showed better retainment of initial activity (more than 97%) than xylanase of *Anoxybacillus sp.* Ip-C but the reported stability ware tested at much lower temperature and shorter time in comparison to ours.

Most of the xylanases used so far for industrial purpose are obtained from the genus *Bacillus* having optimal activity at pH 5.0–pH 6.0 and temperature 40–50°C, and showed stability over a narrow pH range and low temperature (Nakamura *et al*., 1993; Huang *et al*., 2006; Jalal *et al*., 2009). In comparison to those bacillary xylanases, xylanase from *Anoxybacillus* sp. Ip-C has more potentiality for industrial use as it shows stable optimal activity at pH 9.0 and 70 °C temperature.

The incubation period required for maximum xylanase production depends on the growth rate of the microorganism and its enzyme production pattern. Soon after inoculation, *Anoxybacillus* sp. Ip-C started growing and reached a highest biomass at around 52 hour and the xylanase production increased gradually and reached a highest value at around 96 hrs similar to previously reported bacterial systems including *Bacillus licheniformis* and *Bacillus* sp. AR-009 (Archana and Satyanarayana, 1997; Gessesse and Mamo, 1999) whereas comparatively longer incubation periods were required by fungal systems. Xylanase production was maximized after 144 hrs in case of *Sporotrichum thermophile* (Topakas *et al.,* 2003) and the highest enzyme titer was obtained after incubation for a week by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* (Alam *et al*., 1994).

The apparent *K*m and *V*max values for the partially purified xylanase from *Anoxybacillus* sp. Ip-C using oat spelt xylan as a substrate, shows that the xylanase is much more efficient than those reported earlier (Ahmed *et al*., 2012; Anuradha *et al*., 2007).

Previous publications reported that EDTA was inhibitory to some xylanases (Zhang *et al*., 2007) however, in this study, EDTA at 1 and 10 mM concentration did not affect xylanase activity. This suggests that xylanase may have different catalytic mechanisms, and xylanase of *Anoxybacillus* sp. Ip-C is not a metalloenzyme similar to the xylanase (Xyn10) of alkaliphilic *Bacillus* sp. N16-5 (Zhang *et al*., 2010). However *Anoxybacillus* sp. Ip-C shows enhance xylanase activity to 122.45, 119.06 and 118.98% respectively, in presence of Ca^{+2}, Fe^{+2} and Mg^{+2} at 1 and 10 mM concentration. The inhibition of the enzyme activity by Hg^{+2} ions may be due to its interaction with sulphydryl groups of cysteine residues or indole ring of tryptophan residues present in or close to the active site of the enzyme (Zhang *et al*., 2007; Nakamura *et al*., 1993; Khandeparkar *et al*., 2007). The exact reason for the observed inhibitory effect of Hg+2 ions on the enzyme activity is not yet discovered.

SDS-PAGE and Zymogram analysis of the partially purified enzyme indicates that the molecular weight of *Anoxybacillus* sp. Ip-C xylanase is about 45 KDa, similar to xylanase from *B. firmus* (Min *et al.,* 2002).

TLC analysis of enzymatic hydrolysis products showed that Ip-C xylanase catalyzed the hydrolysis of oat spelt xylan, producing xylose and xylooligosaccharides. This hydrolytic pattern might lead to the suspicion that Ip-C xylanase is an endoxylanase, as we know that endo-type xylanases attack the internal glycosidic linkages in xylans or xylooligosaccharides and act by a random attack mechanism, which often results in a mixture of xylooligosaccharides, can be used as ingredients of functional food, cosmetics, pharmaceuticals or agricultural products (Dhillon *et al*., 2008; Andre *et al*., 2009; Hauli *et al*., 2013).

In pulp and paper industries, the ideal xylanase should have optimum activity at higher temperature and alkaline condition, and it should be free from cellulase activity to avoid unwanted hydrolysis of the cellulase fibers (Bajpai, 1999; De Clerck *et al*., 2004). Xylanase of *Anoxybacillus* sp. Ip-C under study showed all these favorable properties which makes pulp and paper industries along with other industries as well.

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