Food Biotechnology

ISSN: 0890-5436 (Print) 1532-4249 (Online) Journal homepage: http://www.tandfonline.com/loi/lfbt20

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To cite this article: Ali Osman Adigüzel & Münir Tunçer (2016) Production, Characterization and Application of a Xylanase from Streptomyces sp. AOA40 in Fruit Juice and Bakery Industries, Food Biotechnology, 30:3, 189-218, DOI: 10.1080/08905436.2016.1199383

To link to this article: http://dx.doi.org/10.1080/08905436.2016.1199383

Published online: 10 Aug 2016.

FOOD BIOTECHNOLOGY
2016, VOL. 30, NO. 3, 189–218
http://dx.doi.org/10.1080/08905436.2016.1199383

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Production, Characterization and Application of a Xylanase from *Streptomyces* sp. AOA40 in Fruit Juice and Bakery Industries

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ABSTRACT

Streptomyces sp. AOA40, which produces halotolerant and thermotolerant xylanase, was isolated from Mersin soil. Various carbon sources were tested for xylanase production with selected fermentation medium. The best carbon source was selected as corn stover. The effect of corn stover concentration and particle size, composition of fermentation medium, fermentation condition such as initial pH and agitation rate on xylanase production was determined. After production, xylanase was partially purified with ion-exchange chromatography and gel filtration chromatography for characterization of xylanase and application in fruit juice and dough improvement. The optimum pH for the activity of xylanase occurred at pH 6.0 in phosphate buffer, while the optimum temperature was 60°C. The relative xylanase activity in the pH ranges of 4–9 remained between 59.93 and 54.43% of the activity at pH 6.0 (100.00%). The xylanase activity showed a half-life of 172 min at 70°C, which was reduced to 75 min at 80°C. The enzyme was highly inhibited by 10–100 mM of Hg^{+2}, EDTA, Mg^{+2}, SDS and 100 mM Cu^{+2}. Clarity of fruit juices increased after enzymatic treatment of apple (17.85%), grape (17.19%) and orange juice (18.36%) with partially purified xylanase and also reducing sugar concentrations of these fruit juices were improved by 17.21, 16.79 and 19.57%, respectively. Also, dough volume was raised 17.06% with using partially purified *Streptomyces* sp. AOA40 xylanase in bread making.

KEYWORDS

Characterization; halotolerant; partial purification; thermotolerant; xylanase

Introduction

Bacteria are well known for their ability to excrete enzymes into the surrounding environment. *Streptomyces* sp., being industrially important organisms, produces a wide variety of extracellular enzymes including xylanases. *Streptomyces* sp. isolated from local soil samples were collected from the Mersin province of Turkey for the production of xylanase enzyme. Recently, there has been an increasing interest in xylanases due to their biotechnological potential in various industrial processes, including the pulp and paper industries (biobleaching),

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bioethanol production, and the feed industry (Nawel et al., 2011). Also, xylanase are used for fruit juice clarification and in the bakery industry (Polizeli et al., 2005). Many xylanases have been identified and researchers are interested in these novel enzymes which resist higher temperatures and salt concentrations due to hard reaction conditions in the industrial process. Thermotolerant and halotolerant xylanases could be used in baking, fruit juice clarification and bioconversation of marine-derived biomass to high added value product such as bioethanol and different organic acids and improvement of fermented foods. There have been a few reports about thermo- and halo-tolerant xylanases. Therefore, isolation of new microorganisms with the capacity of production of enzymes with thermo- and halo-tolerant xylanases is important.

A major part of commercial enzymes are obtained from microbes due to their ease of growth, nutritional requirement, and downstream processing (Prakash et al., 2013). Among these, actinomycetes are the most economically and biotechnologically valuable prokaryotes and produce commercially available enzymes involved in lignocellulose degradation (Porsuk et al., 2013). New strains of the actinomycetes have the potential to produce novel enzymes. In this study, one of the purposes was the isolation of actinomycetes with the capacity of production of xylanases with novel biochemical features like thermo- and halo-tolerances from Mersin soil. Basic soil parameters of Mersin was determined previously (4.0–19.7% soil moisture; 25.0–33.3 $CaCO_3$; 0.027–0.074 dS/m salinity; 0.80–1.2 mS/cm conductivity; 7.5–8.1 pH; 0.82–5.50% total carbon and 0.10–0.58% total nitrogen) (Everest and Seyhan, 2006). In the same research, a high amount of actinomycetes (1.2–3.2 actinomycetes/g dry soil) was also detected (Everest and Seyhan, 2006).

The cost of carbon sources plays a main role in the economics of xylanase production and xylanases are generally produced when the fermentation broth includes a pure xylan or xylan hydrolysate (Sharma and Bajaj, 2005). Alternatively, agricultural wastes can be used for xylanase production from actinomycetes due to the economics of the process.

The aim of this study was to isolate actinomycete strains producing xylanase from soil samples, to characterize xylanase, which is produced by an isolated actinobacterial strain, and detect the availability in fruit juices and dough improvement. The effect of fermentation conditions such as initial culture pH, incubation temperature and agitation rate and nutritional requirements, mainly the type and amount of carbon and nitrogen sources for xylanase production were also studied.

Materials and methods

Isolation of actinomycetes from soil

Soil samples were collected from Mersin (Tarsus, Gözne, Mezitli, Kuyuluk, Güzelyayla, Kale). The 10 g samples were incubated for 1 week with sterile

powdered 1 g calcium carbonate (Quin et al., 2009) at room temperature because calcium ions have the ability to stimulate the formation of aerial mycelia of actinomycetes (Natsume et al., 1989). Incubated soil samples were diluted to 10^{-4}, 10^{-5}, 10^{-6} with ¼ ringer solution (NaCl 2.25 g/l, $NaHCO_3$ 0.05 g/l, KCl 0.105 g/l, $CaCl_2$ 0.12 g/l) and were spread onto ISP4 medium. ISP4 agar, Gause No. 1 agar, and starch casein agar were used for the isolation. Nystatin (fungal inhibitor) was added to each medium at a final concentration of 2.5 µg/ml after sterilization with a syringe filter (Bian et al., 2009). Mediums were incubated for 10 d at 30°C. After incubation, colonies have a powdery texture, dry or folded appearance; branching filaments with or without aerial mycelia were selected and purified on ISP2 medium. Isolates were preserved on ISP2 medium at +4°C. Pure isolates were saved in glycerol stock culture at −80°C.

Screening of xylanolytic isolates

Isolates were screened with spot inoculation on modified ISP2 agar (4 g/l birchwood xylan, 10 g/l malt extract, 4 g/l yeast extract, 15 g/l agar). Plates were incubated at 30°C for 3 d. Plates were stained with 1% (w/v) Congo red solution for 15 min and then destained with 1 M NaCl solution for 15 min after incubation (Ang et al., 2015). Isolates, which produced distinct hydrolytic zones (yellow) around their colonies, were determined. Xylanase production of isolates were checked and compared in shake flask.

Identification of selected isolate with 16S rRNA gene sequencing

Small amounts of mycelia or colonies grown on ISP2 agar were picked up with sterile toothpicks and transferred directly into PCR tubes containing 9 µl sterile Millipore water which were immediately subjected to heating (98°C, 10 min) followed by adding 12 µl PCR mix. The PCR mix comprised 10 µl Dream Taq PCR Master Mix (2×), 1 µl primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1 µl primer 1492R (5′- CACGGATCCTACGGGTACCTTGTTACGACTT-3′). A PCR tube containing water without cell material was used as negative control. The basal PCR condition was adjusted at 94°C for 45 s, 58°C for 60 s, 72°C for 120 s and 72°C for 600 s. After colony PCR, amplified fragments were detected by agarose gel electrophoresis using 1% agarose-1× TAE buffer. The PCR product was purified with E.Z.N.A. Gel Extraction Kit (Omega). Concentration and $A_{260/280}$ rate of purified PCR products were measured and sent to sequencing company (BM Lab., Turkey). The resulting 16S rRNA gene sequence was used to search the GenBank database with the BLAST program to determine relative phylogenetic position. Some biochemical and physiological characterization of isolates such as catalase and oxidase activities, utilization of carbon sources, growth temperature and pH requirements and salt tolerance of

isolate was also determined according to Malviya et al., (2013). Isolates were streaked on ISP2 medium without glucose supplemented with 10% (w/v) casein and gelatine and incubated at room temperature (20–24°C) for 7 d. To determine caseinase activity, the clear zone formation around the colonies was checked. To determine gelatinase, the medium was kept in cold condition (5–10°C) after incubation and then the liquefaction of the medium was checked.

Extraction of crude enzymes

After incubations in shaking flasks, the culture broth was centrifuged at 10,000 *g* for 5 min at 4°C and supernatant was saved at −20°C for xylanase assay.

Enzyme assays and total protein estimation

Crude xylanase activity was determined according to the method of Miller by measuring the amount of reducing sugars liberated from xylan (Miller, 1959). The substrate solution contained 0.5% (w/v) birchwood xylan in 50 mM sodium phosphate buffer (pH 7.0). Substrate and sample were incubated at 30°C for 10 min. The reaction was stopped by the addition of dinitrosalicylic acid (DNS) reagent. The mixture was heated and incubated at 100°C for 5 min. After cooling the mixture to room temperature, absorbance was measured at 540 nm. The final product was calculated according to xylose standard. One unit enzyme activity is defined as the amount of enzyme that releases 1 µmol xylose per 1 min at 30°C under the same assay condition. A blank was calculated with buffer instead of substrate (Tunçer et al., 1999). Similarly, amylase, cellulase and pectinase activities were also measured with the DNS method using starch, carboxymethyl cellulose (CMC) and polygalacturonic acid as substrates instead of birchwood xylan, respectively. The final product was calculated according to glucose (for amylase and cellulase) and galacturonic acid standard (for pectinase). Total protein was estimated according to Bradford, (1976). Protein concentration was determined using bovine serum albumine (BSA) standard curve (A_{595} nm).

Effect of different fermentation medium for xylanase production

Three different fermentation medium was tested for xylanase production (Table 1). Each medium was incubated at 30°C for 6 d. All trials were carried out in 250 ml Erlenmeyer flasks in an orbital shaker. In order to ensure aerobic conditions, the volume of the medium was restricted to 50 ml of the total volume of each flask.

Table 1. Composition of three different fermentation media.

Components (g/l)	Fermentation Medium 1 (ISP2 Liquid medium /FM1)	Fermentation Medium 2 (FM2)	Fermentation Medium 3 (MS-YEM/FM3)
Birchwood xylan[a]	4	4	4
Yeast extract[b]	4	2	6
Malt extract[b]	10	–	–
NH_4Cl	–	5	–
KH_2PO_4	–	1.5	1.98
Na_2HPO_4	–	30	5.03
NaCl	–	2.5	0.2
$MgSO_4.7H_20$	–	0.25	0.2
CaCl	–	–	0.05
Initial pH	7	7	7

[a]Birchwoon xylan was purchased from Sigma. [b]Yeast extract and malt extract were obtained from Merck.

Effect of carbon sources to xylanase production

Different carbon sources (eucalyptus pulp, lentil straw, banana leaf straw, pine sawdust, barley straw, wheat straw, corn stover, *Ricinus communis* straw and xylan) were tested for xylanase production in FM2 (Table 1). Before using, ground and milled lignocellulosic substrate was sterilized by autoclave (121°C, 15 min), washed twice with distilled water and dried at 80°C. To investigate the effect of various carbon sources on xylanase production, *Streptomyces* sp. AOA40 was cultivated for 3 d at 30°C and 120 rpm. Initial culture pH was adjusted to 7.0 ± 0.2. After sterilization of culture media, pH changes were checked and any significant effect of sterilization to pH change was observed.

Effect of corn stover concentration and particle size to xylanase production

Different concentrations of the corn stover ranging from 0–8 g/l were added to the FM2. *Streptomyces* sp. AOA40 was inoculated into these fermentation mediums and incubated at 30°C for 72 h. Different particle sizes (0.25, 0.5 and 0.75 mm) of pretreated corn stover was tested in order to determine their effects on xylanase production (Table 2).

Table 2. Different pretreatment conditions of corn stover.

Chemical	Concentration of chemical	Rate of corn stover dry mass to pretreatment liquid (w/v)	Pretreatment temperature	Pretreatment time
NaOH	1%	1/10	121°C	30 min
HCl	3%	1/10	Room temperature	3 h
H_2O_2	3%	1/10	Room temperature	1 h
Chloroform	99%	1/10	Room temperature	3 h
Lime	1%	1/10	121°C	1 h
H_2SO_4	1%	1/10	121°C	1 h

Effect of medium composition to xylanase production

The effect of yeast extract (0–4 g/l), NaCl (0–10 g/l), NH_4Cl (0–10 g/l), Na_2HPO_4 (0–30 g/l), KH_2PO_4 (0–2 g/l), $MgSO_4.7H_2O$ (0–0.375 g/l) concentrations to xylanase production by *Streptomyces* sp. AOA40 were determined. Each flask was incubated at 30°C and 120 rpm for 3 d. Initial pH of media was adjusted to 7.0 ± 0.2 for all tests.

Effect of initial pH and agitation rate to xylanase production

To evaluate the effects of initial pH of FM2 medium on xylanase production, the initial pH values were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The production of xylanase by *Streptomyces* sp. AOA40 was studied by incubating the shake flasks at 30°C and 120 rpm for 3 d. The effect of agitation rate was assessed by incubating shake flasks at different agitation rates (0–180 rpm) at 30°C for 3 d.

Effect of corn stover pretreated with different chemicals to xylanase production

Corn stover was pretreated with different chemicals under different conditions and used for xylanase production as a carbon source (Table 2) (Adigüzel and Tunçer, 2015). After pretreatment, substrates were washed twice with distilled water and dried at 80°C overnight. Each different pretreated substrate (7 g/l and 0.5 mm particle size) was added to liquid media (pH 7.0 ± 0.2). After inoculation of *Streptomyces* sp. AOA40 to media, xylanase production was performed at 30°C for 3 d. Natural corn stover was used as control.

Partial purification of xylanase

The initial purification step for xylanase was ultra-filtration of the cell-free extracellular liquid cultures. This was carried out using Amicon ultra-filtration cells (Millipore, Watford, UK) using 10 kDa molecular weight cut-off membranes pressurized to 0.21 MPa with nitrogen. The dialysed 20-fold crude concentrated preparation was used for anion exchange chromatography. Anion exchange chromatography was carried out using diethylaminoethyl-sepharose fast flow (Sigma-Aldrich) column material. The material was packed into a Pharmacia-XK50 column (5.0 id × 5.0 cm; Pharmacia, Milton Keynes, UK) according to the manufacturer's instructions. The dialysed 20-fold crude concentrated preparation was loaded onto an anion exchange column and eluted with a NaCl gradient (0–2 M) in Tris-HCl buffer (50 mM, pH 8.5) at a flow rate of 1 ml/min and fractions of 1 ml/tube were collected. Fractions

showing xylanase activity were pooled and used for gel filtration chromatography.

The pooled fractions of anion exchange chromatography was then loaded onto a gel filtration column (1.5 id × 30 cm), which was packed with Sephadex G-100 (Sigma-Aldrich, Missouri, USA) column material according to the manufacturer's instructions. The sample was eluted using 50 mM potassium phosphate buffer (pH 7.0) at a flow rate of 0.5 ml/min. Fractions showing xylanase activity were concentrated by disposable concentration units (Centricon-10; Millipore) and used to evaluate various biochemical characteristics of the enzyme. Enzyme activities and protein contents were assayed at each step by standard assay method and A_{280}, respectively.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and zymogram analysis

SDS-PAGE and native–polyacrylamide gel electrophoresis (Native-PAGE) was performed using an AE-6500 Dual Mini Slab Kit electrophoresis unit (Atto, Japan). The resolving gel was composed of 0.375 M Tris HCl buffer (pH 8.8), acrylamide (10%), bis-acrylamide (0.27%), SDS (10%), ammonium persulfate (0.006%) and tetramethylethylenediamine (TEMED) (0.15%). The stacking gel consisted of 0.125 M Tris HCl buffer (pH 6.8), acrylamide (4%), bis-acrylamide (0.105%), SDS (10%), ammonium persulfate (0.012%) and TEMED (0.30%). The reservoir buffer consisted of 0.25M Trisma-base, 1.92 M glycine and SDS (10%). The sample buffer contained 0.625M Tris-HCl (pH 6.8), glycerol (50%), SDS (10%), 2-mercaptoethanol (25%) and Bromphenol blue (0.05%). Separations were carried out at 16 mA in the stacking gel and at 30 mA in the resolving gel. After electrophoresis, the gel was stained with staining gel solution containing 0.1% (w/v) Coomassie Brilliant Blue (CBB) R-250, 50% (v/v) methanol, 10% (v/v) glacial acetic acid and 40% (v/v) distilled water and destained with a solution containing ethanol, glacial acetic acid and distilled water in the ratio of 1:2:16 (by volume). The resolving gel, stacking gel, reservoir buffer and sample buffer without SDS were used for Native-PAGE. Separations were carried out at 30 mA in the stacking gel and at 20 mA in the resolving gel. For zymogram analysis, the gel was flooded with 3% xylan prepared in 50 mM phosphate buffer (pH 6.0) and incubated at 60°C for 30 min. After, the gel was incubated in Congo red solution (0.1%) for 15 min at room temperature, the gel was destained with 1 M NaCl solution for 30 min (Raj et al., 2013).

Effect of temperature on xylanase activity and thermostability of xylanase

The effect of temperature on xylanase activity was determined by varying the reaction temperature from 30–90°C. The effect of temperature on the stability of xylanase over time was determined by incubating the enzyme preparations

in the absence of substrate over a period of 10 h at 40–80°C. Activity of these samples was then measured by using the standard DNS assay method.

Effect of pH on xylanase activity

The optimum pH of xylanase was determined at the optimum temperature (60°C) by conducting the xylanase assay at different pH using following buffers: sodium acetate (pH 4–6), sodium phosphate (pH 6–8) and Tris-HCl (pH 8–9).

Effect of NaCl concentration on xylanase activity

The effect of NaCl on the xylanase activity was determined at various salinities (0–10% NaCl) at pH 6.0 and 60°C. Enzyme reaction mixture without NaCl was taken as control (100%).

Effect of metallic ions and certain reagents on xylanase activity

Xylanase was incubated in the presence of 10 and 100 mM solution of either Cu^{2+}, K^{+}, Mg^{2+}, Hg^{2+}, Zn^{2+}, Mn^{2+}, Li^{+}, Ca^{2+}, and certain reagents such as ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), SDS and biotin for 1 h at room temperature (25 ± 2°C) and then activity of partially purified xylanase was investigated. The enzyme reaction mixture without metal ions and reagents was taken as control (100%) (Brito-Cunha et al., 2013).

Analysis of the hydrolysis products

The hydrolysis products of birchwood xylan were analyzed by thin layer chromatography (TLC). Partially purified enzyme and substrate was incubated at 60°C for 0, 1, 4, 8, 12 and 24 h. Reaction mixtures were concentrated with heat to approximately 10-fold and then hydrolysis products were detected by TLC. Mixture of formic acid, 2-butanone, *tert*-butanol and distilled water mixture (3: 6: 8:3 by vol.) was used as a solvent system. Compounds were detected by spraying with a phytallic acid (3.25 g), aniline (2 ml) and a water-saturated butanol (100 ml) mixture followed by heating at 95°C for 5–7 min. D-xylose, xylobiose and xylotriose was used as a standard.

Enzyme kinetics

Initial reaction rates of birchwood xylan hydrolysis were determined at different substrate concentrations ranging from 1–30 mg of birchwood xylan/ml of 50 mM phosphate buffer, pH 6 at 60°C. The kinetic constants K_m and V_{max} were estimated following the method of Lineweaver and Burk by using Hyper32 software.

Application of partially purified xylanase in fruit juice process and dough making

Fruits (apples, grapes and oranges) were purchased from the local market and washed. Juices of fruits were extracted and filtered through four layers of muslin. Filtered juices were incubated at 85°C for 3 min (Kareem and Adebowale, 2007). Fruit juices were treated with 12.5 U of xylanase/ml of fruit juice at 30, 40, 50 and 60°C for 30, 60, 90 and 120 min. At the end of treatments, juices were centrifuged at 10,000 *g* for 5 min. Supernatants were analyzed for clarity, reducing sugar and yield. The clarity of the juice was measured by determining the percent transmittance (T %) at a wavelength of 660 nm using UV-Vis spectrophotometer (Nagar et al., 2012). The yield of juices was detected by measuring the volume of the fruit juice after centrifugation. Reducing sugars were determined using DNS method (Miller, 1959). The results were calculated as percentage of control. Untreated fruit juices were use as control (Piemolini-Barreto et al., 2015).

Dough consisted of wheat flour (100 g) and dehydrated yeast (1.5 g), salt (2 g) and water supplemented with partially purified xylanase (40 U/ml) to evaluate effect of *Streptomyces* sp. AOA40 xylanase to oven spring. Dough without xylanase was used as control. Dough was mixed for 4 min and then incubated at room temperature for 20 min. All experiments were performed in triplicate.

Results and discussion

Isolation, screening and identification of xylanolytic actinomycetes

A total of 302 actinomycetes were isolated based on morphology. Among these, 22 isolates which produce zone of clearance on modified ISP2 agar were selected for further studies. The xylanase production of these isolates was measured in FM3 medium (Table 1) at 30°C, 120 rpm and pH 7.0 for 3 d. Isolate AOA40, which produce the highest xylanase, was selected for further xylanase production studies (data was not shown).

The 16S rRNA sequence of the isolate AOA40 was blasted using megablast tool of GenBank (http://www.ncbi.nlm.nih.gov/). This revealed that the isolate was a *Streptomyces* strain (Fig. 1). The physiological and biochemical properties of isolate *Streptomyces* sp. AOA40 are listed in Table 3.

Effect of fermentation medium and carbon sources to xylanase production

The production of extracellular xylanase increased significantly during the growth phase of culture (0–3 d). After the end of the growth phase, production of xylanase decreased constantly until the end of the incubation period (6 d). A typical growth curve and enzyme production by *Streptomyces* sp. AOA40 in different fermentation media are shown in Fig. 2. To determine

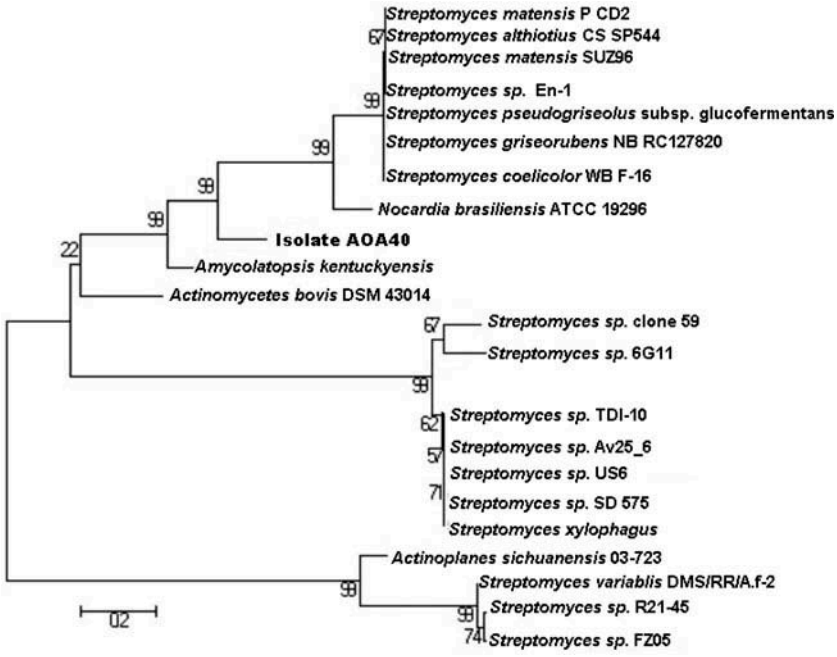

Figure 1. Phylogenetic tree showing the position of the isolate AOA40, based on the partial 16S rRNA sequence comparison, obtained by the neighbor-joining method.

the best medium for xylanase production by *Streptomyces* sp. AOA40, three fermentation broths (FM1, FM2, and FM3) were tested. Similar growth curves and enzyme production patterns were observed for *Streptomyces* sp. AOA40 grown in all fermentation broths; however, maximum xylanase production was obtained when the FM2 was used (Fig. 2). These results indicate that the production of extracellular xylanase was wholly growth-associated in cultures of *Streptomyces* sp. AOA40 grown on fermentation media. This is in accordance with the previous work of Tunçer et al., (1999, 2004), who showed that the production of extracellular xylanase was growth-associated in actinomycete cultures.

Streptomyces sp. AOA40 was capable of growth in FM2 containing either eucalyptus pulp, lentil straw, banana leaf straw, pine sawdust, barley straw, wheat straw, corn stover, *Ricinus communis* straw and xylan (Fig. 3). However, production of extracellular xylanase by *Streptomyces* sp. AOA40 was found to vary between the different carbon sources. The highest production of xylanase was found when *Streptomyces* sp. AOA40 was grown in a medium containing either corn stover (95.68 U/ml) or barley straw (83.22 U/ml), banana leaf straw (79.43 U/ml), and wheat straw (78.39 U/ml), respectively. Pine sawdust was the poorest inducers with maximum production of xylanase (20.92 U/ml) (Fig. 3). Accordingly, different activities for xylan-degrading enzymes from *Streptomyces chattanoogensis* UAH 23 (Fernandez et al., 1995) and *Phanerochaete*

Table 3. The physiological and biochemical properties of isolate *Streptomyces* sp. AOA40. Biochemical and physiological characterization was determined according to Malviya et al. (2013).

Biochemical and physiological characterization	*Streptomyces* sp. AOA40[a]
Oxidase	+
Catalase	+
Gelatinase	+
Caseinase	+
Utilization of carbon sources	
Glucose	+
Raffinose	–
Sucrose	+
Lactose	+
Cellulose	+
Birchwood xylan	+
Mannose	+
Fructose	+
Maltose	+
Galactose	–
Melibiose	+
Trehalose	+
Xylose	+
L-asparaginase	–
Medium pH	
5	+
6	+
7	+
8	+
Growth temperature	
30°C	+
40°C	+
50°C	+
60°C	–
NaCl Concentration in Growth Medium	
4%	+
7%	+
10%	–

[a]Data are presented as means of three replicates.

chrysosporium (Copa-Patino et al., 1993) have been reported with different inducers. Similar results have been reported for corn stover (Kohli et al., 2001; Panagiotou et al., 2003; Kumar et al., 2012), barley straw (Gandarillas et al., 2012), banana leaf straw (Shah et al., 2005) and wheat straw (Singh et al., 2012) to support the enhancements of xylanase productions. The application of agricultural wastes as primary carbon sources in xylanase production reduces the operation cost and also helps in solving problems with their disposal.

The *Streptomyces* sp. AOA40 strain described in this work produced high levels of extracellular xylanase during growth on a simple fermentation medium that did not include chemical inducers, such as β-methyl-D-xylopyranoside, often used to enhance xylanase activity. The levels of xylanase activity is higher than those of most bacteria such as *Thermomonospora fusca* BD25 (Tunçer et al., 1999) and fungi (Panagiotou et al., 2003) and comparable to those reported for *Streptomyces* sp. 2621 (Tunçer et al., 2004).

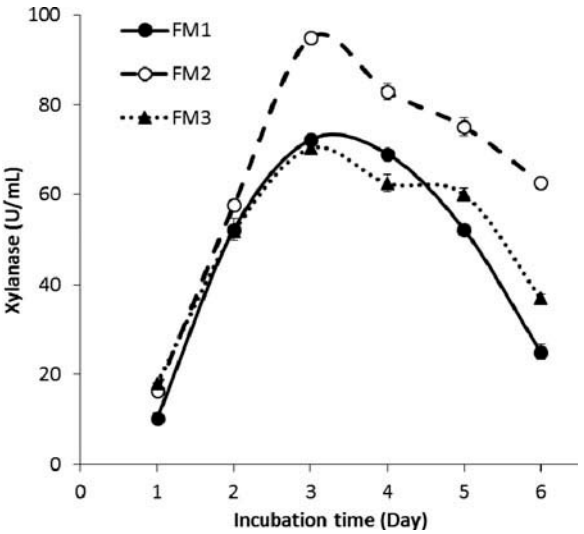

Figure 2. Time-course of xylanase production by *Streptomyces* sp. AOA40 in different fermentation media. Data are presented as means of three replicates with SE. Incubation was carried out at 30°C and 120 rpm.

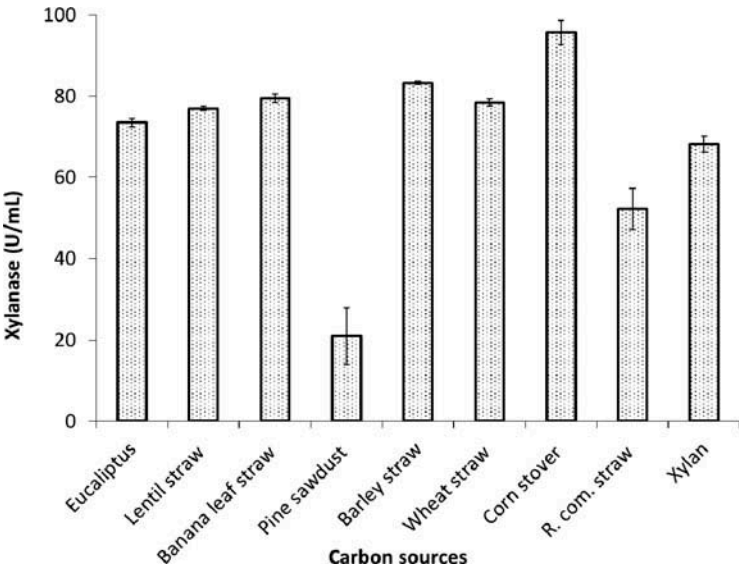

Figure 3. Effect of various carbon sources on xylanase production in FM2 (initial pH 7.0) supplemented with 4 g/l of each carbon source. Fermentation was carried out at 30°C and at 120 rpm for 3 d. Data are presented as means of three replicates with SE. One unit enzyme activity was defined as the amount of enzyme that releases 1 µmol xylose per 1 min at 30°C under same assay condition.

Effect of corn stover concentration and particle size to xylanase production

The effect of corn stover concentration on the production of xylanase by *Streptomyces* sp. AOA40 was investigated by growing the organism in shake

flasks, which containing FM2 supplemented with corn stover concentration ranging from 0–8 g/l as primary carbon and energy source. From the results, corn stover concentration of 7 g/l significantly increased the production of xylanase (136.56 U/ml), when compared to those obtained at 0 g/l concentration (5.99 U/ml) (Fig. 4).

The effect of corn stover particle size of 0.25, 0.50, 0.75 and 1.00 mm on the production of xylanase by *Streptomyces* sp. AOA40 was also investigated. After inoculation, fermentation media were incubated at 30°C and 120 rpm for 3 d. Xylanase production was significantly affected by particle size of corn stover. The highest production of xylanase was found when *Streptomyces* sp. AOA40 was grown in a medium containing either corn stover with particle size of 0.25 mm (98.28 U/ml) or 0.50 mm (81.92 U/ml), 0.75 mm (62.03 U/ml), and 1.00 mm (31.45 U/ml), respectively (Fig. 4). These results indicate that the production level of xylanase was wholly dependent on particle size of corn stover in cultures of *Streptomyces* sp. AOA40 grown on fermentation media.

Effect of medium composition to xylanase production

Different yeast extract contents ranging from 0–4 g/l were added to FM2 medium for the enzyme production. The maximum xylanase activity (118.36 U/ml) occurred at a concentration of 0.5 g/l yeast extract. After this concentration of yeast extract, xylanase production decreased and reached the minimum level at 4 g/l (16.58 U/ml) (Fig. 5a). The effect of increasing concentration of NaCl (0–10 g/l) was also tested. Highest xylanase activity of 114 U/ml was obtained with 1.25 g/l NaCl at 30°C, 120 rpm and pH 7.0 for

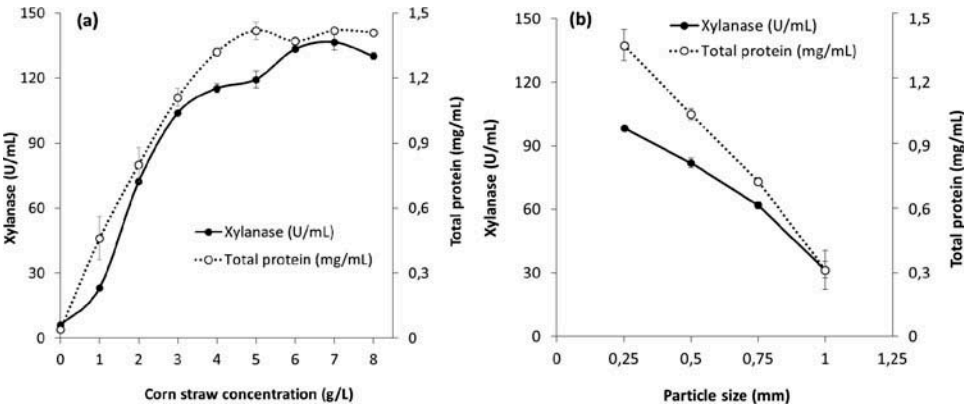

Figure 4. Effect of corn stover concentration (a) and particle size of corn stover (b) to xylanase production. Fermentation was carried out at 30°C and at 120 rpm for 3 d. Data are presented as means of three replicates with SE. Initial culture pH was adjusted to 7.0 ± 0.2. One unit enzyme activity was defined as the amount of enzyme that releases 1 µmol xylose per 1 min at 30°C under same assay condition.

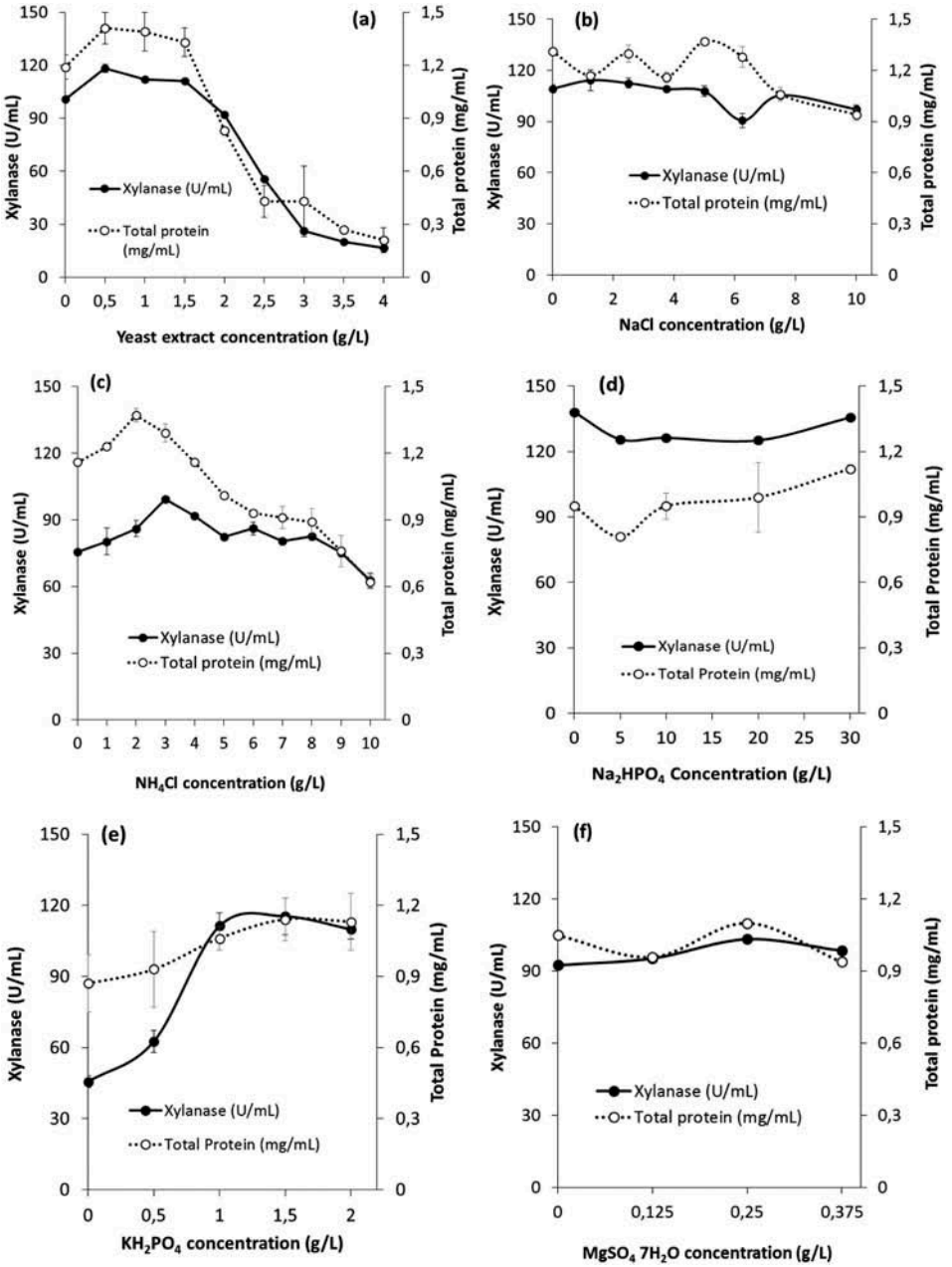

Figure 5. Effect of yeast extract (a), NaCl (b), NH_4Cl (c), Na_2HPO_4 (d), KH_2PO_4 (e), and $MgSO_4$ $7H_2O$ (f) concentrations on xylanase production in FM2 by *Streptomyces* sp. AOA40. Data are presented as means of 3 replicates with SE.

3 d (Fig. 5b). The effect of NH_4Cl and Na_2HPO_4 on xylanase production was also investigated. Maximum xylanase production was obtained at ammonium chloride concentration of 3 g/l (Fig. 5c). Any correlation between xylanase production and Na_2HPO_4 was not detected (Fig. 5d). When 1.5 g/l KH_2PO_4

was added in FM2, 115.32 U/ml xylanase was produced (Fig. 5e). In order to estimate the effect of $MgSO_4.7H_2O$ concentration, the xylanase production was carried out at different concentrations (0–0.375 g/l). Maximum xylanase production was observed at 0.25 g/l (Fig. 5f). Magnesium sulphate had no significant effect on xylanase production. But, total protein level was slightly greater when $MgSO_4.7H_2O$ was increased from 0.125 g/l (0.96 mg of protein per ml) to 0.25 g/l (1.1 mg of protein per ml).

Effect of pH and agitation rate to xylanase production

The effect of initial pH of culture medium on the xylanase production was investigated (Fig. 6a). Maximum xylanase was obtained when the initial pH was 9.0 (106.10 U/ml). The enzyme production was almost comparable at pH 6.0–9.0 (99.72–106.10 U/ml). These results indicate that the organism is alkali-tolerant and produces significant amounts of xylanase under alkaline condition. Generally, fungi produce xylanase at acidic fermentation media while bacteria produce xylanase at neutral or alkali media (Dhiman et al., 2009).

The effect of agitation rate on the production of extracellular xylanase by *Streptomyces* sp. AOA40 was investigated by growing the organism in an agitation range of 0, 60, 120 and 180 rpm. From the analysis of the results, it is evident that the optimum agitation rate for the production of xylanase (119.50 U/ml) occurred at 120 rpm, while at 60 and 180 rpm the *Streptomyces* sp. AOA40 produced considerable amount of xylanase (87.35 and 87.31 U/ml, respectively) (Fig. 6b). Further increase in agitation rate to 180 rpm caused a decrease in the enzyme production, which could be due to

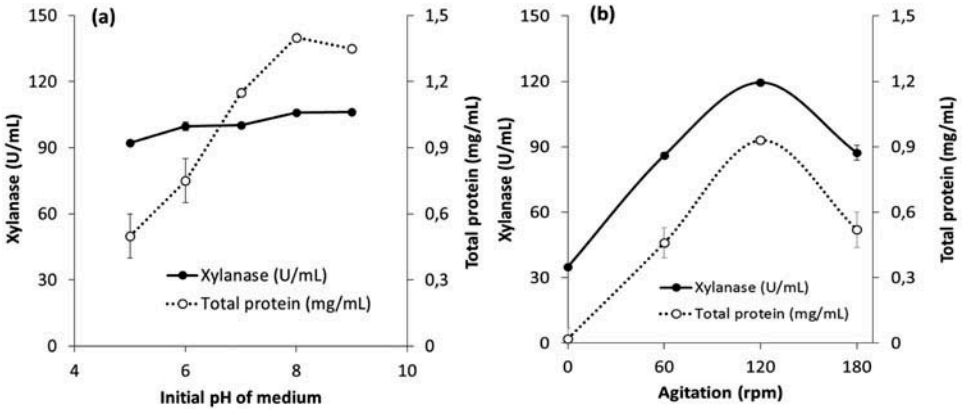

Figure 6. Effect of initial pH of fermentation medium (a) and agitation rate (b) on xylanase production. To evaluate the effect of pH and agitation rate on xylanase production, incubations were carried out at 30°C in FM2 medium supplemented with 7% corn stover (0.25 mm particle size) as primary carbon source. However, agitation rate was kept at 120 rpm for evaluate the effect of pH and initial pH was adjust to 9.0 ± 0.2 for evaluate the effect of agitation rate. Data are presented as means of three replicates with SE.

shorter contact between cells and the medium (Lo et al., 2011). As a result, the optimum agitation rate for xylanase production from *Streptomyces* sp. AOA40 was detected as 120 rpm with 50 ml fermentation medium in a 250 ml shake flask.

Effect of pretreatment methods of corn stover to xylanase production

The effect of pretreatment methods of corn stover on the production of xylanase by *Streptomyces* sp. AOA40 was investigated by growing the organism in shake flasks, which containing FM2 supplemented with 7 g/l corn stover, as primary carbon and energy source, which derived from different pretreatment methods such as NaOH, HCl, H_2O_2, H_2SO_4, lime and chloroform (Adigüzel and Tunçer, 2015). Xylanase production was performed at 30°C, at pH 7.0 and 120 rpm for 3 d. From the analysis of the results, it is evident that the best xylanase production (95.91 U/ml) was occurred with NaOH pretreatment method of corn stover for the production of xylanase, while HCl (81.53 U/ml), H_2O_2 (70.36 U/ml), H_2SO_4 (80.31 U/ml), lime (74.35 U/ml) and chloroform (63.98 U/ml) also supported production of considerable amount of xylanase (Fig. 7). This was probably due to increase

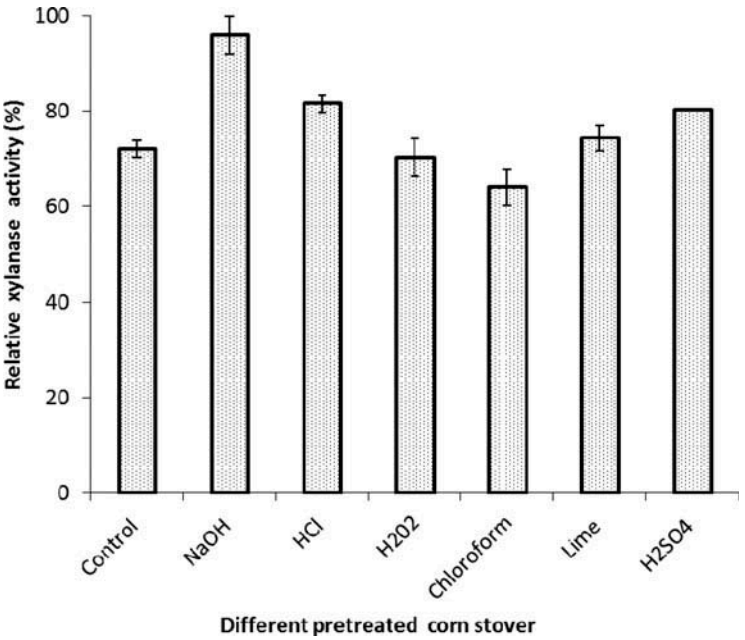

Figure 7. Effect of pretreatment methods of corn stover on xylanase production. Xylanase production was performed at 30°C, and 120 rpm for 3 d in FM2 medium supplemented with 7 g/l corn stover (0.5 mm particle size), as primary carbon source, which pretreated with different pretreatment methods [18]. Initial medium pH was adjust to 7.0 ± 0.2. Data are presented as means of three replicates with SE.

of xylanase and other extracellular lignocellulolytic enzyme to access into pre-treated substrates for hydrolysis. Therefore usability of substrates by microorganism had increased and more fermentable sugars produced to support to biomass increase.

Partial purification of xylanase

The specific activity of xylanase (84.04 U/mg of protein) in culture supernatant increased after ultrafiltration to 101.38 U/mg of protein. After anion exchange chromatography, the specific activity of xylanase, purification-fold and recovery yield were 126.22 U/mg of protein, 1.5-fold and 84.41%, respectively. Xylanase activity was eluted between 0.75 and 1.11 M NaCl and pooled active fractions was applied to gel filtration column and eluted as 3 separate peaks (Fig. 8). Xylanase containing fractions of three corresponding peaks were pooled with specific activity of xylanase, purification fold and recovery yield were 282.10 U/mg, 3.35-fold and 26.99%, respectively. Pooled fractions were concentrated and then used for characterization and showing application of xylanase in bakery and fruit juice clarification. The homogeneity of the enzyme was checked by native and SDS-PAGE; both yielded multiple protein bands (Fig. 9).

Protein bands in partially purified xylanase from *Streptomyces* sp. AOA40 were demonstrated by SDS-PAGE (Fig. 9a). More than 5 bands were visualized on the gel after Coomassie Brilliant Blue R250 staining. Zynogram analysis showed 3 different isoforms of xylanase detected by Congo red staining (Fig. 9b). Such isoforms of xylanases have also been reported in different microorganisms such as *Aspergillus fumigatus* AR1 (Anthony et al., 2003), *Streptomyces* sp. (Nascimento et al., 2002), *Trichoderma atroviride* 676 (Grigorevski-Lima et al., 2011), and *Bacillus mojavensis* A212 (Haddar et al., 2012).

Optimum temperature for xylanase activity and thermostability of xylanase

The effects of temperature on the activity and stability of xylanase were monitored by measuring the activity using the standard DNS assay method. Xylanase assay at different temperatures (ranging from 30–90°C) showed that xylanase from *Streptomyces* sp. AOA40 was active over a broad range of temperature (Fig. 10a). The activity of the enzyme at 60°C was accepted as 100%. Under the conditions applied, maximal xylanase activity (111.23%) was obtained when the incubation temperature was set at 60°C (Fig. 10a). However, the incubation temperature was set at 90°C, the activity was found to be 60.10% of the maximal activity (Fig. 10a). Similar temperature

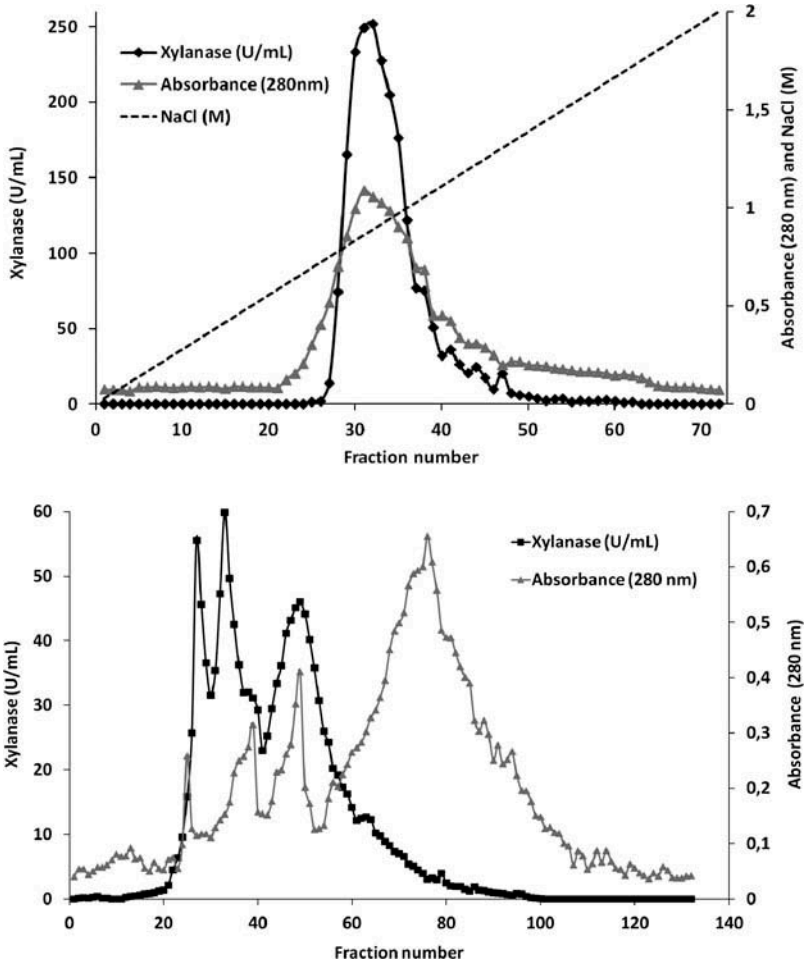

Figure 8. (a) Anion exchange chromatography with DEAE Sepharose. The flow rate was 1 ml/min and the size of fractions was 1 ml. (b) Gel filtration chromatography with Sephadex G-100. The flow rate was 0.5 ml/min and the size of fractions was 1 ml.

optimum for xylanases has also been reported from *Cellulosimicrobium cellulans* CKMX1 (Walia et al., 2014).

To investigate the thermostability, the xylanase was pre-incubated at different temperatures (40, 50, 60, 70 and 80°C) for 0–10 h. The activity of xylanase remained above 50% after incubation at 40, 50 and 60°C for 10 h. The xylanase activity showed a half-life of 172 min at 70°C, which was reduced to 75 min at 80°C (Fig. 10b). Thermostability studies also showed that the crude xylanase activity was remained as 87.83% at 60°C for 1 h and afterwards xylanase activity decreased to 52.35% after 10 h. Xylanase activity also remained as 82.23 and 75.22% at 70°C after 30 and 120 min, respectively (Fig. 10b). The suitable temperature of this xylanase for industrial application lies in between 40 and 70°C.

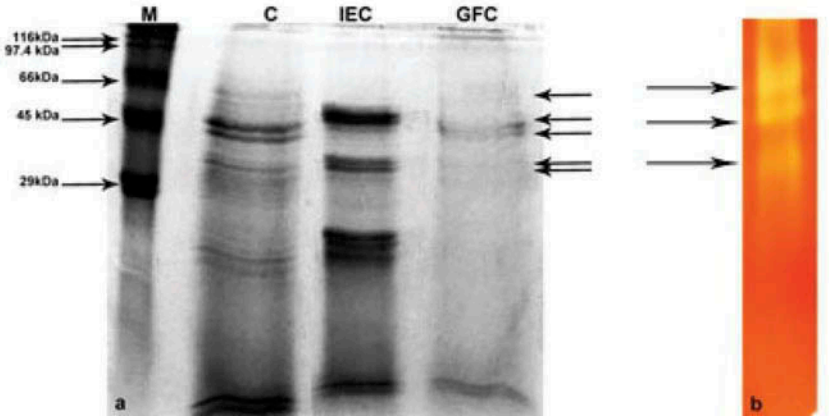

Figure 9. Molecular markers used were β-galactosidase from *E. coli* (116 kDa), phosphorilase b from rabbit muscle (97.4 kDa), albumin from bovine (66 kDa), albumin from egg (45 kDa) and carbonic anhydrase from bovine erythrocytes (29 kDa). (a) SDS-PAGE of purification steps. M, Molecular markers; C, Crude enzyme; IEC, Ion Exchange chromatography fraction; GFC, Gel filtration chromatography fraction. (b) Zymogram analysis of crude enzyme.

Thus, the optimal temperature for the xylanase activity was estimated to be 60°C when the assay was performed at pH 7.0 with a half-life of < 10 h. Similarly in the case of *Streptomyces* sp. RCK-2010, optimum temperature at pH 6.0 was found to be 60°C (Kumar et al., 2012) while the xylanase from *Streptomyes* sp. AMT3 the optimum temperature was found to be 65°C when the assay was carried out at pH 5.0 (Nascimento et al., 2002). In the case of *T. fusca* BD25 (Tunçer, 2000) and *Geobacillus* sp. VSVCF1 (Bhalla et al., 2015),

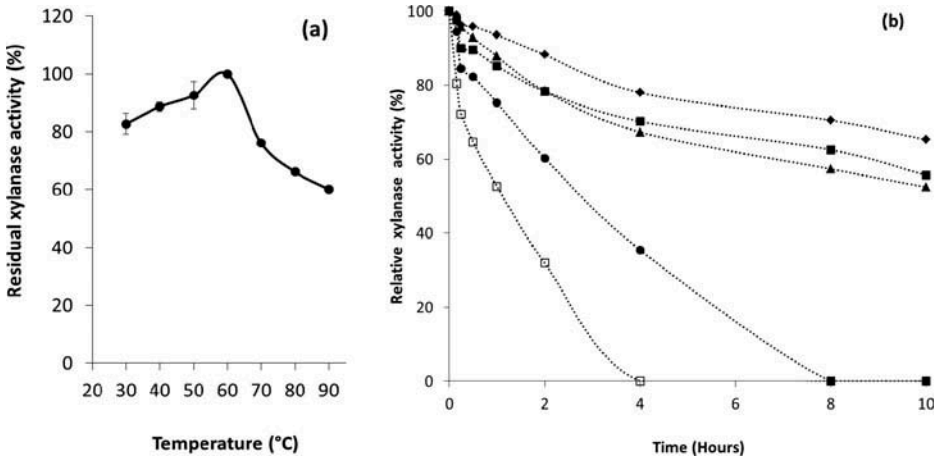

Figure 10. (a) Effect of temperature on partially purified xylanase activity assayed at different temperature range (30–90°C) for 10 min. 100% xylanase activity equivalent to 121.63 U/ml. (b) Thermostability profiles of xylanase. The enzyme wasincubatedat (♦) 40°C, (■) 50°C, (▲) 60°C, (●) 70°C and (□) 80°C for 10 h and residual activity was detected at different intervals. Data are presented as means of 3 replicates with SE.

the optimal incubation temperature for performing the DNS assay was found to be 70°C, justifying the thermophilic nature of the organisms. However, at higher temperatures, the stability of the *Streptomyces* sp. AOA40 xylanase was reduced, with the enzyme having a half-life of 75 min at 80°C. This value is higher than the thermostability of xylanase produced by *Bacillus* sp. HJ14 (Zhou et al., 2014). Xylanase of *Bacillus* sp. HJ14 exhibited half-lives of 4 min at 80°C. However, half-life of *Bacillus subtilis* xylanase at 80°C was 173 min (Saleem et al., 2012).

Optimum pH for xylanase activity

The activity of xylanase over the pH range of 4.0–9.0 was investigated and the maximal xylanase activity (100%) was found to occur at pH 6.0 (Fig. 11). However, at pH 4.0 and 5.0, the activities detected were 59.93 and 63.54%, respectively, of the maximal value; while at pH 7.0 and 8.0, the activities were 66.30 and 59.26%, respectively, decreased with increasing pH (Fig. 11). Xylanase from *Streptomyces viridochromogenes* had a pH optimum of the enzyme was similar to that of xylanase from *Streptomyces* sp. AOA40 which was used in the present study (Liu et al., 2013). Alkali-tolerant features of xylanases are desirable in some industrial application like produce reducing sugar from lignocellulosic wastes and pulp bleaching (Panwar et al., 2014). As xylanase from *Streptomyces* sp. AOA40 has broad pH stability (> 54% activity retained at over a broad pH range of 4–9), it can be also use in many industrial application such as clarification of juices, enhance bread quality and improve maceration.

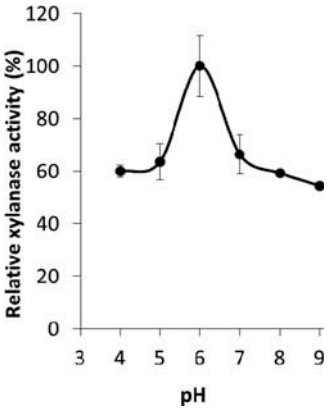

Figure 11. Effect of pH on xylanase activity at 60°C. The ionic strength for all buffers (sodium acetate, sodium phosphate and Tris-HCl) was 50 mM. Relative activity is expressed as a percentage of the original activity at pH 6. Enzyme was obtained after incubation of *Streptomyces* sp. AOA40 at 30°C, 120 rpm in FM2 medium supplemented with 7% corn stover (0.25 mm) as primary carbon source for 3 d. Data are presented as means of 3 replicates with SE.

Optimum NaCl concentration for xylanase activity

The effect of NaCl concentration on partially purified xylanase activity at pH 6.0 was studied. Xylanase from *Streptomyces* sp. AOA40 was active over a broad range of NaCl concentration of 0–10% (Fig. 12). The optimum residual xylanase activity was calculated, when 5% NaCl was presented in reaction mixture. This showed the halophilic feature of the enzyme. Similarly, xylanases which has halophilic nature have been reported earlier (Mamo et al., 2006; Menon et al., 2010). Even though some halotolerant xylanase had been reported earlier, there is no report about halotoerant xylanase from *Streptomyces* sp. to the best of our knowledge. Some research indicated that proteins of halophilic microorganisms and halotolerant proteins might possess more acidic amino acids than the basic amino acids and less hydrophobic core. But, the mechanism of halotolerance of xylanase has not been solved and needs further studies at the molecular and structural level (Ryu et al., 1994; Bai et al., 2012; Kumar and Syal, 2013). The features of salt tolerance suggest the baking potential application of xylanase in the baking industry and bioconversion of marine seaweeds for bioethanol production (Setati, 2010; Liu et al., 2014).

Effect of certain reagents on xylanase activity

The influence of certain reagents on xylanase activity was studied (Table 4). Relative activities were calculated taking the xylanase activities in the absence

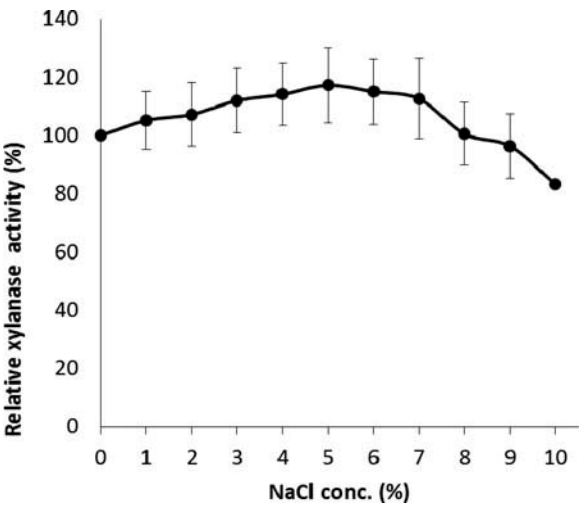

Figure 12. The effect of NaCl concentration on xylanase activity. Enzyme was produced by incubation of *Streptomyces* sp. AOA40 at 30°C, 120 rpm in FM2 medium supplemented with 7% corn stover (0.25 mm) as primary carbon source for 3 d. Data are presented as means of 3 replicates with SE.

Table 4. Effect of certain metal ions and inhibitors on xylanase activity*.

Reagents	10 mM Relative activity (%)	100 mM Relative activity
Cu^{2}+	135.93 ± 4.5	41.27 ± 0.5
K+	75.62 ± 2.0	73.31 ± 0.8
Mg^{2}+	65.44 ± 1.4	45.95 ± 2.0
Hg^{2}+	71.17 ± 0.5	18.43 ± 2.1
Zn^{2}+	86.52 ± 1.2	72.47 ± 0.5
Mn^{2}+	134.87 ± 2.7	122.45 ± 1.8
Li+	74.38 ± 0.7	74.85 ± 0.5
Ca^{2}+	61.70 ± 2.9	98.83 ± 2.3
SDS	58.11 ± 0.5	49.96 ± 3.2
DTT	132.61 ± 2.7	108.36 ± 2.4
EDTA	58.86 ± 0.4	28.39 ± 1.2
Biotin	81.53 ± 1	92.27 ± 2.3

*Enzyme reaction mixture without metal ions and reagents was taken as control (100%).
Data are presented as means of three replicates with SDs.

of reagents as 100% (Nawel et al., 2011). The xylanase activity was stimulated by 10 mM Cu^{2+}, 10 mM Mn^{2+} and 100 mM Mn^{+2}, 10 mM DTT and 100 mM DTT up to 135.93, 134.87 and 122.45, 132. 61 and 108.36%, respectively. The xylanase activity was strongly inhibited by 100 mM Hg^{2+} (18.43%), EDTA (28.39%), Cu^{2+} (41.27%), Mg^{2+} (45.95%) and SDS (49.96%). Similar reports also available, where 10 mM Cu^{2+}, Mn^{+2} (Taibi et al., 2012) and DTT stimulated xylanases. İnhibition of xylanase activity in the presence of Hg^{2+}, EDTA, Cu^{2+} and Mg^{2+} has also reported earlier (Poosarla and Chandra, 2014).

Effect of substrate concentration on xylanase activity

The *Km* value for an enzyme depends on the particular substrate used for the kinetic measurement and also on the environmental conditions such as pH, temperature and ionic strength. The effect of birchwod xylan concentration at a pH of 6.0 and at a temperature of 60°C on the activity of xylanase was investigated. The Km value was calculated using Hyper 32 program. The enzyme gave a typical Michaelis-Menten type response, with K_m and V_{max} of 2.4 mg/ml and 165.4 µmol/min/mg of protein, respectively at 60°C (Fig. 13).

Analysis of enzymatic hydrolysis products

The hydrolysis pattern of birchwood xylan by xylanase from *Streptomyces* sp. AOA40 was studied by TLC. After hydrolysis reaction was carried out for 1 h, a small quantity of xylotriose spot appeared. When hydrolysis time was prolonged to 12 h, spots corresponding to xylotriose and xylobiose were detected on the TLC plate. But, xylose spot was not obtained between 0–12 h of the hydrolysis period. After 24 h of hydrolysis reaction, xylotriose, xylobiose and xylose spots were detected (Fig. 14). These results showed that xylanase produced by *Streptomyces* sp. AOA40 was endo- and exo-lytic.

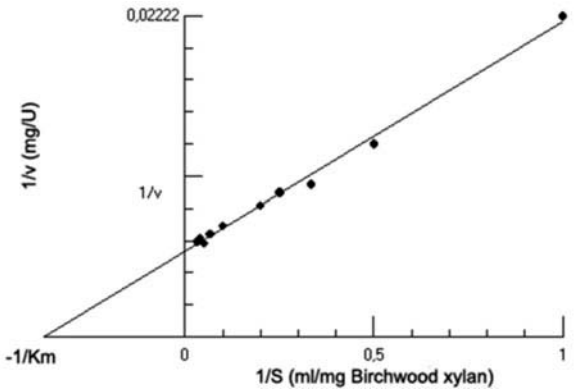

Figure 13. Lineweaver-Burk plot of xylanase activity at pH 6.0 and 60°C with different concentrations of birchwood xylan. Enzyme was produced by incubation of *Streptomyces* sp. AOA40 at 30°C, 120 rpm in FM2 medium supplemented with 7% corn stover (0.25 mm) as primary carbon source for 3 d.

Ninawe et al. had reported that xylooligosaccharides was released in the early stage of birchwood xylan hydrolysis by using xylanase from *Streptomyces cyaneus* SN32. They also detected xylobiose and xylose as the end product after hydrolysis time was prolonged (Ninawe et al., 2008).

Application of partially purified xylanase in fruit juice process and dough making

Partially purified xylanase was used for the application to fruit juice process. Cellulase, amylase and pectinase activities of enzyme source were checked. Any hydrolytic activity, except xylanase, was not detected. The partially purified xylanase produced by *Streptomyces* sp. AOA40 increased juice clarification, reducing

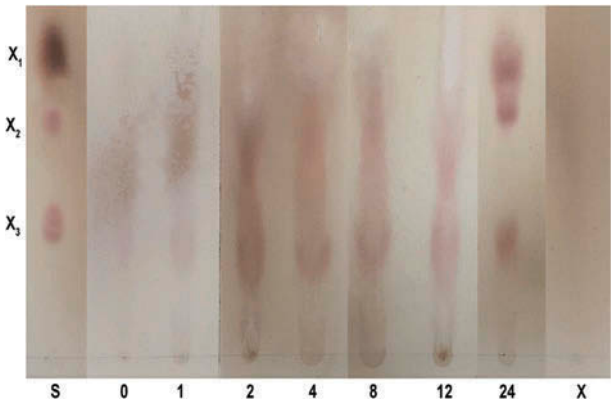

Figure 14. TLC chromatogram of the hydrolysis products of birchwood xylan reacted with partially purified xylanase after 0, 1, 2, 4, 8, 12, and 24 h hydrolysis (X, birchwood xylan; X_1, xylose; X_2, xylobiose; X_3, xylotriose).

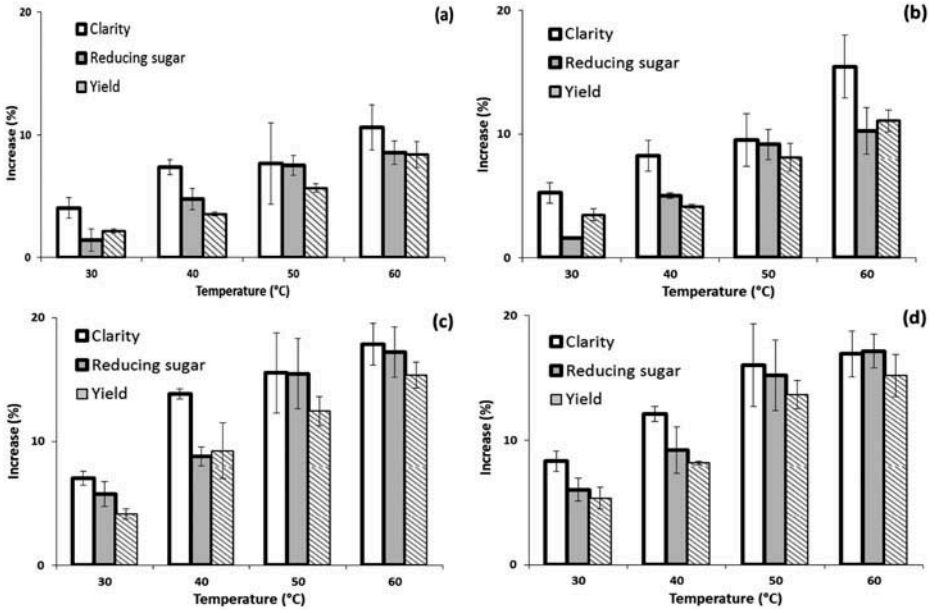

Figure 15. Effect of treatment of apple juice with partially purified xylanase (12.5 U/ml) to clarity, reducing sugar and yield. Incubation time (a) 30, (b) 60, (c) 90 and (d) 120 min. Highest clarity, reducing sugar and yield was obtained when apple juice was treated by xylanase at 60°C for 90 min. Data are presented as means of 3 replicates with SE.

sugar content and yield in apple juice process (Fig. 15). The maximum apple juice clarity (17.85%), reducing sugar (17.21%), and yield (15.35%) was obtained at the incubation temperature 60°C and incubation time of 90 min. Clarity, reducing sugar and yield of the apple juice increased with an incubation temperature from 30–60°C. Similar results were obtained for the treatment of orange juice. However, when the incubation temperature was increased from 50–60°C, the clarity of grape juices decreased. Another significant parameter was the incubation time of treatment. Clarity was influenced positively by increasing incubation time from 30–90 min. A further increase in incubation time did not affect positively to clarity of apple and grape juices. Similarly, some researchers detected that a prolonged incubation time has a negative effect due to the formation of a protein-carbonhydrates and/or protein-tannin complex (Sin et al., 2006). Optimum incubation time was published as 30–90 min in previous research. The optimum incubation temperature and time for treatment of grape juice were 50°C and 90 min, respectively, because maximum clarity (17.19%), reducing sugar (16.79%) and yield (12.35%) increase were determined (Fig. 16). The highest orange juice clarity increase (18.36%) was obtained after treatment at 60°C for 120 min. Sharma and Chand obtained 42.14% clear orange juice after treatment with *Pseudomonas* sp. xylanase at 40°C for 52 h (Sharma and Chand, 2012). Earlier research demonstrated that the treatment of fruit juices with xylanase positively contributes to clarity, reducing sugar and yield (Fig. 17) (Olfa et al., 2007; Nagar et al., 2012;

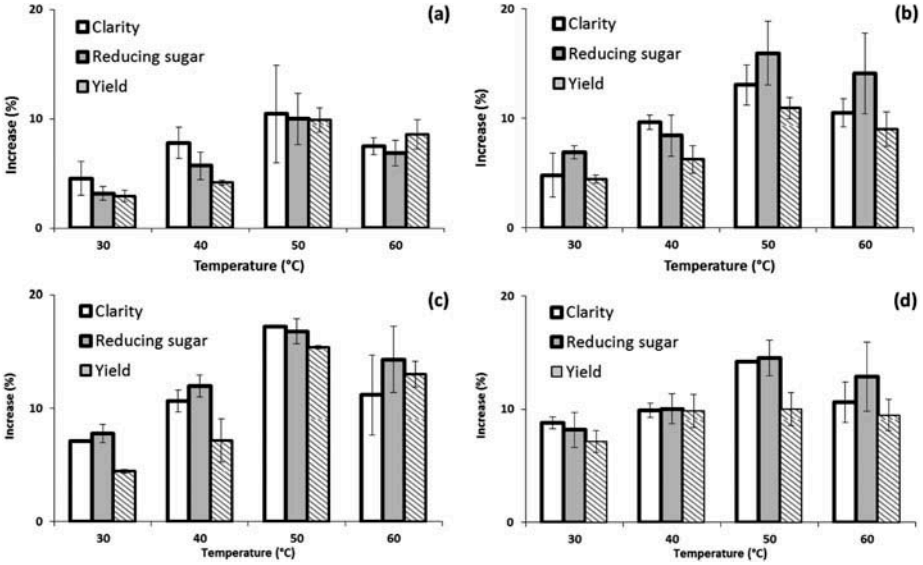

Figure 16. Effect of treatment of grape juice with partially purified xylanase (12.5 U/ml) to clarity, reducing sugar and yield. Incubation time (a) 30, (b) 60, (c) 90 and (d) 120 min. Maximum clarity, reducing sugar and yield obtained by enzymatic treatment of grape juice at 50°C for 90 min. Data are presented as means of 3 replicates with SE.

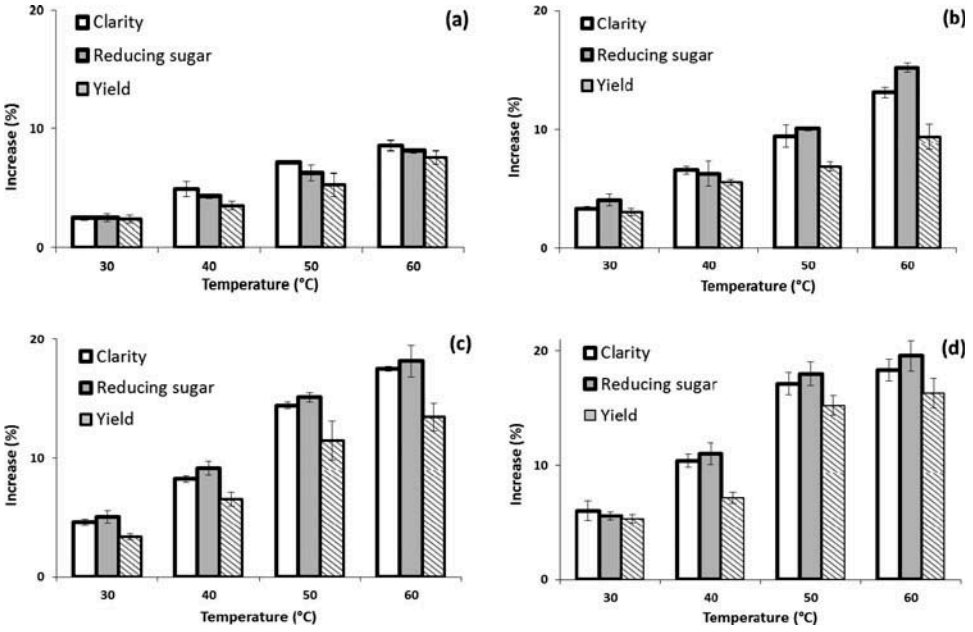

Figure 17. Effect of treatment of orange juice with partially purified xylanase (12.5 U/ml) to clarity, reducing sugar and yield. Incubation time (a) 30, (b) 60, (c) 90 and (d) 120 min. Data are presented as means of three replicates with SEs. Maximum clarity, reducing sugar and yield obtained by enzymatic treatment of orange juice at 60°C for 120 min.

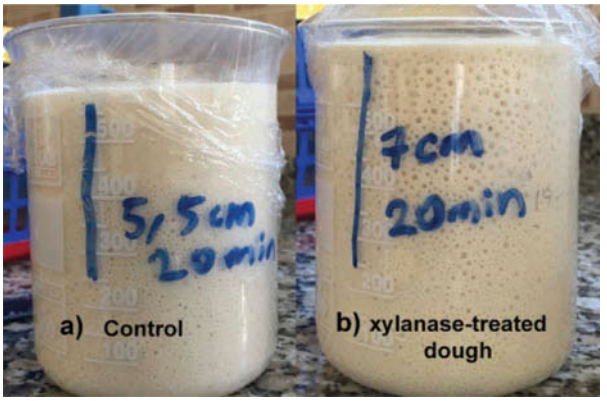

Figure 18. Application of partially purified *Streptomyces* sp. AOA40 xylanase (40 U/ml) for dough-raising in bakery. Dough was prepared with water include any additive for control. Water supplemented xylanase (40 U/ml) was used for determine effect of xylanase to dough-raising. Dough-raising was 17.06% after dough was prepared using water supplemented xylanase.

Sharma et al., 2012; Bajaj and Manhas, 2012; Kumar et al., 2013, 2014). Consequently, our study demonstrated that partially purified xylanase produced by *Streptomyces* sp. AOA40 can be used for apple, grape and orange juice improvement.

In our study, partially purified xylanase treatment of dough also resulted in increased oven spring as compared to the control (Fig. 18). After treatment, increase of dough volume was determined as 17.06%. Similarly, some research has reported that xylanase treatment of dough improved dough volume and handling properties (Jiang et al., 2005; Rouau et al., 2006; Selinheimo et al., 2006; Shah et al., 2006).

Conclusion

From this study, it can be observed that our isolate *Streptomyces* sp. AOA40 was able to grow and produce xylanases using lignocellulosic carbon sources as a primary carbon end energy source. The use of agricultural and industrial lignocellulosic waste as a carbon source for xylanase production may decrease enzyme production cost and may help environmental sustainability and solid waste managements. The pH and temperature optima of the xylanase were 6.0 and 60°C, respectively. The pH optimum of the xylanase is suitable for fruit juice applications. Also the use of thermotolerant xylanase after pasteurization of fruit juices may decrease process cost and time. These results reveal that the xylanase produced by *Streptomyces* sp. AOA40 was halotolerant and thermotolerant. These properties of xylanase may contribute to prevention of contaminations during industrial applications. The halotolerant property of the xylanase made it a

good candidate for marine-derived food processing and biomass conversions to hydrolysis products. As a result, our study showed that partially purified xylanase from *Streptomyces* sp. AOA40 can be used in the fruit juice and bakery industry. These properties of this extracellular xylanase place this enzyme as promising for industrial applications.

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