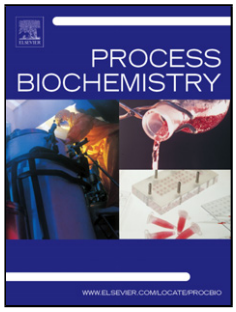
Accepted Manuscript



Title: A novel thermostable cellulase free xylanase stable in broad range of pH from *Streptomyces* sp. CS428

Author: G.C. Pradeep Yun Hee Choi Yoon Seok Choi Chi Nam Seong Seung Sik Cho Hyo Jeong Lee Jin Cheol Yoo<ce:footnote id="fn1"><ce:note-para id="npar0005">Both authors contributed equally to this work.</ce:note-para></ce:footnote>

PII: S1359-5113(13)00276-6
DOI: http://dx.doi.org/doi:10.1016/j.procbio.2013.06.007
Reference: PRBI 9861

To appear in: *Process Biochemistry*

Received date: 14-11-2012
Revised date: 29-5-2013
Accepted date: 1-6-2013

Please cite this article as: Pradeep GC, Choi YH, Choi YS, Seong CN, Cho SS, Lee HJ, Yoo JC, A novel thermostable cellulase free xylanase stable in broad range of pH from *Streptomyces* sp. CS428, *Process Biochemistry* (2013), http://dx.doi.org/10.1016/j.procbio.2013.06.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

A novel thermostable cellulase free xylanase stable in broad range of pH from *Streptomyces* sp. CS428

Pradeep G.C.[#], Yun Hee Choi[#], Yoon Seok Choi, Chi Nam Seong[a], Seung Sik Cho[b], Hyo Jeong Lee[c], and Jin Cheol Yoo*

Department of Pharmacy, Chosun University, Gwangju 501-759, Korea

[a]*Department of Biology, College of Natural Sciences, Sunchon National University, Sunchon, Jeonnam, 540-742, Korea*

[b]*Department of Pharmacy, College of Pharmacy, Mokpo National University, Muan, Jeonnam,534-729, Korea*

[c]*Department of Alternative Medicine, Gwangju University, Gwangju 503-703, Republic of Korea*

[#]Both authors contributed equally to this work.

*Corresponding author: Tel.: +82 62 230 6380, Fax: + 82-62-227-3963, E-mail: jcyu@chosun.ac.kr

Accepted Manuscript

ACCEPTED MANUSCRIPT

Abstract

A cellulase free thermostable xylanase from *Streptomyces* sp CS428 was isolated from a Korean soil sample, purified by single-step chromatography, and biochemically characterized. The extracellular xylanase was purified 26 fold with a 55 % yield by CM Trisacryl cation exchange chromatography. The molecular mass of the enzyme (Xyn428) was approximately 37 kDa. Xyn428 was found to be stable over a broad pH range (3 to ~13.6) and to 50 ºC and have an optimum temperature of 80 ºC. Xyn428 had K_m and V_{max} values of 102.3 ± 1.2 mg/mL and 3225.4 ± 15 mmol/min mg, respectively, when beechwood xylan was used as substrate. N-terminal sequence of Xyn428 was INRTDHNENSYLEIHNNEAR. CS428 was grown on different agro waste xylan and produced 4197.1 U/mL of xylanase activity in 36 h of cultivation in wheat bran without supplements. Xyn428 activity was inhibited by Tris salt at concentrations above 20 mM, and produced xylose and xylobiose as major products. It was found to degrade agro waste materials by small unit of enzyme (20 U/g) as shown by electron microscopy. As being simple in purification, thermo tolerant, pH stabilityin broad range and ability to producexylooligosaccharidesshow that Xyn428 has potential applications in industries as a biobleaching agent and for xylooligosaccharides production.

Keywords: Xylanase, single-step purification, agro-waste, xylooligosaccharides

Accepted Manuscript

ACCEPTED MANUSCRIPT

1. Introduction

Lignocellulosic biomass includes cellulose (35-50 %), hemicellulose (20-35%) and lignin (10-25%). Xylan is the major plant hemicellulose, which constitutes 20–40% of total plant biomass [1, 2]. Xylan consists of a backbone of β-1, 4-linked D-xylose residues having arabinose, glucuronic acid, and/or mannose substitutes. A wide variety of microorganisms including aerobic and anaerobic, mesophiles andthermophiles can produce enzymes that can degrade xylan[2]. The enzymatic hydrolysis of xylanrequires the cumulative actions of endo-β-1, 4-xylanase (EC 3.2.1.8), β- xylosidase (EC 3.2.1.37), and a series of enzymes that degrade side chain groups. Among these, endo- β-1, 4-xylanase is the most crucial enzyme that cleaves glycosidic bonds to produce short chain xylooligosaccharides of various lengths [3].Xylooligosaccharidesproductions from commercial and waste xylan werewell reported [4,5]. Industrial waste materials have become a global challengeand remained as centre of attraction since they create pollution and unnecessarily occupy space and requires high cost for proper management. Thus, the production of value added products, such as, bioethanol and oligosaccharides, offers an attractive solution. Bioethanol production includes two steps, that is, thehydrolysis of biomass to sugar and the fermentation of sugar to alcohol. Furthermore, the alcohol produced by the hydrolysis of industrial waste materials offers an alternative option of limited fossil fuels. In addition, to remove lignin, paper and pulp industries are using xylanase to degrade xylan, which becomes unfriendly to chemical process[6].

In recent days xylanase production using microbes is popular because of its significant role in food, animal feed, pulp, and paper industries [7]. In particular, xylan degrading enzymes from *Streptomyces* are being increasingly reported, such as, from *Streptomyces*

ACCEPTED MANUSCRIPT

lividans [8], *Streptomyces* sp.7b [9], *Streptomyces cyaneus* SN32 [5], *Streptomyces rameus*L2001 [4], *Streptomyces thermocyaneociolaceus* [10], etc. Here, we describe the isolation and identification of a potent xylanase producer - *Streptomyces* sp. CS428. Further purification by single-step column chromatography and characterization of an industrially applicable xylanase from this strain were also performed. Apart from this we have compared the activity and stability of xylanase from CS428 with the commercially available xylanase from *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from *Trichoderma longibrachiatum*.

2. Materials and methods

2.1. Materials

Xylose, Beechwood xylan, Birchwood xylan, xylanase from *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from *Trichoderma longibrachiatum* were purchased from Sigma-Aldrich (St Louis, MO, USA). Thin layer chromatography silica gel plates were purchased from Merck (Darmstadt, Germany). Xylobiose, Xylotriose,and Xylotetrose were from Megazyme (Ireland). CM Trisacryl was purchased from IBF (Villeneuve-la- Garenne, France).All the reagents used were of the highest grade available.

2.2. Bacterial strain, growth conditions, and screening

Ten bacterial strains collected from different Korean provinces were cultivated in xylan medium (pH 6.5) supplemented with 1.25 % beechwood xylan, 0.5% yeast extract, 1% tryptone, 0.75% KH_2PO_4, 0.15% K_2HPO_4, and 0.05% $MgSO_4$. The strains were cultivated in a 250 mL Erlenmeyer flask containing 50 mL medium at 28 ºC and 130 rpm for 5 days. The

ACCEPTED MANUSCRIPT

culture broths were centrifuged at 6,000 ×g for 30 min and supernatants were tested for enzyme activity. Strain CS428,which had good activity, was selected for further study. In addition, comparative xylanase production was studied using agro-waste materials using corn cob and wheat bran with and without supplements (like a nitrogen source, a carbon source, and metal ions). The corncob and wheat bran were bought from local Nam-Gwangju market; grains were removed, washed, dried, and then grounded for use.

2.3. Enzyme assay and protein estimation

Protein concentrations were determined using the Bradford method [11] using bovine serum albumin as standard.

Xylanase activities were assayed at 80 ºC by adding 0.1 mL enzyme (appropriately diluted in 5mM Tris/HCl pH 7.0) sample to 0.1 mL of substrate solution containing 0.5 % (w/v) beechwood xylan prepared in 5 mM Tris/HCl (pH 7.0) for 5 min. A control was run simultaneously with all the reagents in ice cold water (`0ºC). The reducing sugars released were measured using 3,5-dinitrosalicylic acid (DNS) method [12]. A standard xylose curve was constructed to determine enzyme activities. One unit of xylanase activity was defined as the amount of enzyme that releases 1 µmol of xylose per min under standard assay conditions. Cellulose activities were evaluated using sigma cell cellulose, carboxymethyl cellulose, avicel and an artificial substrate such as,paranitrophenyl D- celobioside (pNPC) and paranitrophenyl-β- D glycopyranoside (pNPG).

2.4.Enzyme purification

Accepted Manuscript

ACCEPTED MANUSCRIPT

All purification procedures were carried out at 0ºC unless stated otherwise. *Streptomyces* sp. CS428 was grown for 72 h and the crude supernatant extracellular xylanase was obtained by centrifuging the broth at 6000×g for 1 h. The crude supernatant was then subjected to 30-75 % ammonium sulfate precipitation. Proteins were recovered by centrifugation at 6000×g for 1 h at 4 ºC, dialyzed against 10mM citrate/phosphate (pH 7.0), and concentrated using an ultra filtration membrane (5 kDa, Millipore Corp). The dialyzed enzyme solution was loaded to a CM Trisacryl column (20 cm × 1.1 cm) pre-equilibrated with 10 mM citrate/phosphate buffer (pH 5.5). Proteins were eluted with 0-1 M KCl in the same buffer at 30 mL/h (2 mL/Fraction). Xylanase active fractions were pooled, concentrated, and analyzed for purity. Further characterizations were carried out using the pure enzyme.

2.5. Polyacrylamide gel electrophoresis

The purified enzyme was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% (w/v) polyacrylamide slab gel [13]. For reference proteins, protein size marker (MBI, Fermentas) was used. Proteins were observed by staining with Coomassie Brilliant Blue R-250 and then destaining with a solution containing methanol: glacial acetic acid: distilled water = (1:1:8 by vol). Molecular weight was estimated by comparison with the relative mobility of the reference protein. In addition, xylan zymography was performed as described [14].

2.6. Effect of pH and temperature

Accepted Manuscript

ACCEPTED MANUSCRIPT

The pH and temperature optimum and stability of Xyn428 were compared with the commercially available xylanase from *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from *Trichoderma longibrachiatum*. The optimum pH required for relative xylanase activity were determined at 80 ºC, 70ºC, and 60 ºC respectively for xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* at 5 mM using 100 mM concentrations of various pH buffers (pH values 2.0-13.6). The buffers used were;citric acid/sodium phosphate (2-7.5), Tris/HCl (7.0-9.5), CAPS/NaOH (9-11), sodium bicarbonate/NaOH (9.5-11), glycine/NaOH (9.5-11), and KCl/NaOH (11-13.6). Temperature was optimized at pH 7.0 in the range 50-90ºC. To determine pH stability, enzyme samples were incubated at 10 mMusing various pH buffers at 4 ºC for 24 hr and residual activities were measured at 20mM under standard assay conditions. Similarly, to determine enzyme temperature stability, enzyme samples were pre-incubated at various temperatures (30-90 ºC) for 60 min and residual activities were measured. The assay was done under standard assay conditions.

2.7. Effect of salt concentration

To determine the effect of salt concentration on the activity of Xyn428, different concentrations (2mM, 5mM, 10mM, 20mM, 50mM, 100mM) of Tris salt at different pH values (7.0, 7.5, 8.5, 9.5) were examined. Activities were determined under standard assay conditions.

2.8. Effects of metal ions

Accepted Manuscript

ACCEPTED MANUSCRIPT

Effects of metal ions on the activities of Xyn428 were compared with the commercially available xylanase from *Thermomyces lanuginosus*, and endo-1,4-β xylanase from *Trichoderma longibrachiatum*. For the metal ion effect, the pure enzyme was incubated at 1 mM concentration of Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, K^{+}, Na^{+}, Mn^{2+},and Fe^{2+}. The relative activities were determined under standard assay conditions comparing with control without metal ions (100%).

2.9. Effects of various organic solvents

The enzyme samples were incubated with 10% aqueous solutions of organic solvents that is, methanol, ethanol, iso-propanol, butanol, hexane, heptanes, octane, decane, benzene, toluene, xylene, dichloromethane, acetonitrile, ethyl acetate, acetone, diethyl ether, glycerol, and dimethylsulfoxide (DMSO). Enzyme activities were evaluated under standard assay conditions. In addition, the stability of xylanase in these solvents for two hours at room temperature was measured. Further, enzyme activities were evaluated containing different concentrations of alcohol (0.06-2%). Residual activities were measured under standard assay conditions.

2.10. Effects of detergents and modulators

Detergent and modulator effects were also examined and the activities of Xyn428 were compared with the commercially available xylanase from *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from *Trichoderma longibrachiatum.* The effects of detergents were

Accepted Manuscript

ACCEPTED MANUSCRIPT

evaluated using Triton X – 100, Tween-20, Tween-80, Polyoxylethylene-4-laurylether, Deoxycholic acid (DCA), Sodium Dodecyl Sulfate (SDS) and 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) at 0.25% and the effects of modulators like hydrogen peroxide, sodium perborate, β- mercaptoethanol, Ethylenediamine tetra-acetic acid (EDTA), ethylene glycol-bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) were also evaluated. In all cases, relative enzyme activities were measured under standard assay conditions with the activity without any additive was taken control (100%).

2.11. N- terminal amino acids

The N-terminal amino acid sequence of Xyn428 was determined by Edman degradation using a Procise Model 492 protein sequencer (Applied Biosystems, CA, USA).

2.12. Substrate specificity and kinetic parameters

To evaluate the substrate specificity of the purified enzyme, the enzyme was incubated with 0.5% (w/v) of each substrate such as beechwood xylan, birchwood xylan, wheat bran and corncob xylan. Enzyme activities were measured under standard assay conditions. For kinetic parameters, five different concentrations of beechwood and birchwood xylan (1.25-20 mg/mL) with constant enzyme concentration (0.14 µg) were prepared. Assays were performed under standard assay conditions at optimal conditions. The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were determined from a Lineweaver-Burk plot.

Accepted Manuscript

ACCEPTED MANUSCRIPT

197 *2.13. Enzymatic hydrolysis and xylooligosaccharides production*

198 Enzymatic hydrolysis of Xyn428 was compared with the commercially available
199 xylanase from *Thermomyces lanuginosus* and evaluated using thin layer chromatography
200 (TLC) [5]. Briefly, purified Xyn428 and xylanase from *Thermomyces lanuginosus* (2.44
201 mg/mL) were incubated with commercial beechwood xylan substrate (50 mg/mL) in 100 mM
202 Tris/HCl buffer (pH 7.0) at 50 ºC. At predetermined time 30 µl was withdrawn. The reaction
203 was stopped by adding 5 µl of 10% (v/v) trichloro acetic acid and samples were then spotted
204 on the silica gel plates 60F 254 (E. Merck, Germany). The plates were developed with solvent
205 system of chloroform–acetic acid–water (6:10:2, v/v/v) followed by spraying the plates with a
206 methanol–sulfuric acid mixture (95:5, v/v) and heated for few minutes at 150 ºC. A mixture of
207 xylooligosaccharides consisting of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetrose
208 (X4) (10 mg/mL) were used as the standard.

209

210 *2.14. Xylanase for the degradation of lignocellulose biomass*

211 Untreated corncob and wheat bran was washed with distilled water to a neutral pH.
212 Both biomasses were treated with 20 U/g of purified xylanase from *Streptomyces* sp CS428,
213 *Thermomyces lanuginosus* and endo-1,4-β-xylanase from *Trichoderma longibrachiatum* at
214 Tris/HC pH 7.0, 50 ºC for 2 h. Degradation of biomass by Xyn428 was analyzed by electron
215 microscopy as previously described [4]. Biomass treated with buffer was taken as the control.

216

217 **3. Results and discussion**

Accepted Manuscript

ACCEPTED MANUSCRIPT

3.1. Bacterial strain, growth conditions, and screening

All ten bacterial strains collected from different Korean provinces were cultivated in xylan medium at 28 ºC and 130 rpm for 5days. Among them, strain CS428 showed strong xylan degrading activity and was selected for further study. Based on morphological, biochemical and 16S rRNA sequences, the stain was identified as *Streptomyces* sp. CS428, as we previously described [15]. 16S rRNA sequence of the strain showed 100% similarity with *Streptomyces rameus* LMG20326 (T) (Accession no. AJ781379), *Streptomyces tricolor* LMG20328 (T) (Accession no.781380) and *Streptomyces bangaladeshensis* AAB-4(T) (Accession no. 750056) (Fig. not shown).

3.2. Enzyme production and purification

The comparative study of xylanase production from corncob and wheat bran with or without supplements was performed shown in Fig. 4. Many reports have been issued on xylanase production using wheat bran and corncob [4, 5]. We observed that strain CS428 produced xylanase and achieved a higher yield (4197.14 ± 1.3 U/mL in 36 h) from wheat bran without supplements than has been previously reported. The highest xylanase activity previously reported was 1810.9 U/mL from *S. rameus* L2001 [4]. Interestingly, a higher yield of xylanase production was obtained from wheat bran without supplements than from commercially available beechwood xylan (3329.1 ± 2.6 U/mL), which is encouraging one. In addition, remarkable xylanase production was obtained from corncob with supplements. These findings suggest that Xyn428 is a potent candidate in industrial application.

ACCEPTED MANUSCRIPT

Xyn428 was purified in a single-step by CM Trisacryl cation exchange chromatography. Many xylanase from *Streptomyces* sp. have been purified using multiple column chromatographic steps [4, 16-19]. Our enzyme was purified more efficiently than other available xylanase. A summary of xylanase purification is illustrated in Table 1. Xylanase was purified to 25.9 fold with a recovery yield of 55.1%. Furthermore, xylanase activity recovery and purity fold were greater than those of the xylanase from *Streptomyces cyaneus* SN32 [5], *Streptomyces* sp. 7b [9], and from *Bacillus subtilis* ASH [20] that were separated from single step chromatography.

3.3. Gel electrophoresis

The purified enzymewas analyzed by SDS-PAGE and activity staining of the gel (Zymography) shown in Fig. 1a and 1b respectively. The molecular mass of Xyn428was estimated approximately at 37 kDa, and was seen on gels as a single band. In accord with SDS-PAGE results, the pure enzyme showed a clear band by zymography,confirming that it was a xylanase. Its molecular mass was similar to the xylanases from *Streptomyces thermocyaneociolaceu* (35 kDa) [10] and *Streptomyces* sp TN119 (35.9 kDa) [21] shown in Table 2.

3.4. Effect of pH and temperature

Enzyme activity and stability were markedly affected by pH and temperature. The effects of pH and temperature on the activity and stability of Xyn428 and other xylanase from

Accepted Manuscript

ACCEPTED MANUSCRIPT

Streptomyces are shown in Table 2. The effect of pH on the activity and stability of Xyn428 were also compared with the commercially available xylanase from *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from *Trichoderma longibrachiatum* shown in Fig. 2a and 2b respectively.The xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* were active from pH 6.0 to 13.0 with the highest at 7.0, 7.0, and 12.5 respectively. The enzymes were stable to a broad range of pH Fig. 2b. The xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* remain stable about 85%, 70% and 90 % respectively of the maximal activity at pH approximately 13.6. The reasons behind such a stability on broad range of pH may be due to the reversible denaturation of the protein so that there is no effect on the activity of the enzyme on incubation at different pH. Furthermore, Xyn428 was found to be more stable in acidic media than *Streptomyces cyaneus* SN32 [5], *Streptomyces megasporus* DSM 41476 [22], *Streptomyces matensis* DW67 [19], and to our knowledge the most alkaline stable xylanase reported from *Streptomyces* shown in Table 2. Xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* exhibited a temperature optimum at 80 ºC, 70 ºC, and 60 ºC respectively Fig. 3a. When incubated for 1 h at 60 ºC, xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* retained almost 72%, 90%, and 67 % of the maximal activity shown in Fig. 3b. Xyn428 was found to have the highest optimal temperature reported among the *Streptomyces*, and stability is comparable to that of the xylanase from *Streptomyces* sp. 7b [9], *Streptomyces matensis* DW67 [19] Table 2. Despite the partial stability of xylanase from CS428, *Thermomyces lanuginosus*, and *Trichoderma longibrachiatum* at 80 ºC, Xyn428 showed highest activity at this temperature. This may be due to the protective effects of substrate to Xyn428 for optimum time. Many industrial processes are operated at extremes of

ACCEPTED MANUSCRIPT

pH (either acidic or alikaline) and at elevated temperatures thus the enzyme must suit the process requirements and must be capabale of withstanding such harsh conditions for prolonged periods or at least during the process time which we can find in Xyn428. For every 10°C rise in temperature, reaction rates approximately double. Therefore, assuming the enzyme is stable at the higher temperature, the amount of enzyme needed can be reduced or the conversion time be shortened. There are other benefits from operating at higher temperatures carrying out conversions at increased temperatures (above 60-65°C) markedly reduces microbial infection of the material being processed [23]. In addition, the higher temperatures increase the solubility of polymeric substrates such as carbohydrates, there by improving their mechanical handling characteristics and rendering them more amenable to enzymatic attack. Tolerance to broad range of pH values, a high optimum temperature, and its stability makes Xyn428 a novel among xylanase previously reported from *Streptomyces*.

3.5. Effect of salt concentration

The enzyme activity of Xyn428 was remarkably affected by salt concentration. The purified enzyme showed maximum activity at a salt concentration of 5 mM. At pH 7.0, its activities at concentration of 2 mM, 5mM, 10 mM, 20mM, 50 mM, and 100mM were 37 U, 52 U, 51 U, 43 U, 4 U, and 4.5 U, respectively. However, its activity was substantially inhibited at salt concentrations exceeding 20 mM (Fig. 5).

3.6. Effects of metal ions

Accepted Manuscript

ACCEPTED MANUSCRIPT

The influences of metal ions on the activities of xylanases obtained from CS428, *Thermomyces lanuginosus*, and endo-1,4-β-xylanase from*Trichoderma longibrachiatum* were studied (Table 3). The activity was enhanced 1.33- and 1.23-fold by Ca^{2+} and Co^{2+}, respectively, but was inhibited by Fe^{2+}, Zn^{2+}, Cu^{2+}, and Mg^{2+}, and almost unaffected by K^{+}, Na^{+}, and Mn^{2+} similar to the commercially available xylanase *Thermomyces lanuginosus* and *Trichoderma longibrachiatum* except Mn^{2+} from *Thermomyces lanuginosus.* Cu ions are known to catalyze the auto-oxidation of cysteines to form intra molecular disulfide bridges or the formation of sulphenic acid [24]. The activity was enhanced by $Co^{2+}$is similar to that reported for other xylanases [18, 25]. In addition, Fe^{2+}suppressed the activity like the xylanase from *Streptomyces* sp.SWU10 [16] but against the xylanase from *Streptomyces rameus* L2001 and *Streptomyces matensis* DW67 [4,19]. The result reveals that what kinds of metals should be included or excluded for the industrial applications.

3.7. Effects of organic solvents

The effects of organic solvents on the activity and stability of Xyn428 were examined at 10% concentration (Table not shown). Xylene and ethyl acetate were found to suppress activity, but all other solvents examined have no effect. Xyn428 was stable (2 h) in hexane, heptane, octane and acetone where as unstable in toluene, acetonitrile, and DMSO which suggests that organic solvents have partial or specific effects on activity and stability of Xyn428 that have significant importance on bioindustries. Solvent polarity strongly influences the way in which organic solvents interact with water surrounding enzyme molecules. A highly polar solvent absorbs essential water from enzyme and inactivates it. Actually, solvents with a log *P* value of <4 are toxic because their degrees of partitioning into aqueous layer are higher.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Enzymes that are stable in organic phases may be useful for bioremediation or as biocatalysts [26]. Xyn428 was found to have stability in solvents with a higher log *P* value, that is, 5.2 (decane) to 2.0 (benzene) (with the exceptions of toluene (log *P*=2.5) and xylene (log *P*=3.1)) where as instability in the solvents with lower log *P* value is in correlation (with the exception of DMSO (log *P*=-1.37), acetonitrile (log P=-0.39) and acetone (log *P*=-0.21)). Furthermore, the optimal concentration of ethyl alcohol enhances the activity of Xyn428, but higher concentrations adversely affect its activity, as like the xylanase reported for *Streptomyces rameus* L2001 [4] (Table 4). These data provide the essential data required for the application of purified xylanase reaction in an organic phase.

3.8. Effects of detergents and modulators

The influences of detergents and modulators on the activity of xylanases obtained from CS428, *Thermomyces lanuginosus* and endo-1,4-β-xylanase from *Trichoderma longibrachiatum* were summarized in Table 3. In case of detergents, xylanase activity was not affected by TritonX-100, Tween-20, Tween-80, Polyoxylethylene-4-laurylether and CHAPS but was affected by Deooxycholic acid and SDS, which is similar to that reported for the xylanase from *Burkholderia* sp. DMAX [25]. On the other hand, the modulator β-mercaptoethanol did not affect the activity of Xyn428, but 1, 4-dithiothreitol enhanced its activity. The inhibitions of xylanase activity by EDTA and SDS have been previously reported [17, 27].

ACCEPTED MANUSCRIPT

3.9. N-terminal amino acids

The amino acid sequence of the first 20 N-terminal amino acids of Xyn428 was found to be INRTDHNENSYLEIHNNEAR. This sequence was compared with available sequences in the National Centre for Biotechnology Information (NCBI) protein database using BLAST (basic local alignment search tool;http://blast.ncbi.nlm.nih.gov/Blast.cgi). The BLAST search did not suggest homology with other *Streptomyces* or xylanase sequences, but showed a high degree of homology with sequences from *Streptomyces tendae* TU901 and *Streptomyces clavuligerus* ATCC, and low homology with sequences of the xylanases from *Trichoderma* sp. SY and *Caldicellulosiruptor saccharolyticus* and with a putative β-1, 4-xylanase from *Cochliobolus spicifer*, suggesting that Xyn428 is a β -1, 4 xylanase (Table not shown).

3.10. Substrate specificity and kinetic parameters

Purified Xyn428 was assayed using different natural and synthetic substrates (Table not shown). Its activity was highest for beechwood and commercially available xylan, but least for wheat bran xylan (5% beechwood) and agro-waste materials, which is similar to that observed for xylanase from *S. rameus* L2001 [4]. Xyn428 showed no activity towards Avicel, carboxymethyl cellulose (CMC), p-nitrophenylglucopyranoside (pNPG), and p-nitrophenyl-β-D-celobioside (pNPC). These results show that the xylanase isolated and purified from CS428 is a cellulase free xylanase, similar to a xylanase reported earlier [4, 17, 28]. This is the major property required for the pulp and paper industries.

ACCEPTED MANUSCRIPT

The kinetic constants (K_m) and (V_{max}) of Xyn428 were determined using a Lineweaver-Burk plotto be 102.3 ± 1.2 mg/mL and 3225.4 ± 15 mmol/min mg, respectively, for beechwood xylan, and 60.5 ± 25 mg/mL and 1403.4 ± 55 mmol/min mg, respectively, for birchwood xylan. Comparing V_{max} values, Xyn428 prefered beechwood xylan than birchwood xylan. The K_m and V_{max} using beechwood and birchwood xylan were found 1.8 ± 0.9 mg/mL and 338 ± 66 mmol/minmg, and 18 ± 2.6 mg/mL and 1,100 ± 8 mmol/min mg, respectively [15]. For birchwood xylan as substrate, K_m and V_{max} values were 2.33 mg/mL and 322.69 µmol/min mg, respectively, for *Streptomyces megasporus* DSM 41476 [22]. For beechwood xylan from *Streptomyces rameus* L2001, K_m and V_{max} values were 5.8 mg/mL and 1491 ± 33 µmol/min mg, respectively, and for birchwood xylan, 5.3 ± 0.2 mg/mL and 1643 ± 45 µmol/min mg, respectively [4]. V_{max}value comparisons suggest that Xyn428 is much more efficient than other already reported xylanases.

3.11. Enzymatic hydrolysis and xylooligosaccharides production

Comparisons of xylooligosaccharides productions from CS428 and *Thermomyces lanuginosus* were shown in Fig.6a and 6b respectively. Xylooligosaccharides were the main oligosaccharides released initially which on further hydrolysis by Xyn428 releases xylobiose and xylose whereas xylobiose was the main oligosaccharide from *Thermomyces lanuginosus.* The mode of action of xylanase from CS428 was found to be endo type since it produced xylobiose(X_2) as the predominant end product from beechwood xylan along with higher xylooligosaccharides as intermediates. This finding suggests that Xyn428 could be a strong candidate used to produce xylooligosaccharides. Oligosaccharides were produced from 5 min of incubation, which is similar to that reported for the xylanase from *Actinomadura* sp.

ACCEPTED MANUSCRIPT

S14[14]. Xyn428 was assessed to evaluate whether any cellulase activity was present using various substrates and found that cellulase was completely absent. It is similarto a xylanase recently reported as useful in xylooligosaccharides production [29].

3.12. Xylanase for the degradation of lignocellulose biomass

Corncob and wheat bran were treated with xylanse from *Streptomyces* sp CS428, *Thermomyces lanuginosus* and endo-1,4-β-xylanase from *Trichoderma longibrachiatum* at 50ºC for 2 h. At 400× magnification significant changes were observed versus the untreated control (Fig. 7a and 7b) at 20 U/g. The amount of reducing sugars released by the xylanase from corn cob and wheat bran werere markably increased with time and the release of chromophores were also maximal. Thus, chromophoric material were released as a result of the enzyme action, suggesting that there were a significant decrease in the aromaticity of the residual lignin. Comparing to the commercial xylanase from *Thermomyces lanuginosus* and endo-1,4-β-xylanase from *Trichoderma longibrachiatum*, Xyn428 causes significant decrease in aromicity in corn cob and wheat barn. Xyn428 appeared to be effective at 20U/g, as has been reported for the xylan from *Streptomyces rameus* L2001 [4]. In addition, an ideal xylanase to be useful in biobleaching must be active and stable in high temperatures and alkaline conditions and it must also have lack of cellulase activity [29, 30]. Furthermore, low molecular weight xylanases like Xyn428 more easily penetrate into the re-precipitated xylan on the surfaces of pulp particles [5]. This mitigates the problem of a xylan barrier on the surface of lignin containing pulp during subsequent chemical bleaching steps [31]. Although many microorganisms have been known to produce xylanase, CS428 produces cellulase free xylanase which is stable over wide range of pH and temperatures. Moreover, the xylanase

ACCEPTED MANUSCRIPT

yield from CS428 was higher than the xylanase reported. Thus, Xyn428 favors its potential application in degradation of agro waste biomass for bio bleaching in paper and pulp industries.

4. Conclusions

A thermostable *Streptomyces* sp. CS428 was found to produce an endo-1, 4-β-xylanase (Xyn428), which was purified by one-step chromatography with a yield of 55%. Activity and stability of xylanase from CS428 was compared with the commercially available xylanase from *Thermomyces lanuginosus* and endo-1,4-β-xylanase from *Trichoderma longibrachiatum.* Xyn428 was found to have temperature and pH optima at 80 ºC and 7.0, respectively, and have broad range pH stability (3 to ~13.6) (in fact, it is the most alkaline tolerant xylanase ever reported) and thermostability (50 ºC for 1 h). Its N-terminal amino acid sequence was found to be INRTDHNENSYLEIHNNEAR. Xyn428 activity was inhibited by Fe^{2+}, xylene, EDTA, EGTA, hydrogen perborate, sodium perborate etc. Xyn428 produced xylose and xylobiose as main products, which identifies it as an endoxylanase. In addition, Xyn428 was found by electron microscopy to potently degrade agro waste materials. The thermal and broad range pH stability Xyn428 indicates potential applications in the biofuel, food, and textile industries and for waste treatment.

5. Acknowledgement:

This work was supported by research fund from Chosun University, 2012

ACCEPTED MANUSCRIPT

References:

[1] Saha B. Hemicellulose bioconversion. J Ind Microb Biotechnol 2003;30:279-291.

[2] Wong KK, Tan LU, Saddler JN. Multiplicity of beta-1,4-xylanase in microorganisms: functions and applications. Microbiol Rev 1988;52:305-317.

[3] Mamma D, Kourtoglou E, Christakopoulos P. Fungal multienzyme production on industrial by-products of the citrus-processing industry. Bioresour Technol 2008;99:2373-2383.

[4] Li X, She Y, Sun B, Song H, Zhu Y, Lv Y, Song H. Purification and characterization of a cellulase-free, thermostable xylanase from *Streptomyces rameus* L2001 and its biobleaching effect on wheat straw pulp. Biochem Eng J 2010;52:71-78.

[5] Ninawe S, Kapoor M, Kuhad RC. Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32. Bioresour Technol 2008;99:1252-1258.

[6] Bernier R, Desrochers M, Jurasek L, Paice MG. Isolation and Characterization of a Xylanase from *Bacillus subtilis*. Appl Environ Microbiol 1983;46:511-514.

[7] Collins T, Gerday C, Feller G. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 2005;29:3-23.

[8] Morosoli R, Bertrand JL, Mondou F, Shareck F, Kluepfel D. Purification and properties of a xylanase from *Streptomyces lividans*. Biochem J 1986;239:587-592.

[9] Bajaj B, Singh N. Production of Xylanase From an Alkalitolerant *Streptomyces sp*. 7b Under Solid-State Fermentation, Its Purification, and Characterization. App Biochem Biotechnol 2010;162:1804-1818.

[10] Shin JH, Choi JH, Lee OS, Kim YM, Lee DS, Kwak YY, Kim WC, Rhee IK. Thermostable xylanase from *Streptomyces thermocyaneoviolaceus* for optimal production of xylooligosaccharides. Biotechnol Bioprocess Eng 2009;14:391-399.

[11] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.

[12] Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal Chem 1959;31:426-428.

[13] Laemmli UK. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature 1970;227:680-685.

[14] Sriyapai T, Somyoonsap P, Matsui K, Kawai F, Chansiri K. Cloning of a thermostable xylanase from *Actinomadura sp*. S14 and its expression in Escherichia coli and Pichia pastoris. J Biosci Bioeng 2011;111:528-536.

[15] Simkhada JR, Yoo HY, Choi YH, Kim SW, Yoo JC. An extremely alkaline novel xylanase from a newly isolated *Streptomyces* strain cultivated in corncob medium. Appl Biochem Biotechnol 2012;168:2017-2027.

[16] Deesukon W, Nishimura Y, Harada N, Sakamoto T, Sukhumsirichart W. Purification, characterization and gene cloning of two forms of a thermostable endo-xylanase from *Streptomyces sp*. SWU10. Process Biochem 2011;46:2255-2262.

[17] Elegir G, Szakacs G, Jeffries TW. Purification, Characterization, and Substrate Specificities of Multiple Xylanases from *Streptomyces sp*. Strain B-12-2. Appl Environ Microbiol 1994;60:2609-2615.

[18] Kim DY, Han MK, Lee JS, Oh HW, Park DS, Shin DH, Bae KS, Son KH, Park HY. Isolation and characterization of a cellulase-free endo-β-1,4-xylanase produced by an

ACCEPTED MANUSCRIPT

invertebrate-symbiotic bacterium, *Cellulosimicrobium sp*. HY-13. Process Biochem 2009;44:1055-1059.

[19] Yan Q, Hao S, Jiang Z, Zhai Q, Chen W. Properties of a xylanase from *Streptomyces matensis* being suitable for xylooligosaccharides production. J Mol Catal B: Enzyma 2009;58:72-77.

[20] Sanghi A, Garg N, Gupta VK, Mittal A, Kuhad RC. One-step purification and characterization of cellulase-free xylanase produced by alkalophilic *Bacillus subtilis* ASH. Braz J Microbiol 2010;41:467-476.

[21] Zhou J, Shi P, Zhang R, Huang H, Meng K, Yang P, Yao B. Symbiotic *Streptomyces sp*. TN119 GH 11 xylanase: a new pH-stable, protease- and SDS-resistant xylanase. J Ind Microbiol Biotechnol 2011;38:523-530.

[22] Qiu Z, Shi P, Luo H, Bai Y, Yuan T, Yang P, Liu S, Yao B. A xylanase with broad pH and temperature adaptability from *Streptomyces megasporus* DSM 41476, and its potential application in brewing industry. Enzyme Microb Technol 2010;46:506-512.

[23] Ward OP, Moo-Young M. Thermostable enzymes. Biotechnol Adv 1988;6:39-69.

[24] Vieille C, Zeikus GJ. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. Microbiol Mol Biol Rev 2001;65:1-43.

[25] Mohana S, Shah A, Divecha J, Madamwar D. Xylanase production by *Burkholderia sp.* DMAX strain under solid state fermentation using distillery spent wash. Bioresour Technol 2008;99:7553-7564.

[26] Klibanov AM. Improving enzymes by using them in organic solvents. Nature 2001;409:241-246.

[27] Wang SL, Yen YH, Shih IL, Chang AC, Chang WT, Wu WC, Chai YD. Production of xylanases from rice bran by *Streptomyces actuosus* A-151. Enzyme Microb Technol 2003;33:917-925.

[28] Ruiz-Arribas A, Fernandez-Abalos JM, Sanchez P, Garda AL, Santamaria RI. Overproduction, purification, and biochemical characterization of a xylanase (Xys1) from *Streptomyces halstedii* JM8. Appl Environ Microbiol 1995;61:2414-2419.

[29] Verma D, Satyanarayana T. Cloning, expression and applicability of thermo-alkali-stable xylanase of *Geobacillus thermoleovorans* in generating xylooligosaccharides from agro-residues. Bioresour Technol 2012;107:333-338.

[30] Techapun C, Poosaran N, Watanabe M, Sasaki K. Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review. Process Biochem 2003;38:1327-1340.

[31] Kuhad RC, Singh A, Eriksson KE. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 1997;57:45-125.

ACCEPTED MANUSCRIPT

Figure legends:

Fig. 1: 12 % (w/v) SDS-PAGE (a) and Zymograph (b) of purified xylanase from CS428. Lanes Mr, protein molecular weight marker (Fermentas); lane C, Crude extract; lane A.Sul, 30-75 % ammonium sulfate precipitation fraction; lane P, purified xylanase after CM Trisacryl cation exchange chromatography.

Fig. 2: (a) Optimal pH, (b) pH stability ofxylanase from CS428 (◆),*Thermomyces lanuginosa* (●) and,*Trichoderma longibrachiatum* (▲). To determine pH optimum, the activity of xylanases were determined at 80 ºC using different pH buffers. To examine xylanase stability, xylanases were incubated with different pH buffers (2-13.6) at 4 ºC for 24hr and relative xylanase activity were evaluated under standard assay conditions. Each point represents as mean (n=3).

Fig. 3: (a) Optimal temperature, (b) thermal stability of xylanase from CS428 (◆), *Thermomyces lanuginosa* (●) and,*Trichoderma longibrachiatum* (▲). To determine optimum temperature, the reaction was performed at different temperatures at optimum pH (7.0), whereas to determine xylanase stability purified Xyn428 was stored at different temperatures for one hour. Activitieswere assayed under standard assay conditions. Each point represents as mean (n=3).

Fig. 4: The comparative study of xylanase production using agro-waste materials. Xylanase from commercial xylan (■), corn con in distill water (●), corn cob with supplements (▲), wheat barn in distill water (▼), wheat barn with supplements (◆). All the activity is in U/mL. Each point represents as mean (n=3).

Accepted Manuscript

ACCEPTED MANUSCRIPT

555 **Fig. 5:** Effect of Tris salt on the activity of purified Xyn428 at 80 ºC. The reaction was
556 performed at different salt concentrations. Activities were assayed under a standard assay
557 conditions. Each point represent as mean (n=3).

558 **Fig. 6:**Plot showing xylan degradation after treating beechwoodxylan at 50 ºC and pH 7 with
559 xylanase from CS428 (a), *Thermomyces lanuginosus* (b). M: mixture of Xylose (X1),
560 Xylobiose (X2), Xylotriose (X3) and Xylotetrose (X4).

561 **Fig. 7:** Scanning electron micrographs (× 400) of corncob (a) and wheat bran (b) after
562 treatment with enzyme at concentration20 U/g at 50 ºC for 2 h at pH 7. Control (A), xylanase
563 from CS428 (B), *Thermomyces lanuginosa* (C) and, *Trichodermal ongibrachiatum*(D).

564

565

566

567

568

569

570

571

572

573

Accepted Manuscript

ACCEPTED MANUSCRIPT

574

575

576 **Table 1**

577 Purification summary

Purification Step	Total protein (mg)	Total Activity(U)	Specific Activity (U/mg)	Yield (%)	Purity Fold
Culture Broth	4.7	168196.7	35786.5	100	1
$(NH_4)_2SO_4$ Fractionation	1.48	150448.9	101654.7	89.4	2.8
CM Trisacryl	0.1	92610.3	926103.0	55.1	25.9

578

579

580

581

582

583

584

585

586

587

ACCEPTED MANUSCRIPT

588 **Table 2**

589 Comparison ofXyn428 with other xylanases from *Streptomyces*

Stain Name	Optimum			Stability		Reference
	Mol. Wt	pH	Temp (ºC)	pH	Temp (ºC)	
S. rameus L2001	21.1	5.3	70	2.2-11.3	3-70	[4]
S. cyaneus SN32	20.5	6	60-65	4-9.5	4-60	[5]
S. lividans	43	6	60	-	0-37	[8]
S. sp. 7b	30	6	50	6-9	3-50	[9]
S. thermocyaneociolaceus	35	5	60	4.5-10.5	3-60	[10]
*S.*sp CS802	45	12	60	7.5-13	3-37	[15]
*S.*sp. SWU10 (XynSW2A)	31	6	60	3-9	3-80	[16]
*S.*sp. SWU10 (XynSW2B)	44	6	60	2-9	3-60	[16]
S. matensis DW67	21.2	7	65	4.5-8.0	3-55	[19]
S. spTN119	35.9	7	50	2-11	3-37	[21]
S. megasporus DSM 41476	47.6	5.5	70	4-11	3-60	[22]
S. actuosus A-151	20-45	4-6	60-70	3-9	3-60	[27]
*S. halstedii*JM8 (Xys1L & Xys1S)	45 & 35	6.3	60	4-10	4-50	[28]
S. sp. CS428	37	7	80	3-13.6	0-50	Current study

590

591

592

593

594

Accepted Manuscript

ACCEPTED MANUSCRIPT

595 **Table 3**

596 Effects of Detergents, Modulators, and metal ions on the activities of xylanases from CS428

597 (a), *Thermomyces lanuginosus (b)*, and endo-1,4-β-xylanase from *Trichoderma*

598 *longibrachiatum (c).*

Reagents	Concentration	Type of ion	(a) Relative Activity*(%)	(b) Relative Activity*(%)	(c) Relative Activity*(%)
Triton X - 100	0.25%	Non - ionic	124±4.1	79 ± 3	179 ± 0.9
Tween - 20	0.25%	Non - ionic	111 ± 4.3	83 ± 2.7	185 ± 1.1
Tween - 80	0.25%	Non - ionic	114 ± 4.5	92 ± 2.2	180 ± 3.5
Polyoxylethylene-4-laurylether	0.25%	Non - ionic	108 ± 2.5	104 ± 0.1	111 ± 3.5
Deooxycholic Acid	0.25%	Anionic	74± 3	62 ± 1.9	202 ± 2
Sodium Dodecyl Sulfate	0.25%	Anionic	36 ± 6	45 ± 5.3	76 ± 2.8
CHAPS	0.25%	Zwitterionic	95 ± 3.2	80 ± 3.7	49 ± 3.5
Hydrogen peroxide	5 mM		0	8 ± 2.3	14 ± 5
Sodium perborate	5 mM		0	92 ± 4	55 ± 4
β- mercaptoethanol	5 mM		84 ± 3	87 ± 3	97 ± 2
EDTA	1 mM		0	7.4 ± 5	11 ± 1.5
EGTA	1 mM		0	7.5 ± 6	5 ± 3.4
Ca^{2+}	1 mM		133 ± 0.7	111 ± 0.3	154 ± 1
Mg^{2+}	1 mM		55 ± 2.3	34 ± 3.8	53 ± 5
Cu^{2+}	1 mM		61 ± 2.5	2.4 ± 5	63 ± 3.2
Co^{2+}	1 mM		123 ± 2	105 ± 5	235 ± 1.3
Zn^{2+}	1 mM		53 ± 2	32 ± 6	49 ± 2.7
K^{+}	1 mM		103 ± 2	100 ± 6	138 ± 0.4
Na^{+}	1 mM		104 ± 1	93 ± 2	119 ± 1.6
Mn^{2+}	1 mM		93 ± 2	32 ± 6	101 ± 2

Accepted Manuscript

ACCEPTED MANUSCRIPT

Fe^{2+}	1 mM	38 ± 2.4	32 ± 2	103 ± 1.7
None	-	100 ± 4.7	100 ± 2.5	100 ± 0.2

*The results presented are the averages of three separate determinations (n=3) ±standard deviations.

Accepted Manuscript

ACCEPTED MANUSCRIPT

621 **Table 4**

622 Effect of alcohol concentration on the activity of the purified xylanase from CS428

Final %	Alcohol	Activity (%)*
0.06	Methanol	116 ± 1
0.12		122 ± 0.9
0.25		143 ± 3
0.5		140 ± 2
1		121 ± 3
2		97 ± 5
0.06	Ethanol	112 ± 1
0.12		123 ± 2
0.25		149 ± 2
0.5		139 ± 3
1		110 ± 2
2		109 ± 1
0.06	Iso propanol	120 ± 4
0.12		124 ± 0.95
0.25		145 ± 1
0.5		147 ± 4
1		111 ± 1
2		110 ± 2
0.06	Butanol	112 ± 1
0.12		116 ± 2
0.25		150 ± 2
0.5		143 ± 1

ACCEPTED MANUSCRIPT

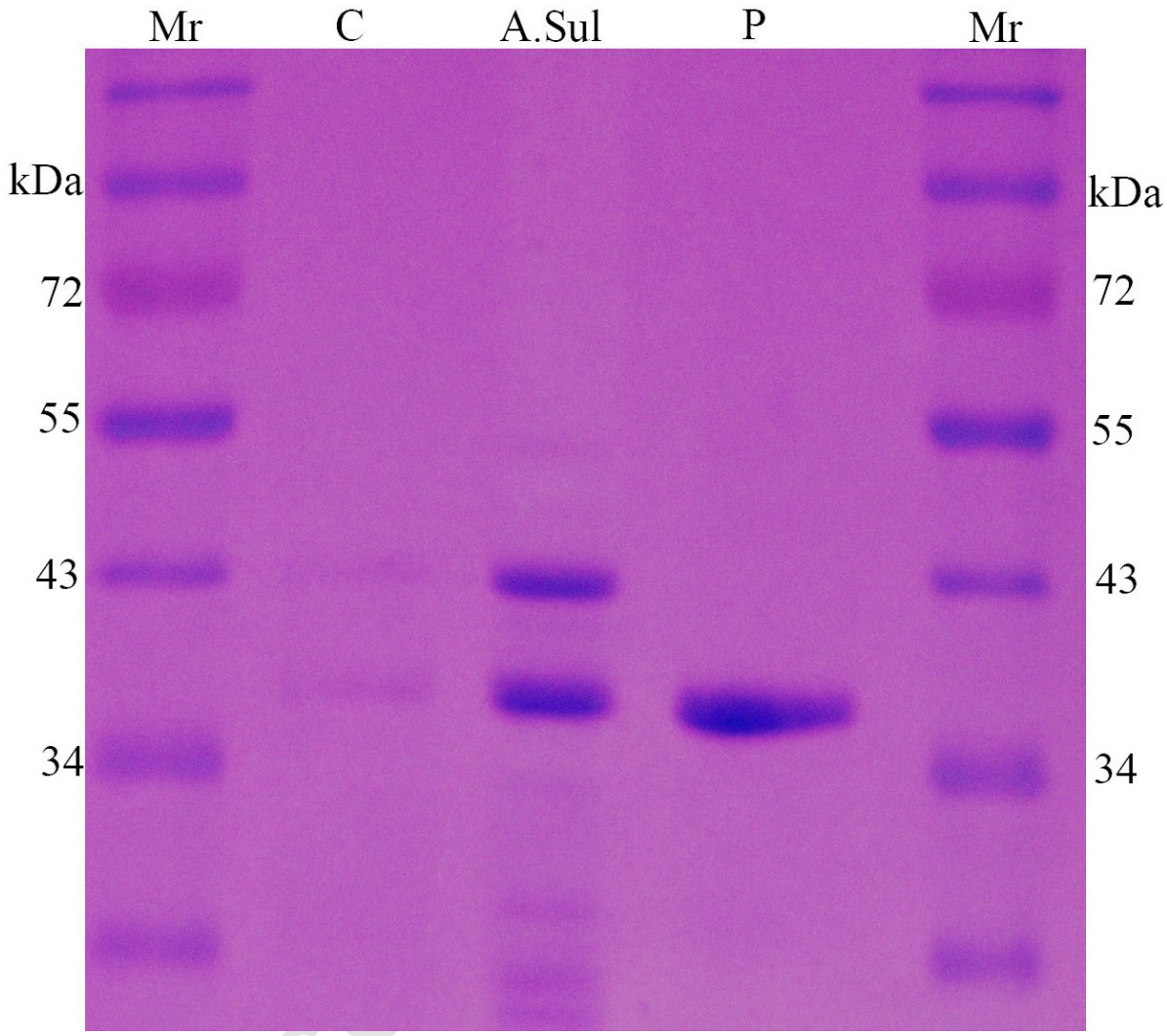
1		69 ± 6
2		53 ± 1
-	None	100 ± 3

* The results presented are the averages of three separate determinations (n=3) ± standard deviations.

Accepted Manuscript

ACCEPTED MANUSCRIPT

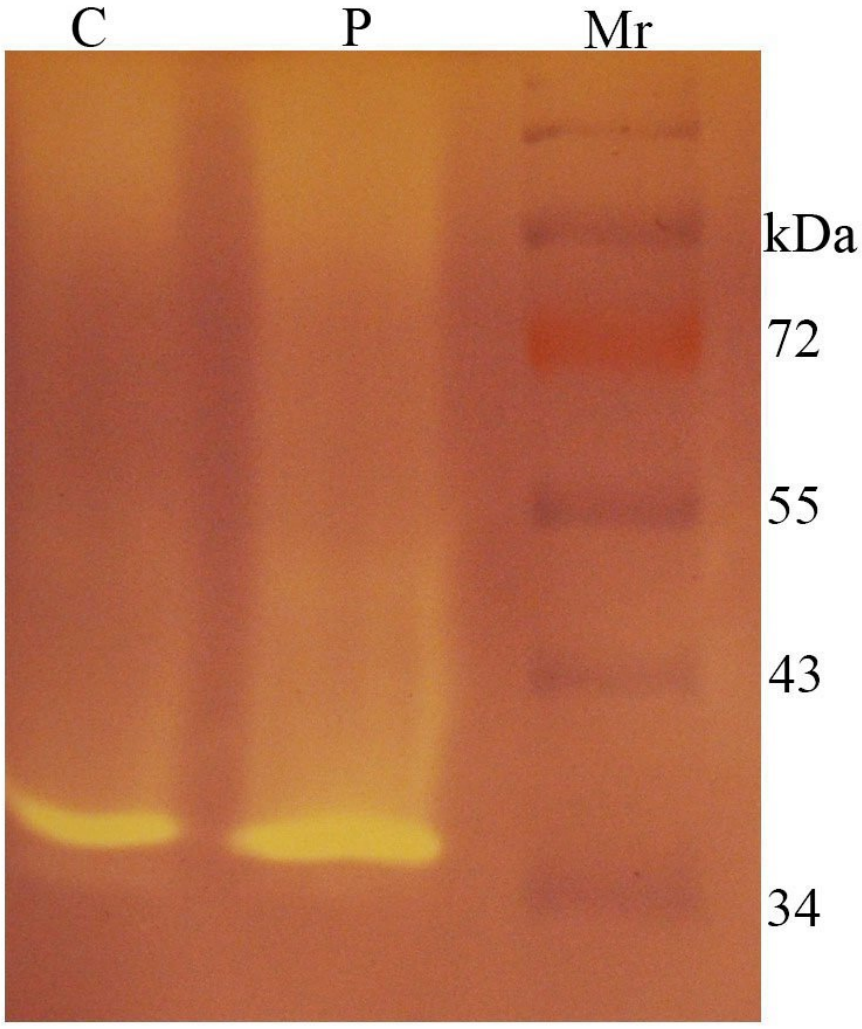
639 **Fig.1**



640

641 (a)

ACCEPTED MANUSCRIPT



642

643 (b)

644

645

646

647

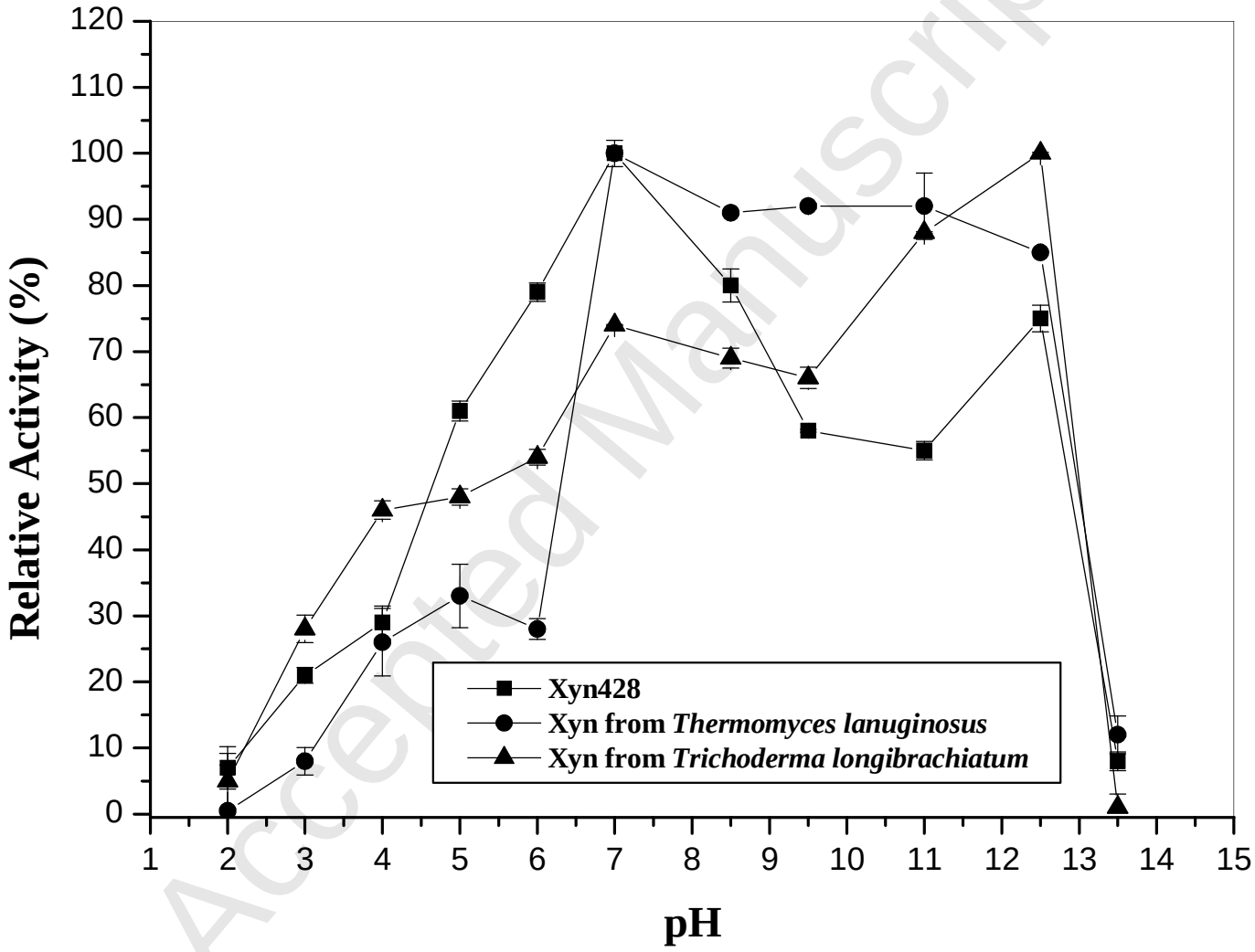
648

ACCEPTED MANUSCRIPT

649

650

651 **Fig.2**



652

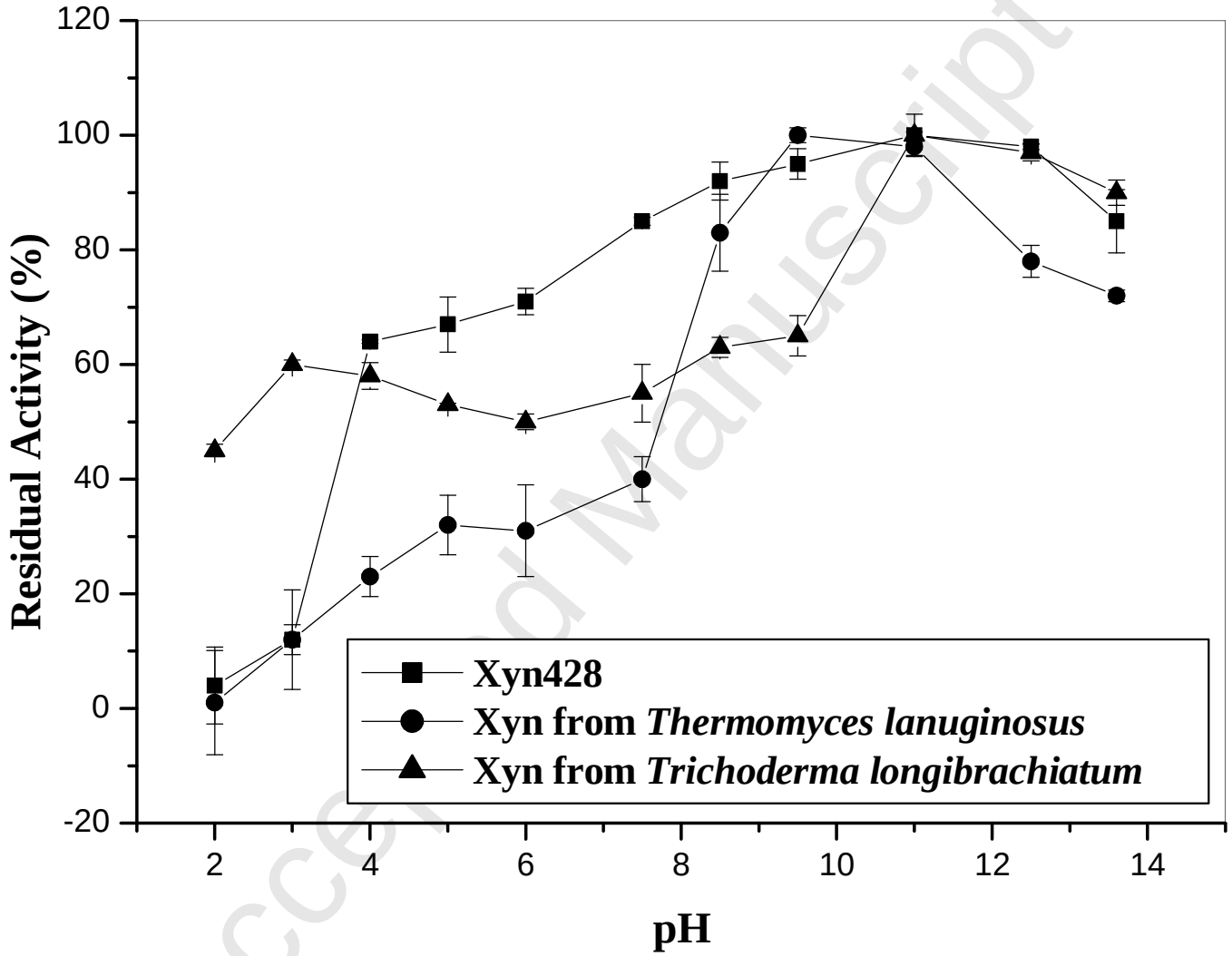
653 (a)

654

655

Accepted Manuscript

ACCEPTED MANUSCRIPT



(b)

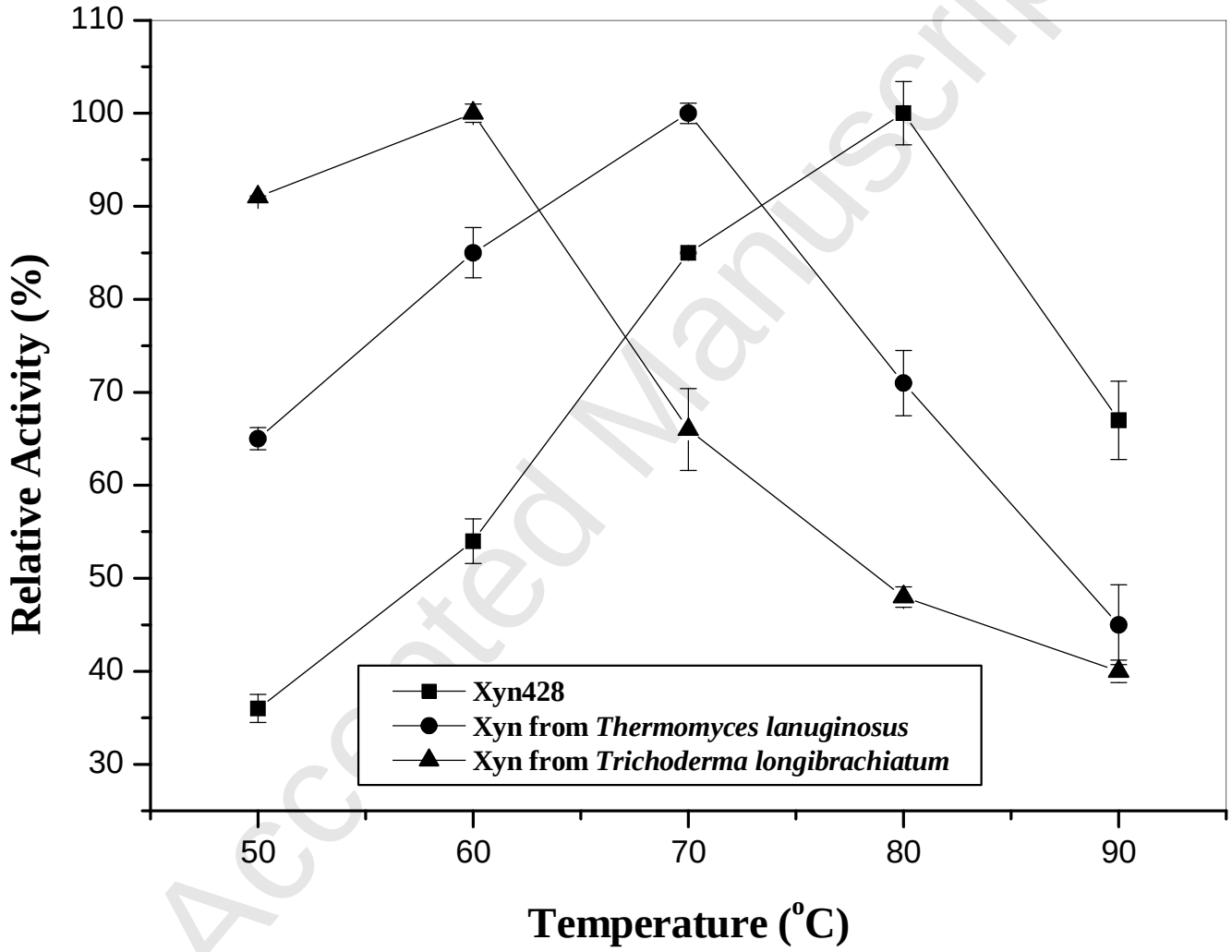
Accepted Manuscript

ACCEPTED MANUSCRIPT

663

664 **Fig.3**

665



666

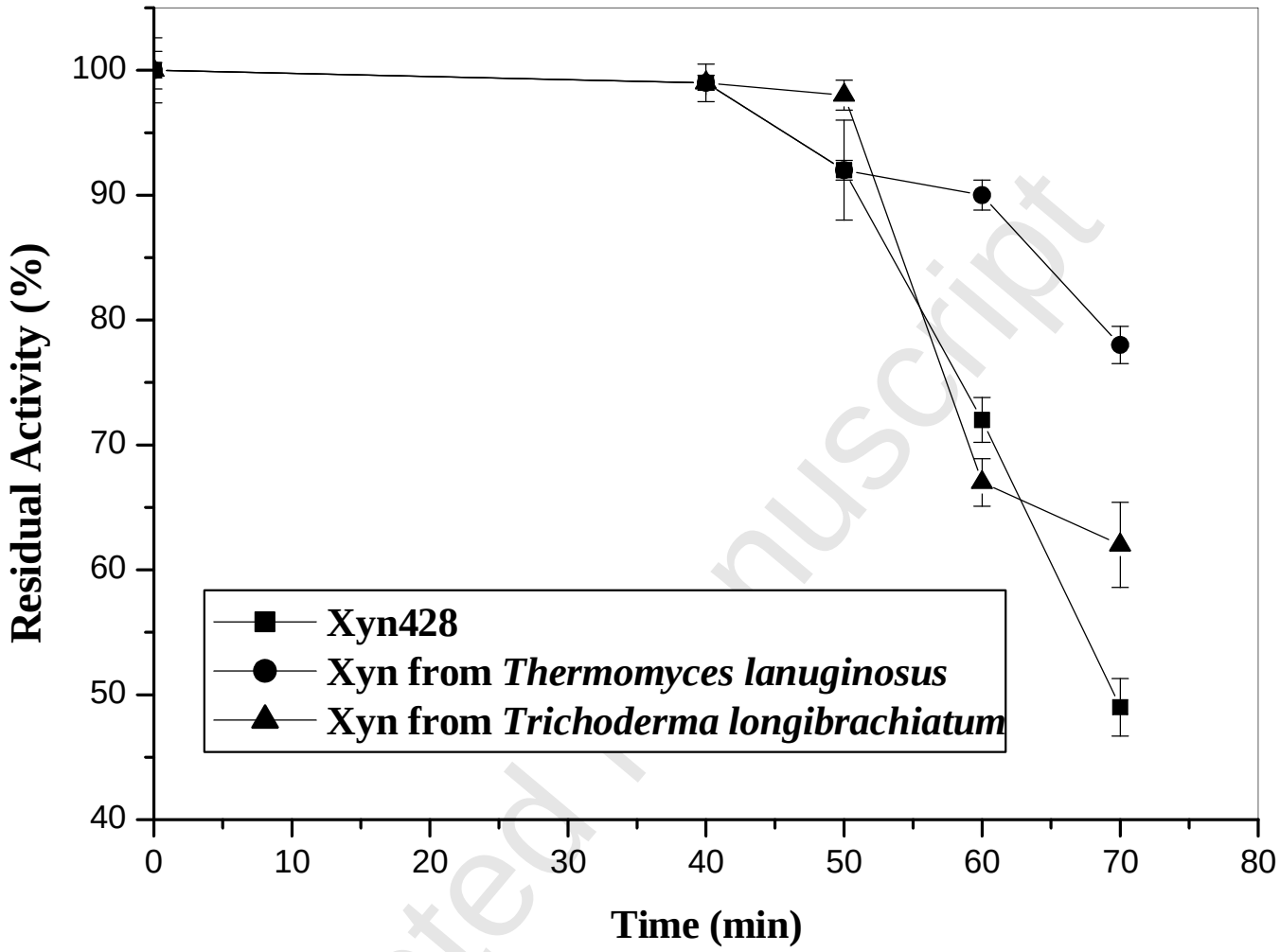
667 (a)

668

669

Accepted Manuscript

ACCEPTED MANUSCRIPT



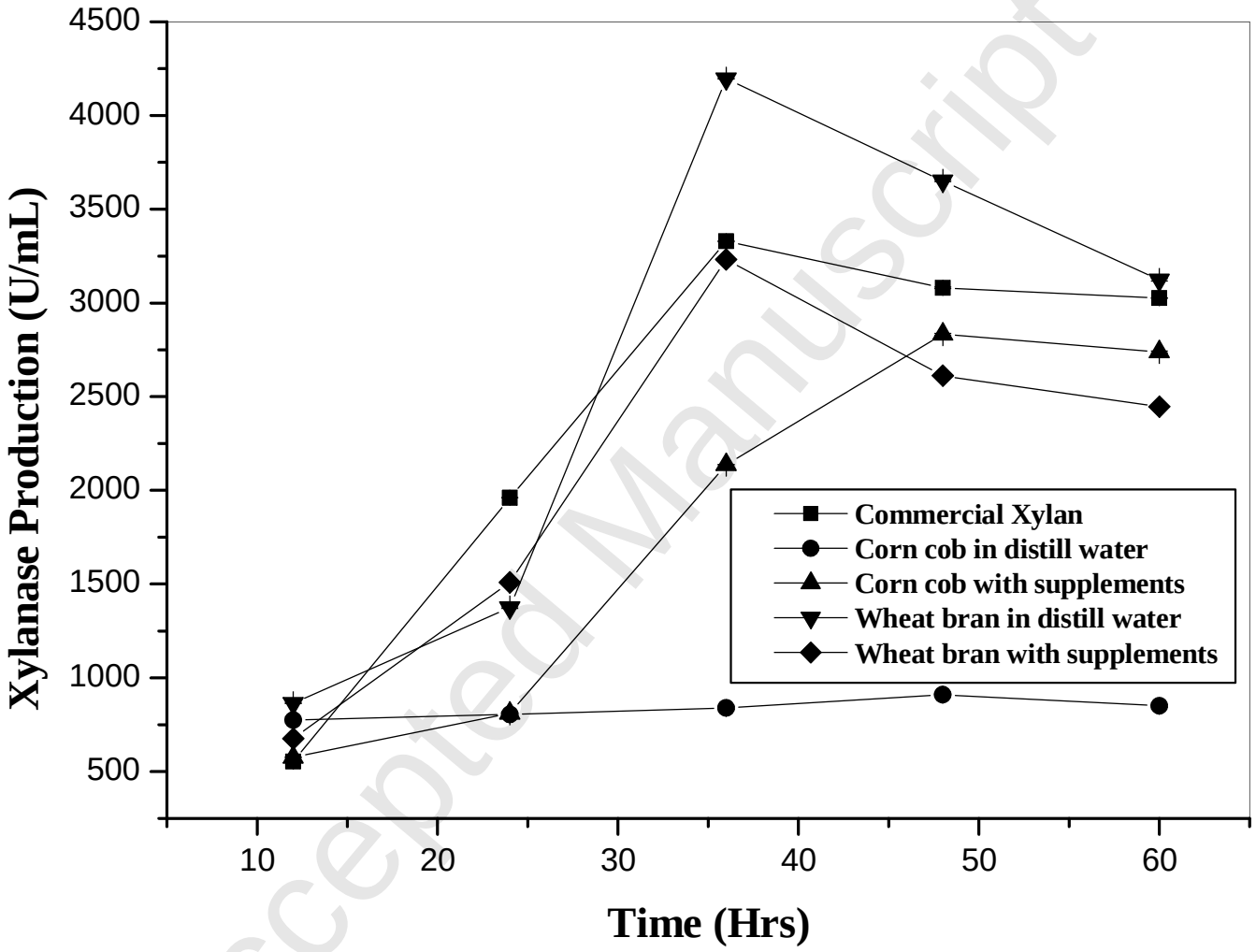
(b)

Accepted Manuscript

ACCEPTED MANUSCRIPT

678 **Fig.4**

679



680

681

682

683

684

685

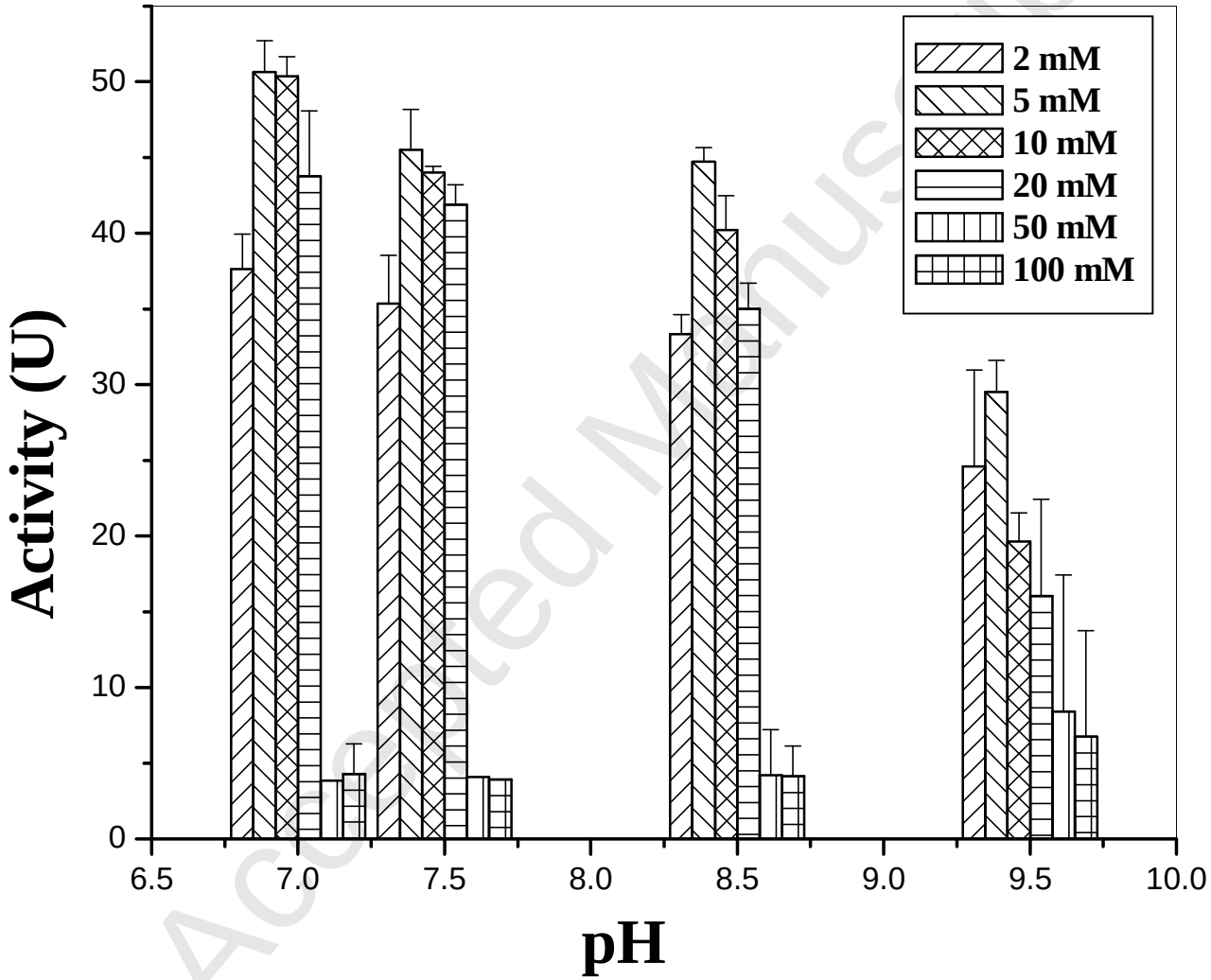
Accepted Manuscript

ACCEPTED MANUSCRIPT

686 **Fig.5**

687

688



689

690

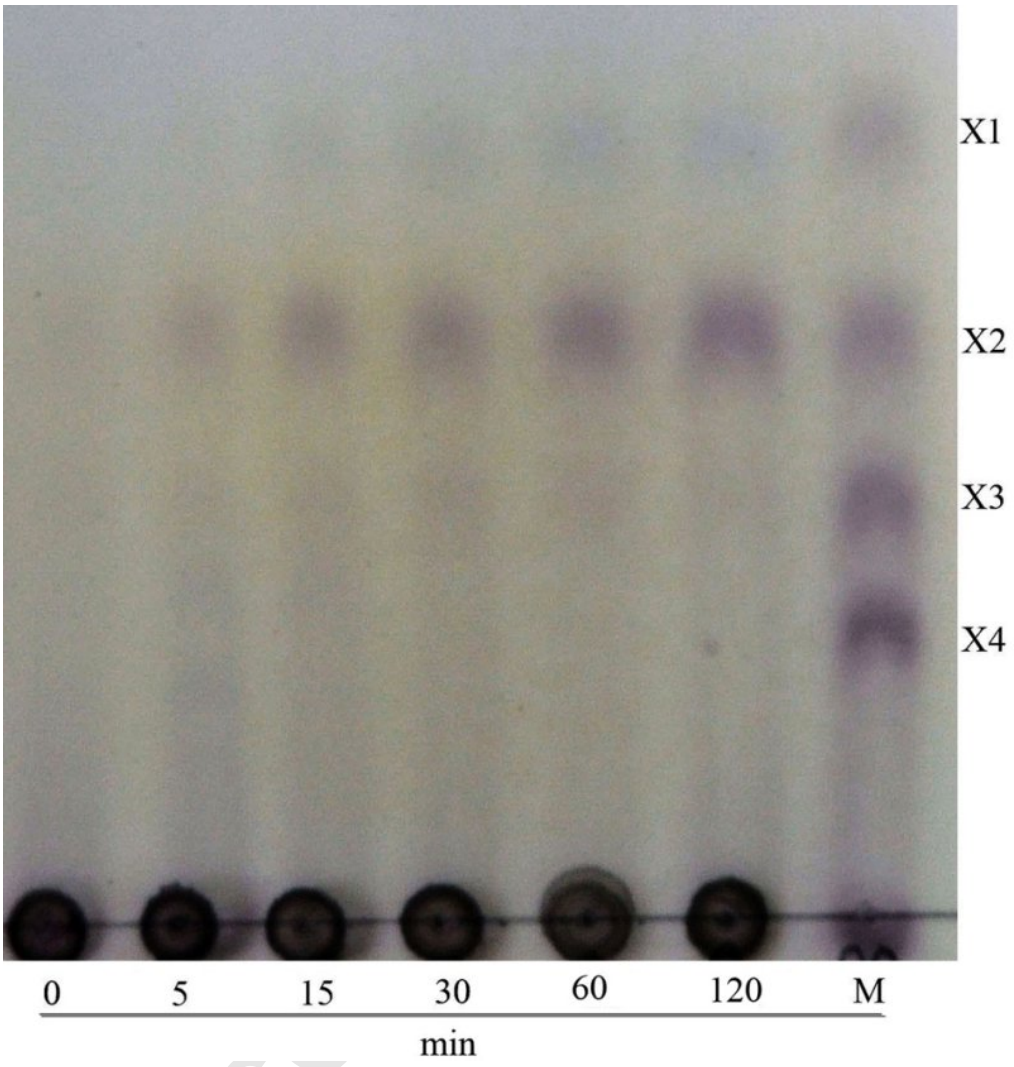
691

692

Accepted Manuscript

ACCEPTED MANUSCRIPT

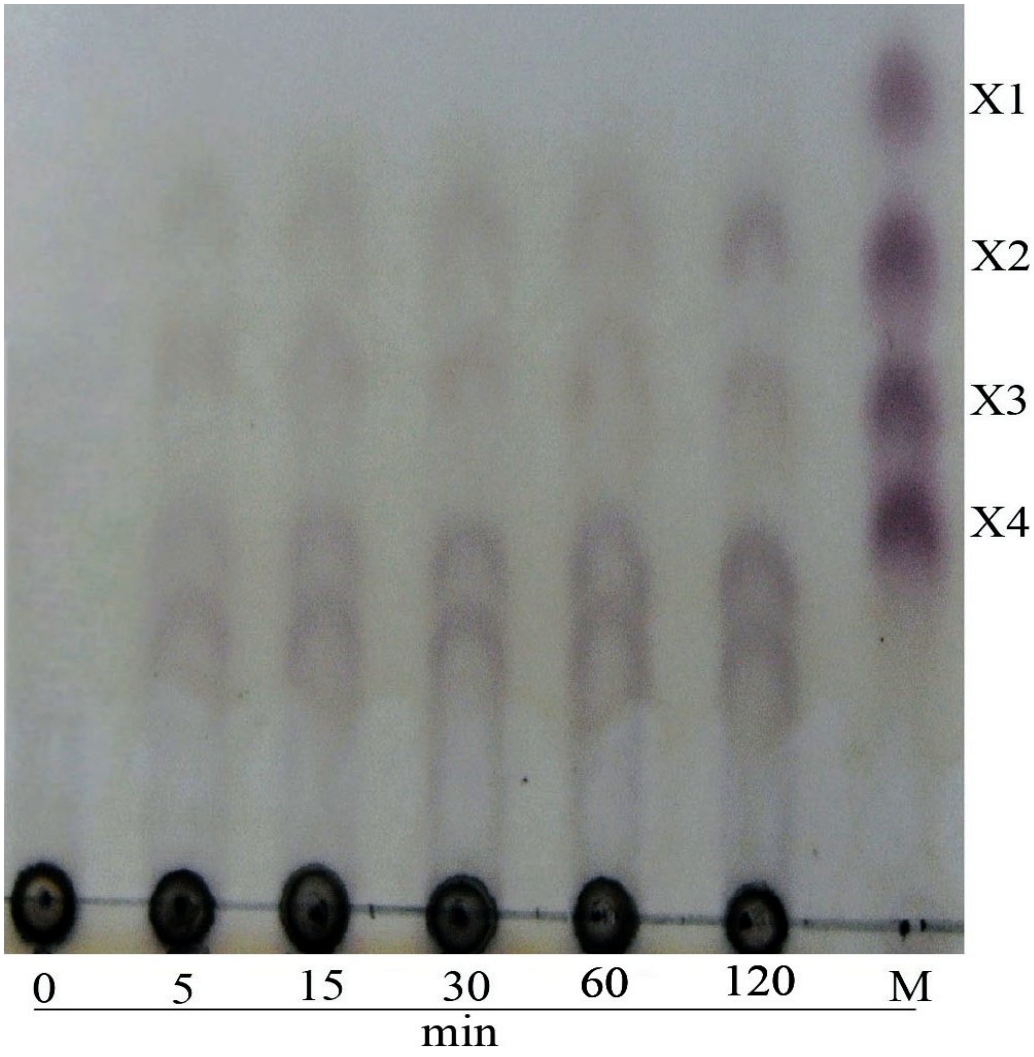
693 **Fig.6**



694

695 (a)

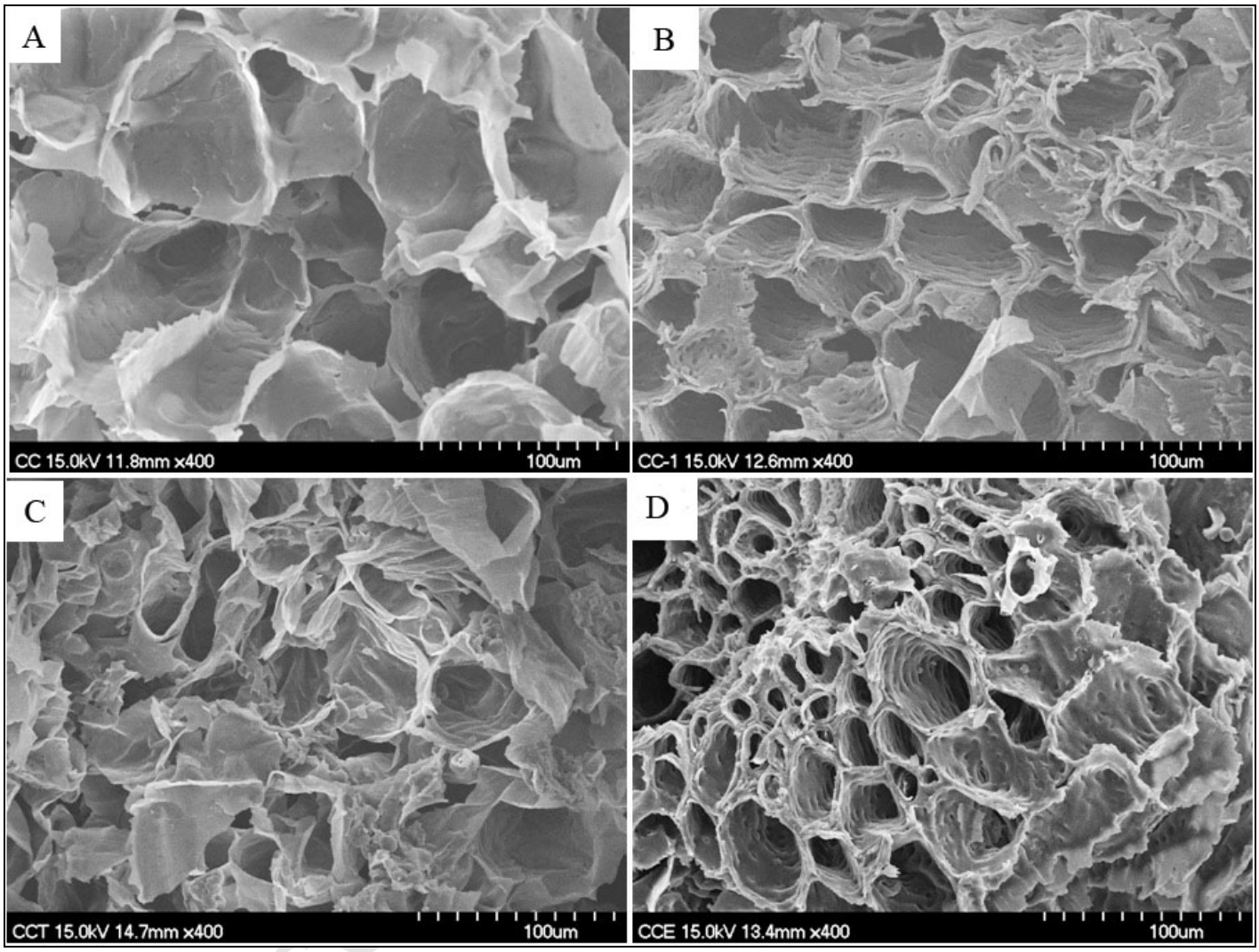
ACCEPTED MANUSCRIPT



(b)

ACCEPTED MANUSCRIPT

703 **Fig.7**



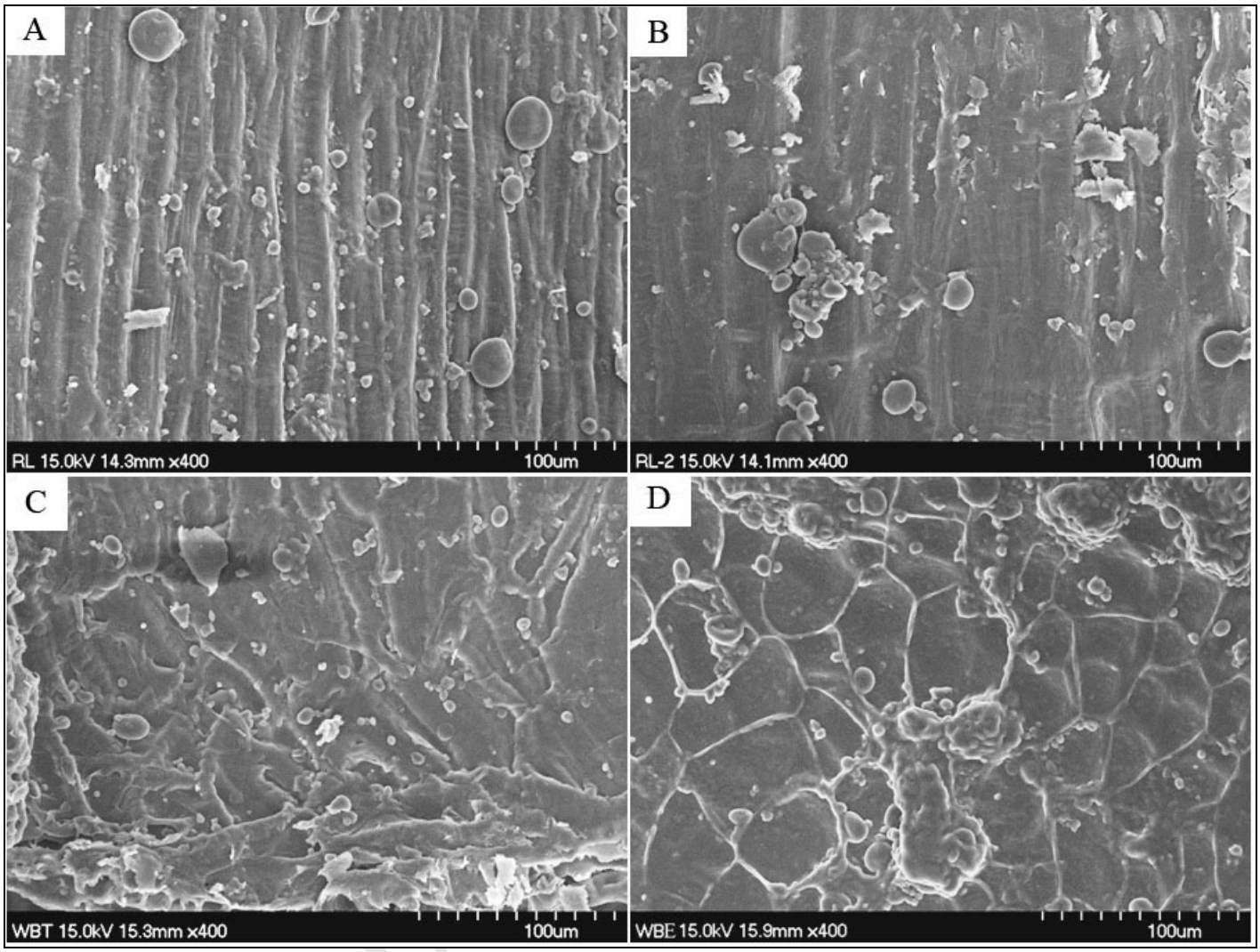
704

705 (a)

706

Acc

ACCEPTED MANUSCRIPT



(b)

Accep

ACCEPTED MANUSCRIPT

709 Highlights

710 An endo β-1, 4 xylanase was isolated, purified with a single-step chromatography.

711 Xyn428 is cellulase free, thermostable, alkaline and stable in broad range of pH.

712 Xyn428 produces xylose and xylobiose as major oligosaccharides.

713 Xyn428 is produced by utilizing industrial biomass like wheat bran, corn cob etc.

714 Agro waste biomass is degraded significantly

715

Accepted Manuscript