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Characterization of Cellulolytic and Xylanolytic Enzymes of *Bacillus licheniformis* JK7 Isolated from the Rumen of a Native Korean Goat

J. K. Seo, T. S. Park, I. H. Kwon[1], M. Y. Piao, C. H. Lee[2] and Jong K. Ha*
Department of Agriculture Biotechnology, Research Institute for Agriculture and Life Sciences,
College of Agriculture and Life Science, Seoul National University, Seoul 151-742, Korea

ABSTRACT: A facultative bacterium producing cellulolytic and hemicellulolytic enzymes was isolated from the rumen of a native Korean goat. The bacterium was identified as a *Bacillus licheniformis* on the basis of biochemical and morphological characteristics and 16S rDNA sequences, and has been designated *Bacillus licheniformis* JK7. Endoglucanase activities were higher than those of β-glucosidase and xylanase at all temperatures. Xylanase had the lowest activity among the three enzymes examined. The optimum temperature for the enzymes of *Bacillus licheniformis* JK7 was 70°C for endoglucanase (0.75 U/ml) and 50°C for β-glucosidase and xylanase (0.63 U/ml, 0.44 U/ml, respectively). All three enzymes were stable at a temperature range of 20 to 50°C. At 50°C, endoglucanse, β-glucosidase, and xylanase had 90.29, 94.80, and 88.69% residual activity, respectively. The optimal pH for the three enzymes was 5.0, at which their activity was 1.46, 1.10, and 1.08 U/ml, respectively. The activity of all three enzymes was stable in the pH range of 3.0 to 6.0. Endoglucanase activity was increased 113% by K^+, while K^+, Zn^+, and tween 20 enhanced β-glucosidase activity. Xylanase showed considerable activity even in presence of selected chemical additives, with the exception of Mn^{2+} and Cu^{2+}. The broad range of optimum temperatures (20 to 40°C) and the stability under acidic pH (4 to 6) suggest that the cellulolytic enzymes of *Bacillus licheniformis* JK7 may be good candidates for use in the biofuel industry. (**Key Words:** *Bacillus licheniformis*, Endoglucanase, β-Glucosidase, Xylanase, Goat)

INTRODUCTION

Lignocellulosic materials are the most abundant resource for the production of renewable bioenergy and fermented products. Cellulosic materials need to be first hydrolyzed into fermentable sugars since they are not useful in their polysaccharide form (Li et al., 2009). The biohydrolysis of cellulose through the use of cellulolytic microorganisms is an attractive approach since the degradation of cellulose by chemical agents produces environmental pollution (Rizzatti et al., 2001). Cellulase, which is produced by fungi and bacteria, can be divided into three major types: endoglucanase (endo-1,4-β-D-glucanase, EC 3.2.1.4), cellobiohydrolase (exo-1,4-β-D-glucanase, EC 3.2.1.91), and β-glucosidase (1,4-β-D-glucosidase EC 3.2.1.21) (Hong et al., 2001). Endoglucanases randomly hydrolyze the internal β-1,4-glysidic bonds of cellulose chains so that new chain ends are produced. In contrast, cellobiohydrolases cleave cellulose chains at the ends to produce cellobiose or glucose. β-glucosidase only hydrolyzes cellobiose, and releases glucose units (Percival Zhang et al., 2006; Kumar et al., 2008).

Fungal species have been primarily used commercially for cellulase production because of their capacity to secrete cellulolytic enzymes into their medium, which allows for easy purification and extraction (Maki et al., 2009). Among the cellulolytic fungi, *Trichoderma* spp. and *Aspergillus* spp. have been extensively investigated since they can produce all three types of cellulose-degrading enzymes (Wang et al., 2008). However, bacterial cellulases have several advantages. First, bacteria have higher growth rates than fungi and can easily grow to high cell densities in inexpensive nutrient sources (Maki et al., 2009). Second,

* Corresponding Author: Jong K. Ha. Tel: +82-2-880-4809, Fax: +82-2-875-8710, E-mail: jongha@snu.ac.kr
[1] Department of Animal Science, University of Illinois, Champaign-Urbana, IL 61801, USA.
[2] Genebiotech Co. Ltd., Gongju, Korea.
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the enzyme expression system of bacteria is more convenient. Third, bacteria can not only survive harsh conditions but can also excrete enzymes that are stable under extreme conditions of high temperature and low or high pH.

Several bacterial genera show cellulolytic activity, including *Bacillus*, *Clostridium*, *Cellulomonas*, *Rumminococcus*, *Alteromonas*, *Acetivibrio*, and *Bacteriodes* (Roboson and Chambliss, 1989). Among these, *Bacillus* species produce a variety of extracellular cellulolytic enzymes. *Bacillus licheniformis* is a facultative and a Gram-positive endospore-forming bacterium (Sneath et al., 1986) which is used extensively in large-scale commercial enzyme production since it can excrete proteins in large quantities of up to 20 to 25 g/L (Schallmey et al., 2004).

Many cellulolytic or xylanolytic *Bacillus* species have been isolated from compost (Archana and Satyanarayana, 1997; Rastogi et al., 2010), milled paper (Geetha and Gunasekaran, 2010), swine waste (Liang et al., 2009), and hot springs (Mawadza et al., 2000). However, the isolation of cellulolytic and xylanolytic *Bacillus* sp. from the rumen of goats has not previously been reported as the rumen environment is a strictly anaerobic environment, which can make it difficult for aerobic bacteria to survive. In this study, we isolated the facultative anaerobic bacteria *Bacillus licheniformis* JK7, which can secrete endoglucanase, β-glucosidase, and xylanase, in the rumen of a native Korean goat which can survive on harsh condition such as provision of low quality roughage as a sole feed source (Son, 1999). The objectives of this study were i) to isolate and identify the microorganism responsible for degrading cellulose and xylan, and ii) to characterize the endoglucanase, β-glucosidase, and xylanase released by selected *Bacillus* sp.

MATERIALS AND METHODS

Materials

All chemicals, media components and reagents used in these experiments were purchased from Sigma (Sigma and Aldrich, St. Louis, USA) and Difco laboratories (Sparks, USA). Azo-CM-Cellulose (Megazyme co. Ltd., Ireland) was used as a substrate to screen cellulolytic bacteria.

Isolation and screening of cellulose-degrading bacteria

The ruminal fluid of goats was collected before their morning feeding from rumen fistulas. The rumen fluid was diluted with modified Dehority (MD) medium (Scott and Dehority, 1965) using 1% carboxymethylcellulose (CMC) as the sole carbon source and anaerobically cultured overnight at 37°C. The fluid was then spread onto MD agar plates containing 1% Azo-CMC and anaerobically cultured overnight at 39°C to screen for bacteria with endoglucanase activity. The colonies forming clear zones were then carefully picked and re-streaked onto Azo-CMC agar plate to check for enzyme activity and isolate single strains. The strains which showed consistent endoglucanase activity were transferred to aerobic conditions and cultured on Luria-Bertani (LB) medium overnight at 37°C. Surviving strains which were facultative anaerobic cellulolytic bacteria were selected. The isolated strain was analyzed by Gram staining as described by Moaledj (1986). Spore formation was examined using phase-contrast microscopy (Nikon Optiphot-2, Japan).

16s rDNA sequencing for strain identification

A total of 1.5 ml of LB culture was centrifuged (10,000 g×1 min) to obtain a cell pellet for DNA extraction, which was performed using a DNeasy Blood & Tissue Kit (Qiagen, Seoul, South Korea). PCR amplification of the 16s rDNA gene fragments was performed using the universal primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3'). The amplified PCR product was visualized by gel electrophoresis. The 16s rDNA band was cut and purified using a Gel DNA extraction kit (Qiagen, Seoul, South Korea). The purified PCR product was then cloned using pGEM-T Easy Vector and transformed into *E. coli* top10 competent cells (Promega, USA) as per the manufacturer's protocol. Plasmids were isolated using a plasmid extraction kit (Bioneer, Korea). A sequence similarity search was carried out using BLAST with the NCBI database (http://www.ncbi.nlm.nih.gov) and alignment was carried out using V-NTI (Life Science Technology, Co. Ltd., USA).

Biochemical analysis of strain identification

Exponentially growing cells were biochemically analyzed using the API 50 CHB Kit (Biomeriux, USA) following the manufacturer's instructions.

Growth curve

The culture medium used in this experiment was liquid LB medium containing 1% CMC. The seed culture was developed prior to measurement of growth phase using same media. The culture media (100 ml) in 500 ml shake flasks was inoculated with 1% of seed culture showing 0.5 of OD_{600} value. Aliquots of the bacterial cultures were taken from the growth medium at two hour intervals, and absorbance was measured at 600 nm. Growth curves were plotted as absorbance vs time. Enzyme activity was also calculated at the two hour intervals.

Enzyme assays

Cellulase and xylanase activity were measured by spectrometric determination of reducing sugars by the 3, 5-dinitrosalicylic acid (DNS) method (Ghose, 1987). Briefly,

a mixture of the enzyme and a 1% CMC solution (1:1) was prepared in 50 mM phosphate buffer (pH 6). Endoglucanase activity was assayed using CMC as a substrate. β-glucosidase activity was determined using salicin (2-hydroxymethyl-phenyl-β-D-glucopyranoside) as a substrate and xylanase activity was determined by measuring the release of xylose from birch wood xylan. For crude enzyme preparation, *Bacillus licheniformis* JK7 was cultured in the basal medium (g/L, 2.5 KH_2PO_4, 2.5 K_2HPO_4, 0.1 NaCl, 0.2 $MgSO_4 \cdot 7H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$, 0.007 $MnSO_4 \cdot 7H_2O$, 0.05 $CaCl_2 \cdot 2H_2O$, 1.0 $(NH_4)_2SO_4$, 2.5 yeast extract, 5.0 CMC, 5.0 birchwood xylan) at 37°C for 24 h. The cultures were centrifuged at 13,000 g×10 min at 4°C and the supernatant was used for the enzyme assay. The reaction mixture was incubated at 37°C for 30 min. After incubation, 300 μl of DNS reagent was added and the mixture was heated to 99°C for 5 min in a boiling water bath. The release of reducing sugars was calculated from the OD measured at 546 nm. One unit of enzymatic activity was defined as the amount of enzyme that released 1 μmol of reducing sugar per minute. All assays were performed in triplicate and average values are reported.

Optimum pH and temperature of cellulase and xylanase and their stability

The optimum pH for crude enzyme preparations was measured in different buffers (50 mM acetate buffer for pH 3 to 5, 50 mM phosphate buffer for pH 6 to 8) at 37°C. The stability of the enzymes at different pH values was determined by pre-incubating crude enzyme in various pH buffer solutions for 4 h at 4°C (Dong et al., 2010). Relative activity was expressed as the percentage of enzyme activity that remained after incubation in comparison to the maximum observed activity at each pH. To determine the optimum temperature for cellulolytic and xylanolytic enzymes, crude enzyme preparations were incubated at a range of temperatures (20 to 80°C) in 50 mM phosphate buffer (pH 6). Thermal stability was determined by incubating crude enzyme at selected temperatures (20 to 80°C) for one hour. The relative activity was calculated in comparison to the maximum observed activity at respective temperature.

All assays were carried out in triplicate, and average values are reported.

Effects of ions and detergents on enzyme activity

The effect of various metal ions and detergents on the activity of the crude enzyme preparations was investigated. The additives used in this study were 5 mM of nine different metal ions ($CaCl_2$, $CoCl_2$, KCl, $MnCl_2$, $NiCl_2$, $MgCl_2$, $FeCl_2$, $CuCl_2$, $ZnCl_2$) and 0.25% detergent (TritonX-100, Tween20). The reaction mixtures were incubated with the additives for 60 min at 37°C and pH 6, and enzyme activities were assayed as described previously. Residual activity was calculated as relative (%) value to control. All assays were performed in triplicate.

Statistical analysis

Data from the characterization of the enzymes at different temperatures and pH values were analyzed statistically using the MIXED procedure in SAS (SAS, 1996). The effects of enzymes, treatments, and the interactions between enzymes and treatments were considered fixed. Significant differences ($p<0.05$) in treatment least square means were reported only if the Tukey-test (SAS, 1996) for treatments was also significant ($p<0.05$). The relative enzyme activities of different chemical additives were analyzed using the GLM procedure (SAS, 1996). Differences between treatments were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Isolation and identification of cellulolytic bacteria

The majority of rumen bacteria are anaerobic as the rumen maintains an obligate anaerobic environment. Representatives of many *Bacillus* strains are known to grow even under anaerobic conditions (Williams and Withers, 1983), but there have been few reports of the isolation of *Bacillus* spp. from the rumen ecosystem. In this study, ten spore-forming facultative microorganisms were screened on LB agar plates containing 1% Azo-CMC. Of these, bacteria JK7 showed maximum endoglucanase activity (data not shown). This strain was found to be a facultative, spore forming, Gram-positive bacteria. The physiological and biochemical characteristics of this organism are listed in Table 1. This bacteria was found to be able to hydrolyze various carbohydrates, including L-arabinose, galactose, fructose, mannose, α-methyl-D-glucoside, N-acethyl-glucosamine, D-turanose, salicin, cellobiose, β-gentiobiose, and D-xylose (Table 1), but did not utilize D-arabinose, erythritol, sorbose, dulcitol, inositol, α-methyl-D-mannoside, Lactose, D, L-arabitol, 2-keto-gluconate, or 5-keto-gluconate (Table 1). Based on these results, JK7 was preliminarily identified as *Bacillus licheniformis*. Strain JK7 was found by 16S rDNA sequence alignment to be closely related to the *Bacillus* genus, with the highest similarity with *Bacillus licheniformis* ATCC14580 (99%). Therefore, this strain was identified as a *Bacillus licheniformis* and designated to *Bacillus licheniformis* JK7 on the basis of biochemical and morphological characteristics and 16S rDNA sequences.

Growth curve

The endoglucanase production and cell growth of

Table 1. Physiologic and biochemical characteristics of *Bacillus licheniformis* JK7

Characteristics	Result	Characteristics	Result
Gram stain	+	Esculine	+
Spore formation	+	Salicin	+
Glycerol	+	Cellobiose	+
Erythritol	-	Maltose	+
D-arabinose	-	Lactose	-
L-arabinose	+	Melibiose	-
Ribose	+	Sucrose	+
D-xylose	+	Trehalose	+
L-xylose	-	Inuline	-
Adonitol	-	Melezitose	-
β-methyl-D-xylose	-	D-raffinose	-
Galactose	+	Starch	+
Glucose	+	Glycogen	+
Fructose	+	Xylitol	-
Mannose	+	β-Gentiobiose	+
L-sorbose	-	D-turanose	+
Rhamnose	+	D-lyxose	-
Dulcitol	-	D-tagatose	+
Inositol	-	D-fucose	-
Mannitol	+	L-fucose	-
Sorbitol	+	D-arabitol	-
α-methyl-D-mannoside	-	L-arabitol	-
α-methyl-D-glucoside	+	Gluconate	+
N-acethyl-glucosamine	+	2-keto-gluconate	-
Amygdaline	+	5-keto-gluconate	-
Arbutine	+		

Bacillus licheniformis JK7 was measured by culturing in a 500 ml shake flask with 100 ml of LB media containing 1% CMC at pH 6 and 37°C (Figure 1). The growth phase of *Bacillus licheniformis* JK7 started at time zero (0 h) and has

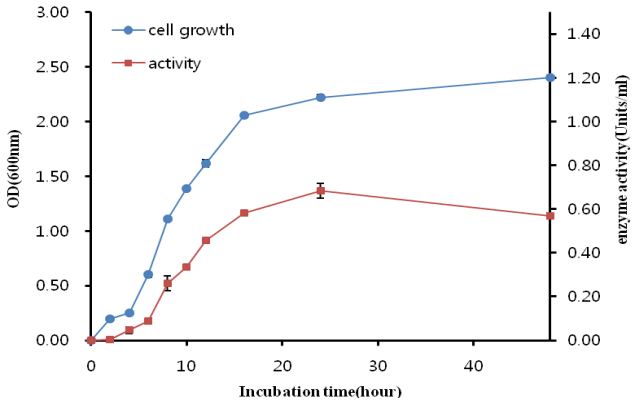

Figure 1. Bacterial growth curve (●) and endoglucanase activity (■) of *Bacillus licheniformis* JK7. The cell growth was determined by measuring the OD600 of the cell culture. Enzyme activity was determined using the culture supernatants. All experiments were performed in triplicate. The data points and error bars indicate the average values and standard errors.

grown to sixteen hours later (16 h). It started faster than previously studied *Bacillus sp.* (Peixoto et al., 2011; Samiullah et al., 2009; Yang et al., 1995). It might be due to different culture condition and the amount of inoculums population.

The stationary began at hour sixteen, which was similar to another *Bacillus* sp. (Peixoto et al., 2011) and *Geobacillus thermoleovorans* (Sharma et al., 2007). Bacterial growth was maintained up to 48. The OD_{600} values were around 2.1 at the stationary phase, and maximum values reached around 2.3 at 48 h. The stationary phase started faster than seen in the growth curves of the previously described *Bacillus licheniformis* 77-2 (Damiano et al., 2003) and *Bacillus licheniformis* SVD1 (van Dyk et al., 2009) and lasted for 30 h, which was longer than other described *Bacillus* sp. (Samiullah et al., 2009) and *Bacillus* sp. V1-4 (Yang et al., 1995).

Endoglucanase production increased rapidly from h 6 up to h 16, with a maximum value of 0.68 U/ml at 24 h and a steady decrease thereafter. The increase in enzyme production was associated with an increase in cell growth, which indicated that cellulose was actively utilized by *Bacillus licheniformis* JK7 during the growth phase. There have been several studies of endoglucanase production which reported similar patterns (Ariffin et al., 2008; Ko et al., 2011; Rastogi et al., 2010; Saratale and Oh, 2011). For example, Rastogi et al. (2010) showed that *Bacillus* sp. DUSELR13 had maximum CMCase activity (0.12 U/ml) at d 9, when the culture had reached the dying phase. The *Geobacillus* strain WSUCF1 also produced maximum CMCase activity (0.13 U/ml) on d 7 at the end of stationary phase (Rastogi et al., 2010). Saratale and Oh (2011) reported that the decrease in cellulolytic enzyme production at the stationary phase was caused by metabolite repression by molecules released after the hydrolysis such as glucose or cellobiose.

Effect of temperature on endoglucanase, β-glucosidase and xylanase activity and stability

The effect of temperature on endoglucanase, β-glucosidase, and xylanase activity of the crude enzyme was determined over a temperatures range of 20 to 80°C at pH 6.0 (Figure 2A). At all temperatures, endoglucanase activity was higher than that of either β-glucosidase or xylanase. In the present study, the xylanase activity of *Bacillus licheniformis* JK7 was lower than those reported by others at all temperatures. According to van Dyk et al. (2009), *Bacillus licheniformis* SVD1 predominantly produced xylanase, and showed minimal production of mannanase, CMCase and avicelase. This difference might be due to the culture conditions (Geetha and Gunasekaran, 2010; Saratale and Oh, 2010). Van Dyk et al. (2009) also used complex

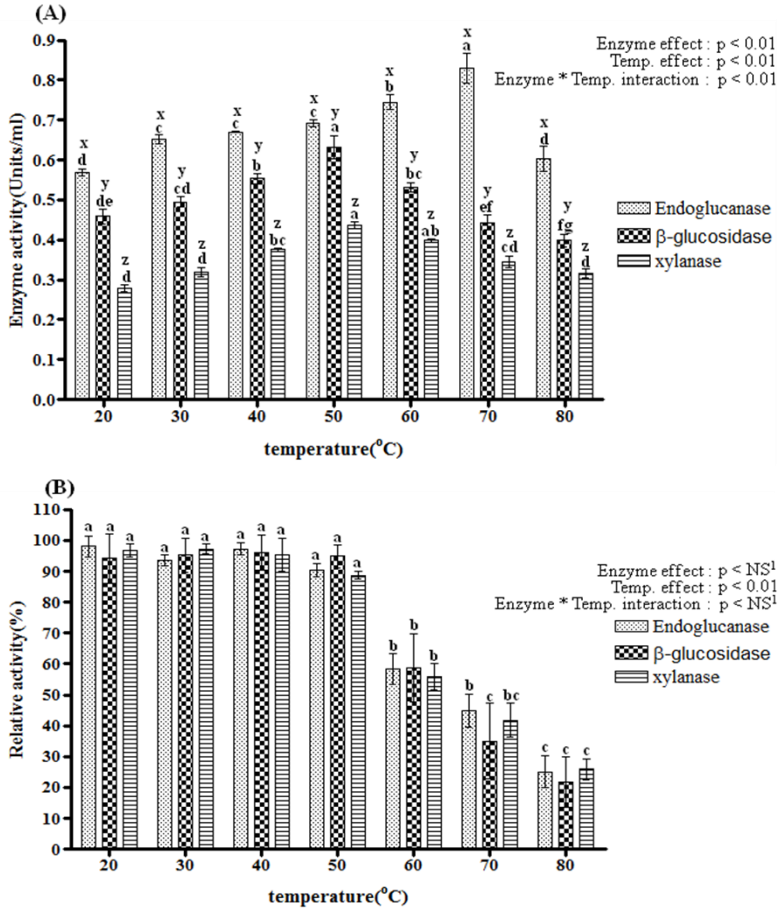

Figure 2. i) Temperature and pH effects on endoglucanase, β-glucosidase and xylanase of *Bacillus licheniformis* JK7 activity (A and C) their stability (B and D). Enzyme activity of the culture supernatants was determined at 24 h. All assays were performed in triplicate. The data points and error bars indicate the average values and standard errors. [a,b,c,d,e,f,g] Indicates a significantly ($p<0.05$) different activity influenced by temperature or pH in the same enzyme group. [x,y,z] Indicates a significantly ($p<0.05$) different activity between different enzymes within same temperature or pH. [1] NS means not significant.

media containing 1% xylan, which could induce increased xylanase production.

The optimum temperature for *Bacillus licheniformis* JK7 endoglucanase activity was 70°C, at which activity was 0.75 U/ml. Activity increased linearly with increased temperature, up to 70°C, and declined thereafter. The previously described *Bacillus* sp. CH43 (Mawadza et al., 2000) showed a similar optimal temperature for endoglcucanase. In another study, *Bacillus* DUSELR13 (Rastogi et al., 2009) also showed maximum endoglucanase activity at 75°C. However, in that study, endoglucanase activity was very low at low temperatures (20 to 40°C). In comparison, *Bacillus licheniformis* JK7 showed endoglucanase activity at broad range of temperatures in our study. Thermophilic cellulose degrading enzymes have great potential for the biofuel, leather, textile, food and agriculture industry, since high temperatures are often required in these processes (Rastogi et al., 2009; Trivedi et al., 2011).

Bacillus licheniformis JK7 showed maximum β-glucosidase and xylanase activity (0.63 U/ml, 0.44 U/ml respectively) at 50°C (Figure 2A). This is consistent with the β-glucosidase of *Bacillus licheniformis* KCTC1918 (Choi et al., 2009), which showed a similar optimal temperature of 47°C. The optimum temperature of various *Bacillus* sp. xylanases have also been reported in the literature, with similar results (Archana and Satyanarayana, 1997; Ko et al., 2010; Ko et al., 2011, Yang et al., 1995; Yin et al., 2010).

The thermo-stability of endoglucanase, β-glucosidase, and xylanase was assessed at selected temperatures ranging from 20 to 80°C, as shown Figure 2(B). The relative activity was calculated as the relative enzyme activity compared to the maximum value observed across the range of temperatures. All three enzymes were stable at a range from 20 to 50°C. At 50°C, endoglucanse, β-glucosidase and xylanase had 90.29, 94.80, and 88.69% residual activity, respectively. However, the residual activity of endoglucanse, β-glucosidase and xylanase declined after 50°C. In the case of endoglucanase, maximum activity was observed at 70°C,

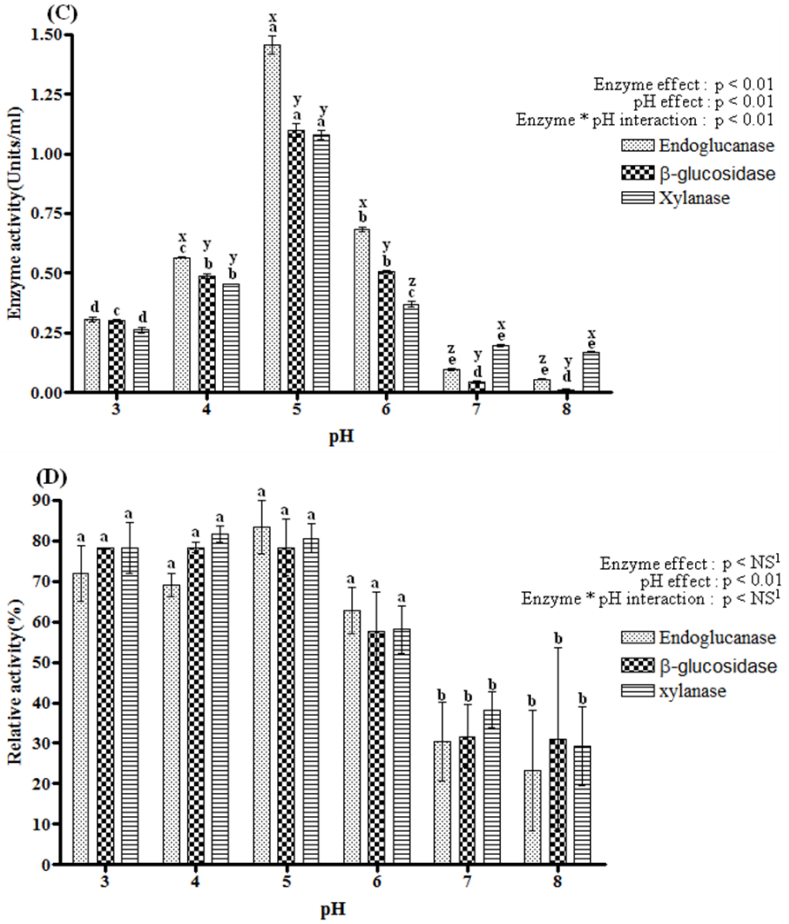

Figure 2. ii) Temperature and pH effects on endoglucanase, β-glucosidase and xylanase of *Bacillus licheniformis* JK7 activity (A and C) their stability (B and D). Enzyme activity of the culture supernatants was determined at 24 h. All assays were performed in triplicate. The data points and error bars indicate the average values and standard errors. [a,b,c,d,e,f,g] Indicates a significantly ($p<0.05$) different activity influenced by temperature or pH in the same enzyme group. [x,y,z] Indicates a significantly ($p<0.05$) different activity between different enzymes within same temperature or pH. [1] NS means not significant.

but it maintained 44.68% residual activity after one hour of pre-incubation.

Effect of pH on endoglucanase, β-glucosidase and xylanase activity, and stability

The effect of pH on enzyme activity was investigated at various pH levels ranging from a pH of 3.0 to 8.0 as shown Figure 2(C). The optimal pH for all three enzymes was 5.0 and their activity at that pH was 1.46, 1.10 and 1.08 U/ml, respectively. Endoglcucanase retained 39% and 46% of its maximum activity across the pH range of 4.0 to 6.0, and β-glucosidase retained more than 45% residual activity in the same range. Xylanase maintained 41% and 34% of its maximum activity between pH 4.0 and 6.0. This is consistent with Bishoff et al. (2007), who reported on a cloned glycoside hydrolase family 5 endoglucanase gene from *Bacillus licheniformis* B-41361; their recombinant gene had maximum endoglucanase activity at pH 5.5. The optimum pH of xylanase in the multi enzyme complex of *Bacillus licheniformis* SVD1 was also 5.0 (van Dyk et al., 2010). Many industrial processes involving cellulase need to use extreme pH conditions to reduce contamination by other bacteria (Dong et al., 2010). Since these processes often require acidophilic enzymes to degrade fiber efficiently under low pH conditions, the relatively high acidophilic nature of the enzymes examined in this study might be considered beneficial for industrial application. All three enzymes were strongly inhibited at pH 7.0 to 8.0. Figure 2(D) shows the pattern of pH stability of selected enzymes. Relative activity was calculated as the percentage of the maximum observed activity for each enzyme. Endoglucanase, β-glucosidase and xylanase activities were found to be stable in the pH range of 3 to 6. They maintained more than 58% of their maximum activity at selected pH after four hour pre-incubation at 4°C. At pH 7 and 8, relative enzyme activity of all three enzymes dramatically declined, with remaining endoglucanase β-glucosidase and xylanase activity of only 23, 30, 29% of their maximum activity, respectively.

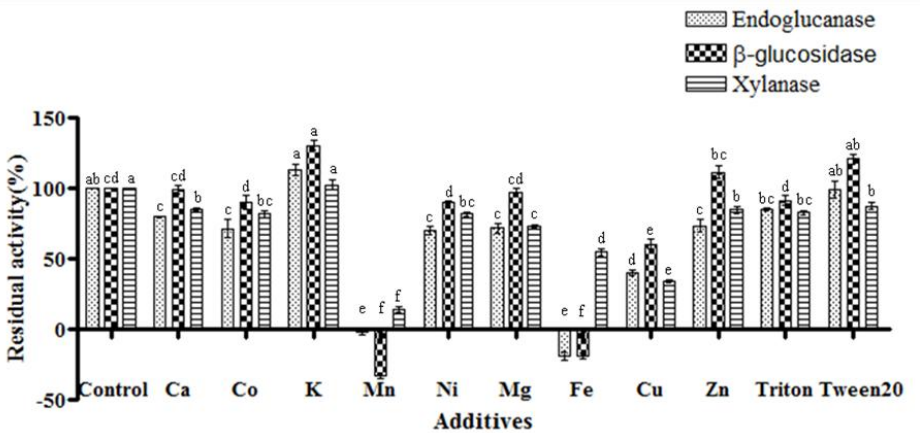

Figure 3. Effect of chemical additives on endoglucanase, β-glucosidase, xylanase activity of *Bacillus licheniformis* JK7. Residual activity was calculated as relative (%) considering control as 100%. All assays were performed in triplicate. The data points and error bars indicate the average values and standard errors. [a,b,c,d,e,f] Indicates a significantly ($p<0.05$) different enzyme activity compared to control.

Effects of various chemical additives on endoglucanase, β-glucosidase and xylanase activity

The effects of various chemicals on endoglcucanase, β-glucosidase, and xylanase were investigated by the DNS assay method (Figure 3). Crude enzyme was incubated for one hour with 5 mM of each of the metal ions and 0.25% of TritonX-100 and Tween20 before determining the residual activity of the three enzymes. Residual activity was expressed as the relative amount (%) compared to control (100%). Understanding the effect of various metal ions and reagents on enzyme activity is important since many industrial applications need to increase enzyme activity, which is often accomplished by addition of chemical additives at various stages of the process (Dong et al., 2010). The majority of *Bacillus* spp. which produce cellulase showed responses ranging from stimulation to inhibition depending on the specific cation (Christakopoulos et al., 1999).

In this study, endoglucanase activity was found to be stimulated by K^+ to 113% of the control (Figure 3). GH5 endoglucanase from *Martelella mediterranea* (Dong et al., 2010) was previously reported to show increased relative activity when K^+ was added. K^+ may stimulate enzyme activity due to its ability to alter the structure of the enzyme itself (Kui et al., 2009). However, in our study the enzyme activity was inhibited by Ca^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and TritonX-100. In particular, Mn^{2+} and Fe^{2+} both strongly inhibited endoglucanase activity (2.18, and 19.0%, respectively). The strong inhibitory effect of Mn^{2+} on endoglucanase activity is consistent with previous reports of *Bacillus amyloliquefaciens DL-3* and *Bacillus flexus* (Lee et al., 2008; Trivedi et al., 2011). An inhibitory effect on enzyme activity by metal ions usually suggests the presence of a sulfhydryl group in the active site, where oxidation by the metal ions destabilizes the conformational folding of the enzymes (Karnchanatat et al., 2007).

In present study, the relative activities of β-glucosidase with 5 mM Ca^{2+}, Co^{2+}, K^+, Mn^{2+}, Ni^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, and 0.25% of TritonX-100 and tween20 were 99, 90, 130, -33, 90, 96, -19, 60, 111, 91 and 120%, respectively (Figure 3). Detergents such as tween20 have been implicated in altering the conformational or structural characteristics of selected enzymes (Bajaj et al., 2009). Xylanase was not influenced by selected chemical additives, with the exception of Mn^{2+} and Cu^{2+} (Figure 3). The strong inhibition of Mn^{2+} on the xylanase activity of *Bacillus* species was also reported in a previous study (Mamo et al., 2006). The slight stimulatory effect of K^+ and the inhibition of the xylanase enzyme activity of *Bacillus licheniformis* by tritonX-100 is also similar to what was observed by Archana et al. (2003).

CONCLUSION

The broad range of optimum temperatures (20 to 40°C) and the stability under acidic pH (4 to 6) suggest that the cellulolytic enzymes of *Bacillus licheniformis* JK7 may be good candidates for use in the biofuel industry, which requires that celluloses be able to be hydrolyzed by acids at high temperature (Gao et al., 2008).

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