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Full Length Research Paper

Production and improved bleaching abilities of a thermostable xylanase from a newly isolated *Streptomyces chartreusis* strain

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A *Streptomyces chartreusis* strain L1105 capable of producing extra-cellular xylanase was isolated and identified in this study and the production conditions optimized. The xylanase activities obtained were 121, 137 and 334 U/ml, when using birch wood xylan, beech wood xylan and corncobs xylan respectively, as substrates. Production of xylanase by L1105 on corncobs xylan was enhanced by optimizing the nitrogen source, growth temperature and initial pH of the culture medium. The effect of surfactants (Tween 80) on xylanase production was investigated and the maximum production was observed at a concentration of 0.2% (v/v). After optimization of various production parameters, the maximum xylanase yield (731 U/ml) was obtained at 40°C using 2.5% corncobs xylan as substrate for 7 days of cultivation. Furthermore, the potential application of the xylanase was evaluated in wheat straw pulp, from which the amount of reducing sugars released by the xylanase increased significantly with time. Enzymatic pre-bleaching of wheat straw pulp showed a 12% reduction in the kappa number of the pulp. We are the first to demonstrate that the *S. chartreusis* strain has the capacity for xylanase production, although, many other xylanase-producing microorganisms have been reported.

Key words: *Streptomyces chartreusis*, xylanase, submerged fermentation, production parameters.

INTRODUCTION

Xylans account for 20 to 35% of the total dry weight of hardwoods and annual plants and represent a vast resource that can be used for production of fermentable sugars and fuels as described previously (Filho, 1998). The complete cleavage of xylan is carried out by the synergistic action of β-xylanase and its accessory enzymes, including β-xylosidase, α-arabinofuranosidase, α-methylglucuronidase andacetyl xylan esterase (Beg et al., 2001). In recent years, increasing concern over preserving resources and environment has initiated a growing interest in producing microbial enzymes. Xylanases from microorganisms have attracted a great deal of attention because of their biotechnological potential in various industrial processes such as food, feed and paper-pulp industries.

Xylan-degrading enzymes produced by a wide variety of fungal species have been reported to be used in biobleaching of kraft pulps, such as *Thermomyces lanuginosus* (Li et al., 2005), *Penicillium janthinellum* (Oliveira et al., 2006), *Arthrobacter* sp. (Khandeparkar and Bhosle, 2007), *Streptomyces cyaneus* (Ninawe et al., 2008) et al. However, little research on xylanase from *S. chartreusis* has been reported.

In this study, we aimed to isolate and identify actinomycete species from a soil sample under a layer of decaying tree fibers in Anhui Province, China and to evaluate their

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Abbreviations: AQ, Anthraquinone; **CMC,** carboxymethylcellulose; **MES,** 2-(N-Morpholino)-ethane sulfonic acid; **MOPS**, 3-(N-morpholino)-propanesulphonic acid; **CHES,** 2-(cyclohexylamino)-ethanesulfonic acid; **CAPS,** (cyclohexylamino)-1-propanesulphonic acid.

capacity to produce xylan-degrading enzyme activity during growth in a liquid medium containing xylan as the carbon source. We also optimized various parameters for the enzyme production under submerged fermentation and investigated the storage stability of the crude xylanase.

MATERIALS AND METHODS

Chemicals and statistical analysis

All chemicals used were of analytical grade. Birchwood, oat spelt, and beechwood xylans were purchased from Sigma (Sigma Aldrich Co Ltd, Germany). Lignocellulosic substrates such as bagasse and corncobs were obtained from local farms. Corncob xylan and bagasse xylan were prepared according to the method of Kusakabe et al. (1976). All other chemicals were of analytical grade. Unbleached wheat straw pulp was used for this study. The unbleached wheat straw (*Triticum sativum*) NaOH-AQ pulp was kindly supplied by the China National Pulp and Paper Research Institute (Beijing, China). Wheat straw was cooked under the following conditions: temperature 150°C, NaOH concentration 14.0% (w/v), AQ concentration 0.025% (w/v), liquid/solid ratio 6 and time 60 min. Then, pulp samples were washed thoroughly with deionized water to remove soluble reducing sugars and any free soluble residual lignin before they were used. Pulp samples were thoroughly washed after each treatment step with water until a neutral pH was obtained.

The results given here are the mean of triplicates performed independently. The standard deviation in all the experiments was within 10%.

Screening and identification of strain L1105

Actinomycetes was isolated from soils collected at twenty different locations in China and screened on agar plates containing 1.0% corncobs xylan, 0.5% yeast extract, 1.0% tryptone, 0.75% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 1.5% agar. The plates were incubated at pH 6, 40°C, for 3 to 5 days. Those colonies that grew well under such condition and showed a clean zone around the colonies were isolated. The isolated strains were cultured in liquid media containing 1.5% corncobs xylan, 0.5% yeast extract, 1.0% tryptone, 0.75% KH_2PO_4, 0.15% K_2HPO_4 and 0.05% $MgSO_4 \cdot 7H_2O$ at pH 6 in Erlenmeyer flasks. After incubation on a rotary shaker (40°C, 140 rpm) for 6 days, the culture broth was centrifuged and the supernatants were collected for enzyme assay. The newly isolated actinomycete strain L1105 from Anhui Province in China showed very high capacity for producing xylanase. This strain was maintaine on potato dextrose-agar and used throughout the study for the production of xylanase. Stock cultures were maintained on potato dextrose-agar slants at 4°C and were transferred every 6 to 7 weeks. PDA plates were incubated at 38 to 40°C for 4 to 5 days and stored at 4°C until use. Cultures were also maintained as spore suspension in 20% (v/v) glycerol at -20°C.

The actinomycete strain L1105 was identified by comparison of its morphological, cultural, biochemical and physiological characteristics with those of *Streptomyces chartreusis* ATCC 21999 and ATCC 23336. *S. chartreusis* ATCC 21999 and ATCC 23336 were obtained from the China General Microbiological Culture Collection Center (CGMCC), Beijing, China. International Streptomyces Project (ISP) medium (Shirling and Gobblicb, 1966) was used for strain characterization. The sequence of strain L1105 was amplified by PCR with the primer A: 5'-GGTTACCTTGTTA CGACTT-3' and primer B: 5'-AGAGTTGACCCTGGCTCAG-3'. The amplification was performed using the TC-512 PCR instrument (TECHNE) with the following cycling parameters: 94°C for 4 min, followed by 30 cycles of 30 s at 94°C, 80 s at 52°C and 90 s at 72°C with final extension at 72°C for 8 min. The amplified construct harboring the 16S rDNA was sequenced. Then, the 16S rDNA sequence was compared with GenBank and a neighboring phylogenetic tree was constructed with related strains. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method with 1000 replicates.

Cultivation conditions parametric optimization of xylanase production

For submerged fermentation, the basal medium of flask culture contained (g l^{-1}): corncobs xylan, 25; yeast extract, 5; tryptone, 10; KH_2PO_4, 7.5; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 1.5. The initial pH of the medium was adjusted to 6.0 and not further controlled thereafter. Shake flasks, prepared in triplicate, with each containing 50 ml of the medium in 250 ml Erlenmeyer flasks, were inoculated with an agar block (0.5 cm^2) from a 5 day old plate culture. Inoculated flasks were incubated on a rotary shaker at 140 rpm for 7 days at 40°C. Samples were withdrawn at regular intervals and filtered through Whatman filter No. 1; the resulting filtrate was used for analysis of extracellular xylanase, cellulase and total soluble protein.

To investigate the effect of various carbon sources on xylanase production, various carbohydrates and xylans were tested as the sole carbon source for xylanase production at various concentrations.

To determine their effects on xylanase production, various inorganic and organic nitrogen sources were added separately to the basal medium with a fixed total concentration. The cultivation was carried out under the general conditions described earlier.

For studies on the effect of pH on xylanase production, the isolated *S. chartreusis* L1105 was grown in a medium containing 2.5% corncobs xylan with different initial pH values in the range 4.5 to 7.5. The production of xylanase by *S. chartreusis* L1105 was evaluated by incubating the flasks at 40°C for 7 days. Xylanase production was also studied at different temperatures, including 30, 35, 40, 45 and 50°C. Tween 80 at different concentrations was supplemented in the medium to determine the effect on production.

After optimization of these parameters, xylanase production was carried out under optimized nutritional and fermentation conditions for maximum yield of the enzyme to be demonstrated over time for applications in the pulp and paper industry.

Enzymatic assays

Xylanase activity was assayed according to Bailey et al. (1992) by determining the release of reducing sugars from a 1% (w/v) Birchwood xylan (Sigma) solution prepared in 50mM sodium citrate buffer (pH 5.3) at 50°C. One unit (U) of enzymatic activity corresponded to 1 µmol of xylose equivalents released per minute under the assay conditions.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and zymogram

SDS-PAGE was performed using 12.5% (w/v) acrylamide in gels by the method of Laemmli (1970). Protein spots were visualized by Coomassie brilliant blue R-250 staining. The molecular weight standard used was the low molecular weight calibration kit for SDS electrophoresis (Fermentas SM031): β-galactosidase, 116.0 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; lactate dehydrogenase, 35.0 kDa; REase Bs p981, 25.0 kDa; β-

Table 1. The usable carbon sources for strain L1105.

Carbon source		Carbon source		Carbon source	
Inositol	+	Inulin	+	D-glucose	+
Mannitol	+	Glycerin	+	Xylose	+
Salicin	+	Sodium gluconic	+	Lactose	+
Raffinose	+	Galactose	+	Melezitose	+
Rhamnose	+	Sodium citrate	+	L-arabinose	+
Starch	+	Sodium succinate	+	Melampyrit	+
Sorbitol	+	Sodium malate	+	Erythritol	+
Sucrose	+	Cellobiose	+	Sodium acetate	+
Melibiose	+	Sodium malonate	+	Sodium propionate	+
Fructose	+	Asparagine	+	Sodium hippurate	+
Mannose	+	Laetrile	+	Tyrosinase	+
Maltose	+	Lodium tartrate	+	Peptonized milk	+
Amylase	+	Liquefied gelatin	+	Peptonized milk	+

lactoglobulin, 18.4 kDa; lysozyme, 14.4 kDa.

Zymogram analysis of xylanase was performed by the method of Tseng et al. (2002) with slight modification. 0.1% of Birchwood xylan was incorporated into running polyacrylamide gel (12.5%).
Once the electrophoresis was finished, the gel was washed twice for 15 min each) at 4℃ in a solution containing 25% isopropanol and four times for 8 min each in a solution of 20 mM phosphate buffer (pH 6.5) to remove SDS, then incubated in the same buffer for 20 min at 40℃. The zymogram was prepared by soaking the gel in 0.1% Congo red solution for 15 min at room temperature (25±2℃), then washed with 1 M NaCl and 0.5% acetic acid introduced to expose the xylanase active bands that contrasted against the dark background.

Determination of pH and temperature optima, pH stability and thermostability

The optimum pH was determined by measuring the activity at 50℃ using the following buffers: 50 mM citrate buffer (pH 2.2 to 4.2); 50 mM acetate buffer (pH 3.8 to 5.8); 50 mM MES buffer (pH 5.2 to 7.2); 50 mM MOPS buffer (pH 6.2 to 8.2); 50 mM Tris-HCl buffer (pH 7.0 to 9.0); 50 mM CHES buffer (pH 8.15 to 10.15) and 50 mM CAPS buffer (pH 9.30 to 11.3). pH stability was determined by incubating the enzyme in the buffers for 30 min at 50℃. Temperature optimum was determined by assaying the enzyme activity at several temperatures at pH 7.2. The enzyme thermostability was determined by measuring residual activity, after incubation of the enzyme at varying temperatures and pH 7.2 in the absence of substrate, for different periods of time.

Biobleaching of wheat straw pulp

The wheat straw pulp was first treated with xylanase in Tris-HCl buffer, pH 7.0 at 50℃. The enzyme dosages ranged from 0 to 120 U/g of the dried pulp. The pulp samples were mixed with suitably diluted enzyme in sealed polyethylene plastic bags and then incubated for 3 h with intermittent kneading. Control sample was treated under the same conditions with inactivated (boiled) enzyme. Following the incubation period, the enzyme-mediated release of lignin-derived compounds and chromophoric material from pulp was monitored in filtrates by measuring their absorbances at 237, 254, 280 and 465 nm, respectively. The amount of reducing sugars released from pulp was determined spectrophotometrically at 540 nm according to the DNS method (Miller, 1959). The pulp obtained was then washed with distilled water and analyzed for kappa number, according to the TAPPI Test Methods T236 cm 85.

RESULTS AND DISCUSSION

Isolation and identification of *S. chartreusis* L1105

After the first screening, 102 candidates were obtained and further cultured in liquid media. The media of actinomycete strain L1105 showed comparative higher xylanase activity among all the selected strains. Further identification was conducted as follows: The newly isolated strain L1105 grew well in general culture media. The aerial mycelium appeared white to pale blue and the vegetative mycelium showed a brown color. Excellent spore formation was seen in every culture medium tested. There were no soluble pigments when strain L1105 was cultured in media. The usable carbon sources for strain L1105 are shown in Table 1.

After carefully comparing the features of strain L1105 with the characteristics of *S. chartreuses* ATCC 21999 and ATCC 23336, its physiological and cultural properties were found to be similar to only those of *S. chartreusis*. The total length of 16S rDNA fragment was 1404 bp through PCR amplification. Our results showed that 16S rDNA sequence of *Streptomyces* sp. L1105 displayed up to 99.65% homology with that of *S. chartreusis* DSM41255. Combining the traditional characteristics from physiological and biochemical tests, strain L1105 was identified as *S. chartreusis* L1105. *S. chartreusis* L1105 was able to produce high titres of xylanase activity when grown at 40℃ in liquid medium containing xylan as the sole carbon source (Table 2). We are the first researchers to demonstrate that *S. chartreusis* L1105 has the capacity for xylanase production, although, many microorganisms were shown to produce xylanases.

Table 2. Extracellular enzyme activities in *S. chartreusis* L1105 culture supernatants after 7 days of growth at 40℃ on growth medium containing different substrates.

Substrate	Xylanase activity (U/ml)
Sucrose	4.17±1.15
D-glucose	4.25±1.04
Soluble starch	5.17±1.33
Lactose	8.17±1.03
D-xylose	9.08±2.25
Mannose	8.72±1.44
Oat spelt xylan	45.16±6.62
CMC	66.89±4.06
Bagasse xylan	69.71±8.33
Birchwood xylan	121.05±11.12
Beechwood xylan	137.57±9.09
Corncobs xylan (soluble)	189.57±12.85
Corncobs xylan (insoluble)	334.34±10.09

The basal medium consisted of 0.5% yeast extract, 1.0% tryptone, 0.75% KH_2PO_4, 0.15% K_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$ (pH6) in distilled water. The actinomycetes were cultured at 40℃ for 7 days on an oscillatory shaker at 140 rpm. Results presented in the table are the mean of three parallel experiments ± S.D.

Effect of carbon source on xylanase production

Xylanases are produced by many bacterial and fungal genera such as *Bacillus, Aspergillus, Penicillium, Schizophyllum, Aureobasidium, Thermomyces* and especially by *Trichoderma* sp. as described previously (Wong et al., 1988; Gomes et al., 1992, 1993; Beg et al., 2000; Li et al., 2005). The carbon source used in the production medium is one of the major factors affecting enzyme production and level. In this study, different xylans such as Birchwood, oat spelt, beechwood, bagasse and corncobs xylan as well as various carbohydrates were used as the sole carbon source for xylanase production by *S. chartreusis* L1105 and the results are shown in Table 2. When xylan in the basal medium was replaced with different carbon sources, such as D-xylose, lactose, sucrose, D-glucose, soluble starch, CMC and mannose, maximum xylanase activity occurred with xylans, followed by CMC and D-xylose. Among the carbon sources tested, 2.5% xylan was found to be the most effective and suitable for xylanase production by *S. chartreusis* L1105 (Table 2). Actually, different xylanase-producing microorganisms may require different suitable carbon sources. Several other substances has been also reported as the suitable carbon sources, including oat wheat (Carmona et al., 1998), Birchwood xylan (Tseng et al., 2002), oat spelt xylan (Chivero et al., 2001; Saha, 2002), bagasse xylan (Breccia et al., 1998) and wheat bran arabinoxylan (Bataillon et al., 2000).

The xylanase production by *S. chartreusis* L1105 reached 334.34 U/ml after 7 days at 40℃. With regard to the other *Streptomyces* species of strain AMT-3, the xylanase concentrations obtained after 10 days of incubation with various carbon sources (1%), were 70.0 U/ml with Larchwood xylan, 36.4U/ml with oat spelt xylan and 10.3 U/ml with Birchwood xylan, respectively (Nascimento et al., 2002).

Maximum xylanase production (334.34 U/ml) was achieved in 7 days of incubation at 40℃ (pH 6.0) (Table 2). Xylanase production increased with an augment in concentration of xylan from 0.5 to 2.5% (w/v) and it declined considerably with further increases in xylan concentration (data not shown). This may be due to the formation of a thick suspension of the substrate which, in turn, did not mix freely in shake flasks. The results shown in Table 2 demonstrate that birchwood xylan, beechwood xylan and corncobs xylan act as efficient substrate for xylanase production because the obtained enzyme titers were 121.05, 137.57 and 189.57 U/ml, respectively. This indicates that xylanase production is strongly induced by xylan. CMC was also able to induce a level of xylanase activity (66.89 U/ml) similar to that obtained from Oat spelt xylan (45.16 U/ml) and bagasse xylan (69.71 U/ml). When xylose, glucose, lactose and sucrose were used, xylanase activity in much lower levels was also detectable, at enzyme titers of 9.08, 4.25, 8.17 and 4.17 U/ml, respectively (Table 2). The data suggests that basal levels of xylanase are always present in *S. chartreusis* L1105 cultures. These findings confirm that microorganisms constitutively produce xylanases in basal levels to allow the provision of low molecular weight xylan fragments that induce xylanase production as described previously (Kulkarni et al., 1999). Because the xylanase activity observed during *S. chartreusis* L1105 growth on

Table 3. Effect of nitrogen sources on xylanase production by *S. chartreusis* L1105 at 40°C under submerged fermentation.

Nitrogen source (%)	Xylanase activity (U/ml)
NH_4Cl(1.5)	9.78±0.85
KNO_3(1.5)	14.57±1.11
$(NH_4)_2SO_4$ (1.5)	15.22±1.07
Yeast extract (1.5)	42.54±2.68
Tryptone (1.5)	60.51±2.09
Tryptone (0.5%) + yeast extract (1.0%)	270.64±11.22
Tryptone (1.0%) + yeast extract (0.5%)	351.40±12.01

The basal medium consisted of 2.5% corncobs xylan, 0.75% KH_2PO_4, 0.15% K_2HPO_4, 0.05% $MgSO_4·7H_2O$ (pH 6) in distilled water. The actinomycetes were cultured at 40°C for 7 days on an oscillatory shaker at 140 rpm. Results presented in the table are the mean of three parallel experiments ± S.D.

corncobs xylan (insoluble) was much higher than those observed on the other substrates, corncobs xylan (insoluble) was used as the sole carbon source in subsequent experiments.

Effect of nitrogen source on xylanase production

Among the various inorganic and organic nitrogen sources tested (Table 3), a combination of tryptone and yeast extract stimulated the highest xylanase production. The xylanase production was maximized (351.40 U/ml) at a concentration of 1.0% (w/v) tryptone and 0.5% (w/v) yeast extract (Table 3), whereas it was significantly decreased at 0.5% (w/v) tryptone and 1.0% (w/v) yeast extract. When inorganic nitrogen sources such as NH_4Cl, KNO_3 and $(NH_4)_2SO_4$ were used as sole nitrogen source for xylanase production by *S. chartreusis* L1105, much lower xylanase activities were shown as 9.78, 14.57 and 15.22 U/ml, respectively, which corresponds to the fact that organic nitrogen sources stimulate xylanase production (Gaikaiwari et al., 1996).

Effect of the initial culture pH and temperature on xylanase production

The initial pH of the medium is an important factor significantly affecting the production of xylanases. By incubating the flasks at 40°C for 7 days, *S. chartreusis* L1105 was grown in a medium containing 2.5% corncobs xylan with different initial pH values to evaluate the effect on xylanase production. Xylanase production by the current strain was observed in the range pH 4.5 to 7.5, while no xylanase activity was observed in the medium at pH 4.0. The optimum initial culture pH experiment showed that the maximum activity was achieved at pH 6.0 (Figure 1). Our findings agree well with earlier studies that showed that xylanase production by various bacteria and fungi is markedly dependent on pH (Wong et al., 1988).

Apparently, the environmental temperature not only affects growth rates of organisms but also exhibits remarkable influence on the levels of xylanase production. Xylanase production at different temperatures was also examined for 7 days keeping the other fermentation conditions constant. Xylanase yield increased with increasing temperature from 30 to 40°C and decreased from 40°C. The optimum incubation temperature for enzyme production was found to be 40°C (386 U/ml). A significant decline (72%) in xylanase activity was observed at 50°C, while no xylanase production was observed above 55°C (Figure 2). Growth and xylanase production totally ceased at higher temperature (55°C) and similar observations were shown by Oliveira et al. (2006) for *Penicillium janthinellum*.

Effect of Tween 80 on xylanase production

The effect of surfactant Tween 80 on xylanase production was investigated and the maximum increase in xylanase production was observed in the presence of Tween 80 at a concentration of 0.2% (v/v) (Figure 3). Similar situations have been reported by others, while using a concentration of 0.2% (v/v) of olive oil as an additive for enzyme production and such compounds probably increase the permeability of the cell membrane and cause rapid secretion of the enzymes (Battan et al., 2007).

Time course of xylanase production by *S. chartreusis* L1105

After optimization of various production parameters,

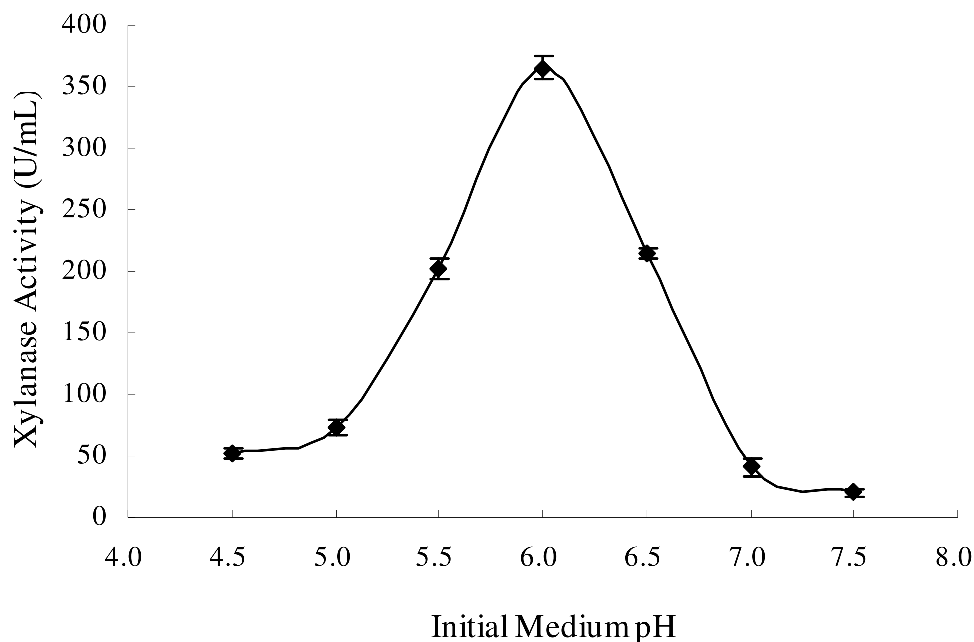

Figure 1. Influence of pH of the medium on xylanase production by *S. chartreusis* L1105 grown on 2.5% (w/v) corncobs xylan (insoluble) at 40℃ under submerged fermentation.

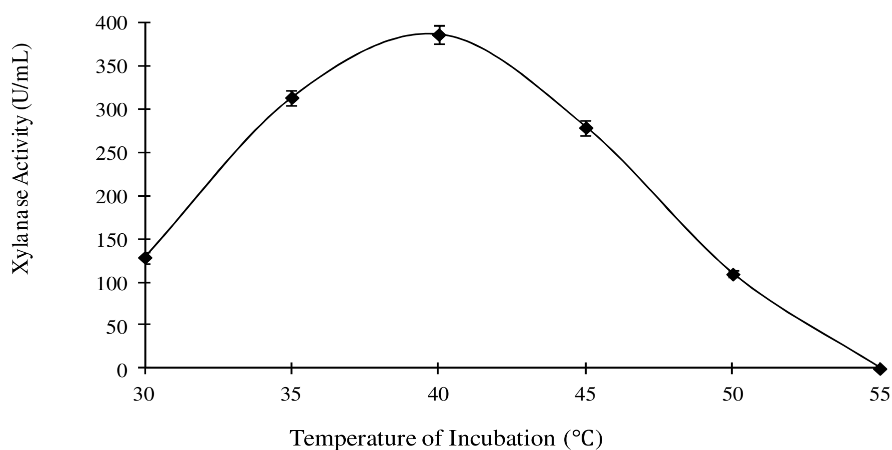

Figure 2. Influence of temperature on xylanase production by *S. chartreusis* L1105 grown on 2.5% (w/v) corncobs xylan (insoluble) at 40℃ under submerged fermentation.

xylanase production was carried out at 40℃ for 7 days in a liquid medium containing 2.5% corncobs xylan as substrate by *S. chartreusis* L1105. The fermentation time-course for xylanase production is shown in Figure 4. Under the current optimized nutritional and fermentation conditions, the xylanase production (731.3 U/ml) was almost 2 fold the initial level (334.34 U/ml). In addition, it was ten- and fifty-fold higher in comparison to the data reported by Nascimento et al. (2002) and Georis et al. (2000), respectively. Xylanase production started from the second day and reached a maximum on the seventh day. From the fourth to the sixth day, a significant

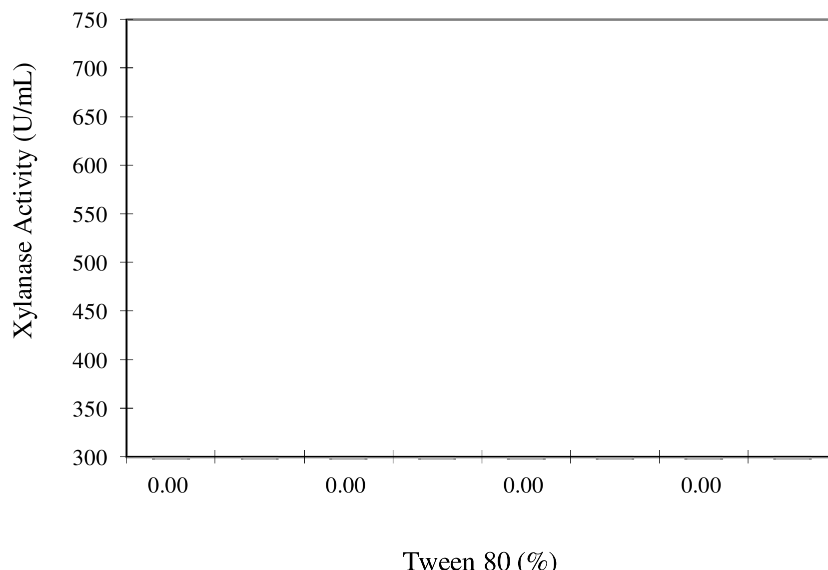

Figure 3. Influence of Tween 80 on xylanase production by *S. chartreusis* L1105 grown on 2.5% (w/v) corncobs xylan (insoluble) at 40°C under submerged fermentation.

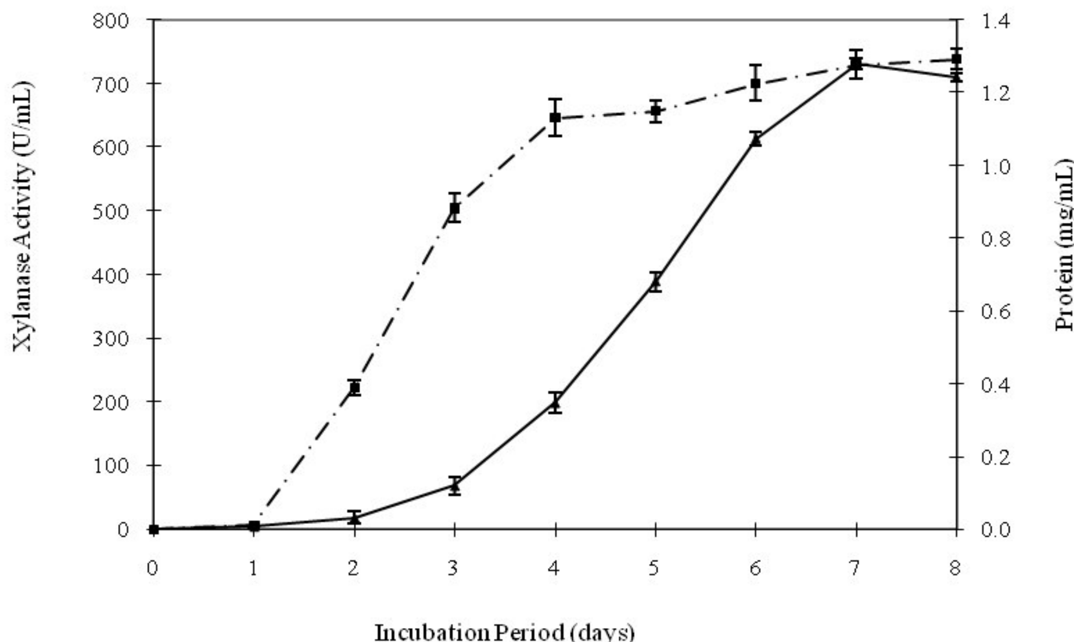

Figure 4. Fermentation time-course for xylanase (closed triangles) and protein (closed squares) by *S. chartreusis* L1105 grown on 2.5% (w/v) corncobs xylan (insoluble) at 40°C under submerged fermentation.

increase in enzyme production (nearly 75% of total production) was observed and from the seventh day onwards, a slight decrease in production was observed. Xylanases are usually expressed at the end of the exponential phase and harvesting time is correlated to the medium under consideration (Kulkarni et al., 1999). The phenomenon of sudden increase and subsequent decrease in enzyme activities during the cultivation period has also been noted in xylanase produced by *Streptomyces* sp. CH-M-1035 (Flores et al., 1996).

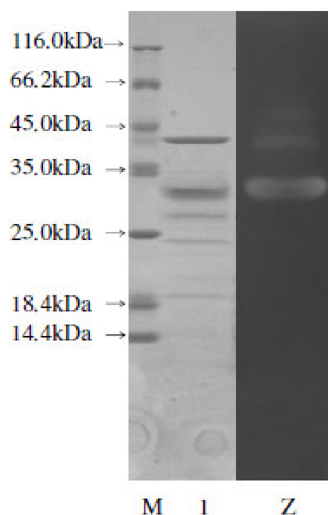

Figure 5. SDS-PAGE and zymogram analysis of the crude xylanase from *S. chartreusis* L1105. Lane M, the low molecular weight calibration kit; lane 1, crude extract; lane Z, zymogram of the crude extract.

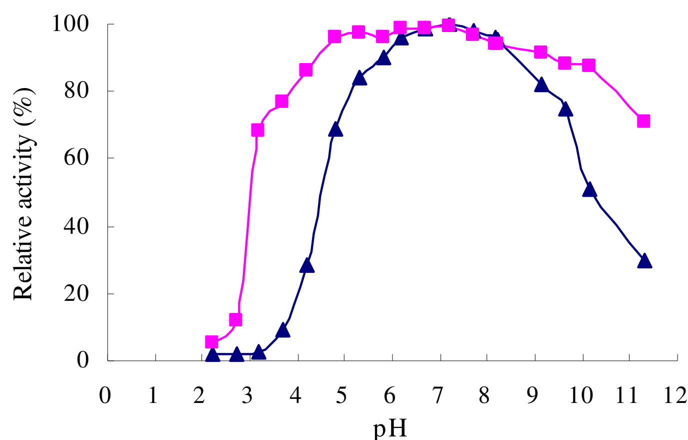

Figure 6. Optimum pH (closed triangles) and stability pH (closed squares) of the xylanase from *S. chartreusis* L1105. The optimum pH of xylanase was measured at 55℃ using 50 mM of different buffers. For pH stability determination, the xylanase was diluted in 50 mM buffers at different pH and incubated at 50℃ for 30 min.

Although, many microorganisms are known to produce xylanases, this study shows, for the first time, that *S. chartreusis* L1105 has the capacity for xylanase production, with very high activity. The xylanase was analyzed by SDS-PAGE and activity staining of gel electrophoresis (zymogram) (Figure 5). The molecular mass of the primary xylanase was estimated to be 31.6 kDa. The result shows that the enzyme belongs to the family of F/10 xylanases. The molecular mass was similar to the molecular mass of the xylanase from fungal species such as *Aspergillus kawasachii* (35 kDa) (Ito et al., 1992) and *Aspergillus nidulans* (34 kDa) (Fernandez-Espinar et al., 1994), but it was different from *Streptocarpus cyaneus* SN32 (20.5kDa) (Ninawe et al., 2008).

Effects of pH and temperature on the activity and stability of xylanase

The xylanase from L1105 was most active at a neutral pH, namely pH 6.7 to 7.7 and retained 82% of its activity at pH 9.2 (Figure 6). It retained 90% of its activity at 50℃ for 30 min when tested in the pH range of 5 to 9 (Figure 6). Like xylanases from different strains of *Streptomyces* sp., the xylanase was also stable over a wide pH range. However, the pH stability (pH 5 to 10) of the xylanase was slightly shifted to the neutral and alkaline range compared with pH 5.0 to 9.0 from *Streptomyces actuosus* A-151 and *Streptomyces olivaceoviridis* A1 (Wang et al., 2003, 2007). Considering the potential application in bleaching pulp at high pH values, the tolerance to alkaline conditions of this xylanase is crucial.

The xylanase exhibited its optimal activity at 70℃ (Figure 7). It was stable up to 50℃ and retained more than 68% of its activity after heating at 65℃ for 30 min (Figure 7). Compared with 60℃ of xylanases from most strains of *Streptomyces* sp., the xylanase was optimally active at 70℃. The optimal temperature was similar to values for the xylanase from the strains *S. actuosus* A-151 as described previously (Wang et al., 2003). The thermal stability of xylanase in this study was similar to another xylanase from the *Streptomyces* strain sp. S38 (Georis et al., 2000), which was almost completely stable up to 60℃. The thermal stability of the xylanase from various strains differed depending on the experimental conditions and the xylanase in this study was stable up to 50℃ and retained 68% of its activity at 65℃ after 0.5 h of incubation. Its thermal stability is also an attractive feature with regard to industrial applications.

Effect of xylanase treatment on wheat straw pulp

Unbleached wheat straw pulp was pretreated with *S. chartreusis* L1105 xylanse under the optimal pH using various enzyme doses (Figure 8). Release of chromophoric material and reducing sugar from pulps were determined. From Figure 8, it can be seen that most of the ultraviolet light and visible light absorbing material was released within 3 h. The amount of reducing sugars released by the xylanase from wheat straw pulp was significantly greater with increasing time (Figure 9). The peak at 280 nm in the UV spectrum indicates the presence of lignin in the released colored compounds after the enzyme treatments. The correlation between the release of chromophores and hydrophobic compounds (A254, A280 and A465 nm) and the reduction in kapp

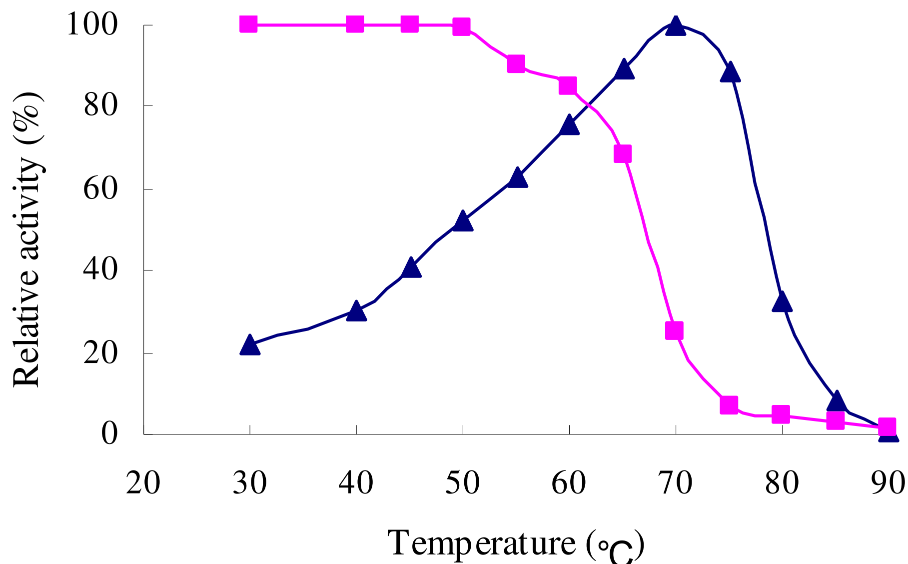

Figure 7. Optimal temperature (closed triangles) and thermostability (closed squares) of the xylanase from *S. chartreusis* L1105. The optimal temperature was determined at different temperatures and pH 7.2, MOPS buffer. To measure the thermostability, the xylanase in 50 mM MOPS buffer, pH 7.2, was incubated for 30 min at different temperatures.

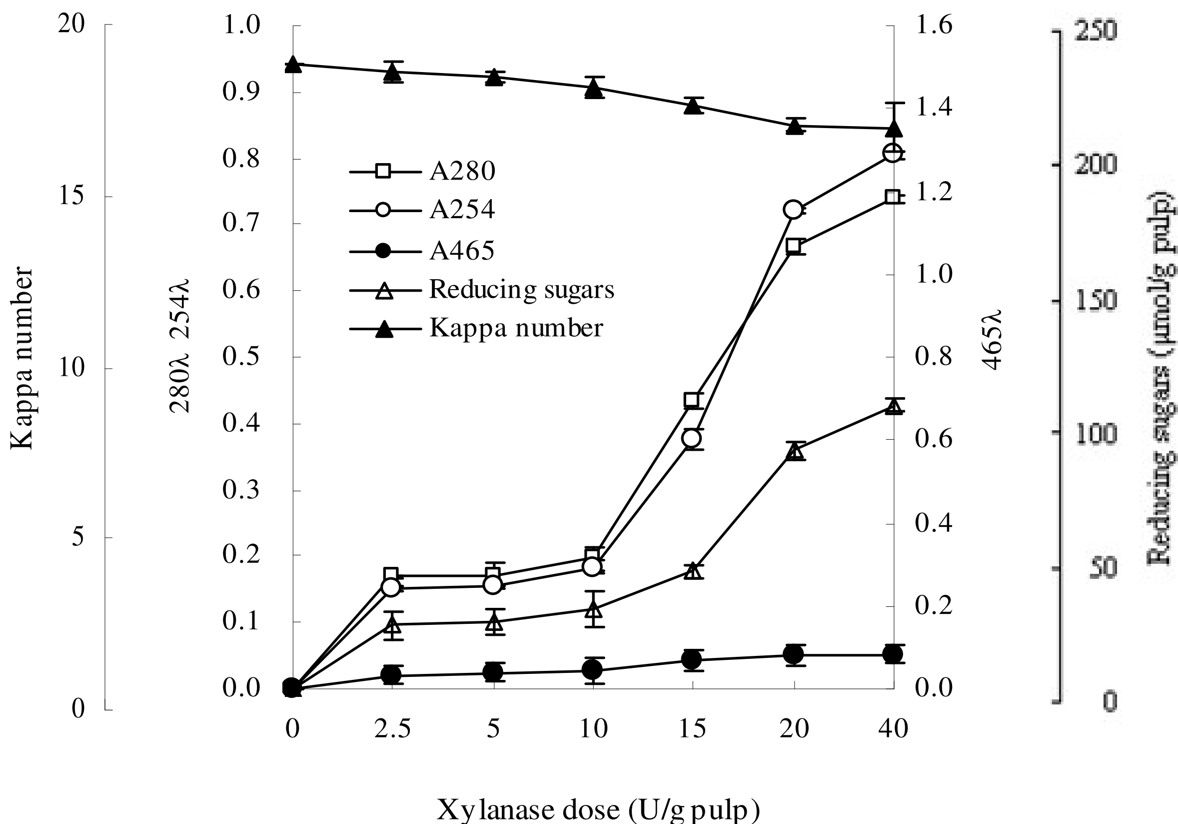

Figure 8. Optimization of xylanase dose for biobleaching of pulp. These experiments were performed at pH 7.0, temperature 50°C, at a retention time of 60 min and a pulp consistency of 5%. Symbols: open square, A280; open circle, A254; closed circle, A465; open triangle, reducing sugars; closed triangle, Kappa number.

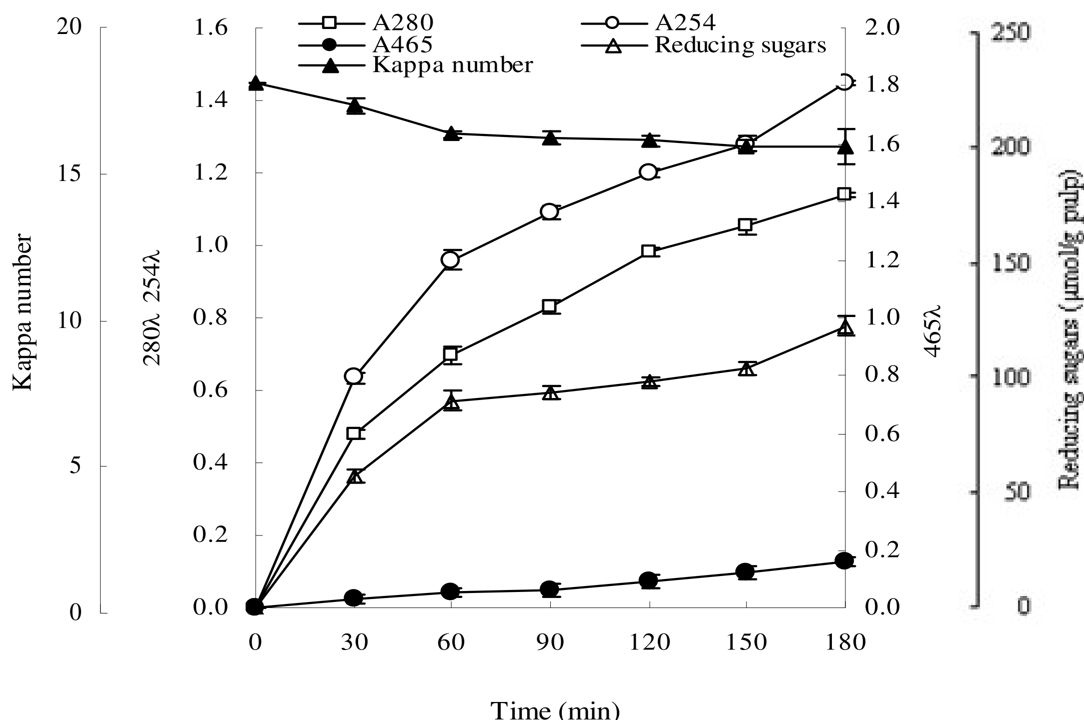

Figure 9. Optimization of retention time for biobleaching of pulp. These experiments were performed at pH 7.0, temperature 50℃ and a pulp consistency of 5%. Symbols: open square,A280; open circle, A254; closed circle, A465; open triangle, reducing sugars; closed triangle, Kappa number.

number coupled to the release of reducing sugars suggested the dissociation of LCC from the pulp fibers (Beg et al., 2000). The release of phenolic compounds (A254 nm) and hydrophobic compounds (A465 nm) were also maximal after 2 h of reaction time (Figure 9). Thus, chromophoric material was released as a result of the enzyme action and these data suggest that there was a significant decrease in the aromaticity of residual lignin, which was confirmed by the decrease in kappa number (Figure 8). Kappa number of untreated wheat straw pulp was 18.08. After treatment with enzyme, it decreased to 16.25. It was effective on wheat straw pulp at a charge of 2.5 U/g dry pulp even after 1 h, where decreases greater than 1.3 kappa number points were observed. The kappa number reductions obtained were 9.7 and 10.1% with 20 U/g and 40 U/g pulp, respectively. The result indicates that the kappa number of the pulp decreased by ~12.2% during the first stage itself. Higher enzyme dose or longer periods of incubation did not enhance the extent of biobleaching benefits significantly (Figures 4 and 5).

Several reports described the reduction in kappa number by different xylanases. The xylanase from *Streptomyces thermoviolaceus* showed a reduction in kappa number by 16% when treating Birchwood kraft pulp. Xylanase from *Streptomyces* sp. QG-11-3 showed a 23% reduction in kappa number when treating eucalyptus kraft pulp (Beg et al., 2000). *Bacillus pumilus* xylanase showed a 14% reduction in kappa number of kraft pulp (Bim and Franco, 2000).

The physicochemical properties of the pulp revealed a 12% reduction in kappa number, as evident from the release of chromophores (A254 nm) and hydrophobic compounds (A465 nm) (Figures 8 and 9). The release of ultraviolet light absorbing material and visible light absorbing material (lignin and phenolic compounds) and the release of reducing sugars are interrelated phenomena. Xylan is a part of hemicellulose and is sandwiched between lignin and cellulose layer. When xylan is degraded by the xylanase, the xylose and other reducing sugars are released from the hemicellulose layer that then results in an increase in the free sugar content in the pulp sample. When the pulp was treated with xylanase, it also resulted in the release of lignin and phenolic compounds from the pulp fibers, which caused the enhancement in absorbance.

The newly isolated *S. chartreusis* L1105, capable of producing xylanase, has been reported for the first time in this study. Maximum xylanase production was obtained using corncobs xylan as a carbon source under optimized nutritional and fermentation conditions. The high level of xylanase production by *S. chartreusis* L1105 indicates that it would be an interesting source of the new xylanase and may have potential economic advantages. The characterization of the xylanase, showing alkaline tolerance and thermal stability, suggests its potential use in biobleaching processes, which require xylanases that

are active at high temperature and pH. The application test results of the new xylanase for pretreatment of wheat straw pulp demonstrated that the additive effects of the action of xylanase rendered the fibers more accessible to chemical bleaching agents, assisting in lowering the concentration of the latter in the subsequent bleaching process. Further studies on the process of bleaching pretreated pulp with the xylanase from the newly isolated strain are in progress.

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