β-Xylosidase from *Streptomyces* sp. CH7 and Its Gene Cloning and Expression in *Escherichia coli*

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β-Xylosidase activity of about 0.9 U/mg of protein was produced from *Streptomyces* sp. CH7 when grown in a medium containing 1% xylan as a carbon source at pH 7.0 and 40°C for 24 h. The enzyme had pH and temperature optima of 6.5 and 55°C, respectively. It was stable at temperatures up to 50°C and to pH values in the range of 4.5 to 9.5. Its gene has been cloned in *E. coli* with pUC18 as a cloning vector. A recombinant plasmid containing a 3.6-kilobase insert was found to express β-xylosidase activity. Subcloning of the insert into pUC19 indicated that the cloned gene also carried its own promoter which was able to function in *E. coli*.

Key words: Gene cloning, *Streptomyces*, β-xylosidase.

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บีตา-ไซโลสิเดสจาก *Streptomyces* sp. CH7 และการโคลนยีนกับการแสดงออกใน *Esherichia coli*

สุมาลี อึ้งใจธรรม จิราวรรณ ธนะ ยุวดี ตาลาวณิช และไพเราะ ปิ่นพานิชการ (2544) *วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย* **26(2)**

Streptomyces sp. CH7 ผลิตบีตา-ไซโลสิเดสได้ประมาณ 0.9 หน่วยต่อมิลลิกรัมโปรตีน เมื่อเลี้ยงในอาหารเลี้ยงเชื้อที่มี 1% ไซแลน เป็นองค์ประกอบที่ pH 7.0 อุณหภูมิ 40°ซ เป็นเวลา 24 ชั่วโมง เอนไซม์นี้ทำงานได้ดีที่สุดที่ pH 6.5 และอุณหภูมิ 55°ซ มีความเสถียรต่ออุณหภูมิได้สูงถึง 50°ซ และต่อ pH ในช่วง 4.5-9.5 งานวิจัยนี้ยังได้โคลนยีนบีตา-ไซโลสิเดสโดยมี *E. coli* เป็นเซลล์เจ้าบ้าน และ pUC18 เป็นพลาสมิดพาหะ พบว่าได้รีคอมบิแนนท์พลาสมิดที่มีชิ้นดีเอ็นเอสอดแทรกขนาด 3.6 กิโลเบส ซึ่งสามารถแสดงออกให้แอคติวิตีของบีตา-ไซโลสิเดสจากการโคลนชิ้นดีเอ็นเอต่อไปโดยเข้าพลาสมิด pUC19 พบว่า ยีนนี้มีโปรโมเตอร์ของตัวเองและสามารถแสดงออกได้ใน *E. coli*

คำสำคัญ การโคลนยีน *Streptomyces* บีตา-ไซโลสิเดส

INTRODUCTION

Xylan is the major component of the hemicellulose found in plant cell walls. It consists of a main chain of xylopyranosyl residues linked by β-1,4-glycosidic bonds and often has O-acetyl, arabinosyl, and methyl glucuronosyl substituents.[1] Hydrolysis of the xylan backbone requires at least two enzymes, namely endo-β-1,4-xylanase and β-xylosidase. Endo-xylanases hydrolyze xylan mainly into xylo-oligosaccharides, and β-xylosidases then hydrolyze the xylo-oligosaccharide to D-xylose.[2] β-Xylosidases are essential for complete degradation of xylan to the final product, xylose, and have been purified and characterized from many bacteria and fungi.[3-6] *Actinomycetes* are emerging as an important source of lignocellulose degradation and *Streptomyces* is among such microorganisms being widely investigated.[7-10]

While a number of β-xylosidase genes have been cloned and characterized from a wide range of bacteria and fungi, there has been very limited information on *Streptomyces* so far.[11-14] The present work, therefore, describes the isolation of β-xylosidase-producing *Streptomyces* and a preliminary study of the enzyme properties, including its gene cloning.

MATERIALS AND METHODS

Bacterial strains and plasmids

Streptomyces sp. CH7 was isolated from a soil sample collected from a sugar cane field in Chonburi province, Thailand, on humic acid-vitamin agar medium[15] adjusted to pH 9.0 and under the incubation temperature of 45^{O}C. *E. coli* DH5α was a host for gene cloning with pUC18 and pUC19 as cloning or subcloning vectors.

Culture conditions for enzyme preparation and transformation

Cultivation of *Streptomyces* was performed by inoculating 100 μl of spore suspension (10^8-10^9 spores/ml) into 25 ml of medium containing (w/v) 1.0% xylan, 0.1% yeast extract, 0.5% polypeptone, 0.5% corn steep liquor, 0.4% K_2HPO_4, 0.02% KCl, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.002% $FeSO_4 \cdot 7H_2O$ adjusted to pH 7.0 or as indicated in the results. Incubation was at 40^{O}C or as otherwise indicated with shaking at 200 rev/min for 24 h. The mycelium was harvested, washed twice with 0.1 M acetate buffer pH 6.5 and mechanically disrupted in a chilled mortar in the presence of an approximately equal amount of alumina. The cell paste was extracted with the same buffer. The supernatant obtained after removing the cell debris by centrifugation at 10,000 g for 10 min was used for β-xylosidase assay. Cultivation of *E. coli* for β-xylosidase assay was in Luria broth (LB) in the presence of 0.5% (w/v) xylan for 15 hr. The enzyme was prepared as described for *Streptomyces*. For transformation, *E. coli* was grown in LB and the cell preparation and transformation were carried out by standard techniques as described by Sambrook *et al*.[16]

β-Xylosidase assay

β-Xylosidase was assayed as described by Poutanen and Puls[3] with minor modifications. The reaction mixture contained 0.05 ml of 50 mM *p*-nitrophenyl -β-D-xylopyranoside (*p*NPX), 0.2 ml of 100 mM sodium acetate buffer, pH 6.5, and 0.25 ml of appropriately diluted enzyme solution. The reaction mixture was incubated at 55^{O}C for 15 min. The reaction was stopped by addition of 2.5 ml of 500 mM Na_2CO_3 and absorbance of the released *p*-nitrophenol was immediately measured at 405 nm.

One unit of the enzyme was defined as the amount of enzyme that catalyzes the release of 1 micromole of *p*-nitrophenol per min under the assay conditions.

Effect of temperature and pH on enzyme activity

β-Xylosidase activity was determined under standard conditions except at different temperatures from 35^{O}C to 70^{O}C. It was then determined at different pHs from 4.0 to 9.5 at the optimal temperature.

Effect of temperature and pH on enzyme stability

Enzyme solution was preincubated in the absence of a substrate at different temperatures from 40^{O}C to 70^{O}C or at different pHs from 4.0 to 9.5 at 0^{O}C for 30 min; thereafter the residual activity was determined under optimal conditions.

Protein content

Protein content was determined by the method of Lowry *et al.*[17] using bovine serum albumin as a standard.

Construction of gene library and screening for β-xylosidase expression

Chromosomal DNA from *Streptomyces* sp. CH7 was isolated, partially digested with *Sau*3AI and fractionated into fragments of 1-4 kb as described by Kieser *et al.*[18] Plasmid isolation and manipulation were performed by standard protocols as described by Sambrook *et al.*[16] DNA fragments were ligated into the *Bam*HI site of pUC18 at the molar ratio of DNA insert to vector of 5:1. The recombinant plasmids were transformed in *E. coli* DH5α and the transformed colonies were screened on agar plates containing 10 μg of 4-O-methyl umbelliferyl-β-D-xylopyranoside (MUX) per ml. Transformants expressing β-xylosidase exhibited blue-white fluorescence under UV light after overnight incubation at 37^{O}C due to the umbelliferone released by the enzyme cleavage.

RESULTS AND DISCUSSION

Isolation of β-xylosidase-producing *Streptomyces*

Streptomyces spp. capable of growing in alkaline pH at 45^{O}C were isolated and screened for β-xylosidase activity. From 261 isolates, *Streptomyces* sp. CH7 was found to have the highest β-xylosidase activity. However, the optimal cultivation temperature and pH for the enzyme production were 40^{O}C and 7.0, respectively (Figure 1). As was the case for *Streptomyces lividans* as reported by Kluepfel *et al.*,[7] the maximal β-xylosidase production by *Streptomyces* sp. CH7 was obtained from the 24-h culture (Table 1). The amount of β-xylosidase produced by this organism was considerably higher when compared with those of the reported amounts of 0.43, 0.37, and 0.055 U/mg of protein from *Aspergillus terreus*,[19] *Streptomyces lividans*,[7] and *Streptomyces olivochromogenes*,[20] respectively.

Properties of the crude β-xylosidase

The enzyme had temperature and pH optima of 55^{O}C and 6.5, respectively (Figure 2 A&C). Its optimal temperature was slightly higher than those of the β-xylosidases from *Streptomyces* sp. 3137[21] and *Streptomyces* sp. CH-M-1035[10] which were 45-50^{O}C. It was thermally stable up to 50^{O}C but above this value its stability decreased sharply though it retained about 50% of its activity at 55^{O}C over 30 min (Figure 2B). It was stable in the pH range of 4.5 to 9.5 when pre-incubated for 30 min (Figure 2D).

Figure 1. Effects of temperature (A) and pH (B) on β-xylosidase production by *Streptomyces* sp. CH7 cultivated for 24 h.

Table 1. β-Xylosidase activity of *Streptomyces* sp. CH7 at different cultivation times.

Cultivation Time (h)	Specific Activity (U/mg protein)
24	0.90
48	0.58
72	0.45

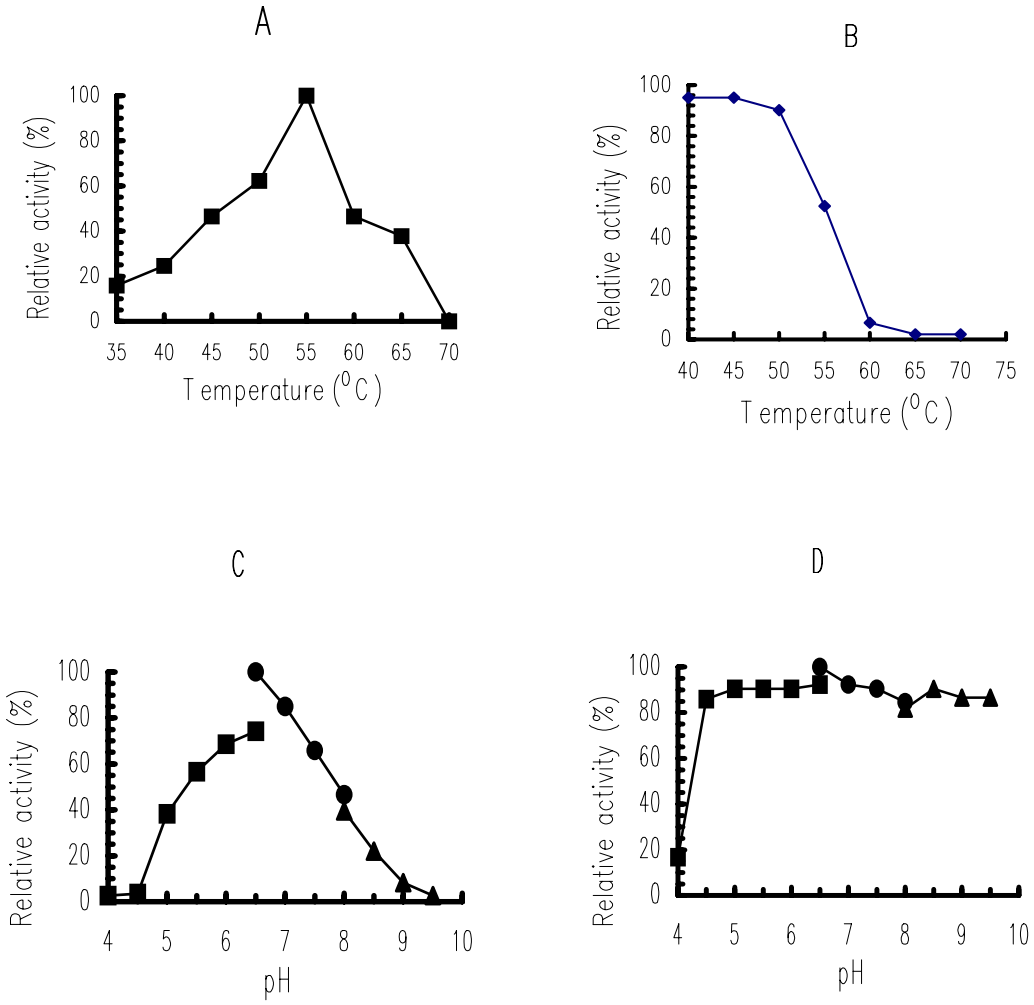

Figure 2. Effects of temperature on activity (A) and stability (B) and effects of pH (■ acetate; ● phosphate; ▲ tris.HCl) on activity (C) and stability (D) of β-xylosidase from *Streptomyces* sp. CH7. The maximum activity in each experiment was specified as 100% except for the temperature stability experiment in which the activity from the enzyme kept at 4°C was specified as 100%.

Cloning of β-xylosidase gene in *E. coli* DH5α

One β-xylosidase-positive clone was obtained from approximately 5,000 transformants. A plasmid, designated pCH7-1, having the size of 6.3 kb and containing a 3.6 kb-DNA insert, was isolated from this clone. Re-transformation of pCH7-1 into *E.coli* DH5α resulted in all transformants exhibiting fluorescence, indicating the presence of the β-xylosidase gene in pCH7-1 (Figure 3). Subcloning of

the 3.6 kb-*Eco*RI-*Hin*dIII fragment into pUC19 gave a plasmid, designated pCH7-1(19), which also resulted in all transformants showing fluorescence when it was transformed in *E. coli* DH5α (data not shown). This result indicated that the cloned gene also contained its own promoter which was able to function in *E. coli*.

Figure 3. Transformants of *E. coli* DH5α harbouring pCH7-1 exhibiting blue-white fluorescence on an agar plate containing MUX.

Assaying for β-xylosidase activity of *E.coli* DH5α harboring pCH7-1 or pCH7-1(19) also confirmed the capability of the β-xylosidase gene expression in *E.coli* under *lac* or its own promoter, although expression under its own promoter was of slightly lower efficiency (Table 2). This finding was in agreement with Hindle *et al.*[22] who showed that a promoter of the *arg CJB* gene from *Streptomyces coelicolor* A3(2) was able to function in *E. coli*. Deng *et al.*[23] also reported that there was certain similarity between promoters from *E.coli* and *Streptomyces*. Even though the β-xylosidase gene from *Streptomyces* sp. CH7 was able to express in *E. coli,* the enzyme specific activity was still lower than that from the original strain (Figure 1 and Table 2). Recently, Choa *et al.*[24] reported the construction of a novel expression vector for *E.coli* based on the phase- and growth rate-dependent *rmf* (ribosome modulation factor) promoter. With this vector using the *lacZ* gene as a model for gene expression, a high level of β-galactosidase was produced when the recombinant cells were subject to temperature downshift or substrate famine. Therefore, if the β-xylosidase gene from the present work is cloned in this vector,

overexpression of the gene may be obtained under controlled growth conditions.

A preliminary restriction map of pCH7-1 is shown in Figure 4. Further study on the nucleotide sequence of the cloned gene including purification and characterization of β-xylosidase from *Streptomyces* sp. CH7 and the gene expression product are being investigated.

Table 2. Expression of β-Xylosidase activity in *E. coli* DH5α harbouring plasmid pUC18, pUC19, pCH7-1 or pCH7-1(19).

Plasmid	Specific Activity (U/mg protein)
pUC18	0.01
pUC19	0.01
pCH7-1	0.32
pCH7-1(19)	0.10

Figure 4. Restriction endonuclease map of pCH7-1 and location of the DNA insert containing β-xylosidase gene. The thick line represents the DNA insert.

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