

**PARTIAL PURIFICATION AND SOME PROPERTIES OF XYLANASES
FROM *Streptomyces* Ab106.3**

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**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE
IN BIOTECHNOLOGY**

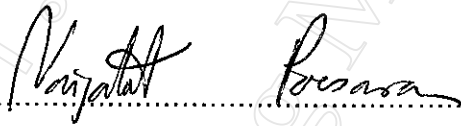
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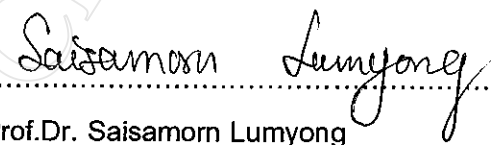
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ABSTRACT

Streptomyces Ab 106.3 produced multiple forms of xylanases. It was found that baggase was the best carbon source for xylanase production by *Streptomyces* Ab106.3. Xylanase of 15.9 U/ml could be obtained from a 5-L fermentor after fermentation for 144 hr at 55 °C, agitation rate of 200 rpm, aeration rate of 1vvm, and pH of the medium was kept at 7. The xylanase activity obtained from 5-L fermentor was about 2 folds compared to the shake flask culture. Tween 80 enhanced enzyme production, but it caused purification difficulty. The partial purification of xylanases was carried out by ammonium sulfate precipitation, DEAE-cellulose ion-exchange column chromatography, and Sephadex G-100 gel-filtration column chromatography, respectively. After passed both column chromatographies the crude xylanase was separated into six fractions, A1-A3 and B1-B3. The partial purified xylanases have the optimum pH for xylanase activity, optimum temperature for xylanase activity, and the pH stability of 6.0-7.0, 60-70 °C, and 5.0-7.0, respectively. The SDS-PAGE and zymogram results, *Streptomyces* Ab106.3 might produce at least 3 forms of xylanases, first xylanase had the largest molecular weight in the ranges of 45-66 kDa, second xylanase had the medium molecular weight in the ranges of 30-45 kDa, the last one had the smallest molecular weight in the range of 20.1-30 kDa.

ชื่อเรื่องวิทยานิพนธ์	การเตรียมเอนไซม์กึ่งบริสุทธิ์และสมบัติบางประการของไซลาเนสจากเชื้อสเตรปโตมัยซิส เอบี106.3	
ชื่อผู้เขียน	นายเอกลักษณ์ เหมจินดา	
วิทยาศาสตร์มหาบัณฑิต	สาขาวิชาเทคโนโลยีชีวภาพ	
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บทคัดย่อ

Streptomyces Ab106.3 ผลิตไซลาเนสได้หลายชนิด จากผลการทดลองพบว่าชานอ้อยเป็นแหล่งคาร์บอนที่เหมาะสมในการผลิตไซลาเนสโดย *Streptomyces* Ab106.3 การผลิตไซลาเนสในถังหมักขนาด 5 ลิตรเป็นเวลา 144 ชั่วโมงที่อุณหภูมิ 55 องศาเซลเซียส ความเร็วรอบของการกวน 200 รอบต่อนาที อัตราการให้อากาศที่ 1vvm และค่าความเป็นกรด-ด่างของอาหารเลี้ยงเชื้อเท่ากับ 7 ได้ไซลาเนส 15.9 U/ml ซึ่งสูงกว่าการผลิตในฟลasks พบว่า Tween80 ช่วยเพิ่มปริมาณการผลิตไซลาเนสได้แต่รบกวนขั้นตอนการแยกเอนไซม์ให้บริสุทธิ์ ได้ทำการแยกเอนไซม์ให้บริสุทธิ์ขึ้นบางส่วนโดย การตกตะกอนโปรตีนด้วยแอมโมเนียมซัลเฟต การใช้คอลัมน์แลกเปลี่ยนประจุ DEAE-cellulose และ คอลัมน์คัดแยกขนาด Sephadex G-100 ตามลำดับ ขั้นตอนดังกล่าวสามารถแยกไซลาเนสออกเป็น 6 ส่วนคือส่วน A1-A3 และ B1-B3 พบว่าไซลาเนสที่บริสุทธิ์ขึ้นบางส่วนมีค่าความเป็นกรด-ด่างที่เหมาะสมในการทำงานในช่วง 6.0-7.0 อุณหภูมิที่เหมาะสมในการทำงานอยู่ในช่วง 60-70 องศาเซลเซียส และ ไซลาเนสที่บริสุทธิ์ขึ้นบางส่วนแล้วสามารถคงสภาพได้ที่ค่าความเป็นกรด-ด่างในช่วง 5.0-7.0 จากผลของโครมาโตแกรม SDS-PAGE และ Zymogram ที่ความเข้มข้นของเจล 12 เปอร์เซ็นต์ พบว่า *Streptomyces* Ab106.3 ผลิตไซลาเนสได้อย่างน้อย 3 ชนิดที่มีขนาดของโมเลกุลแตกต่างกัน โดยมีขนาดโมเลกุลในช่วง 45-66 kDa 30-45 kDa และ 20.1-30 kDa ตามลำดับ

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ABBREVIATION AND SYMBOLS

μ	Micro
l	Liter
β	Beta
α	Alpha
min	Minute
h	Hour
g	Gram
$^{\circ}$	Degree
C	Celsius
U	Unit
p	Para
k	Kilo
Da	Dalton
MW	Molecular weight
IU	International Unit
D	Dextro
L	levo
T $\frac{1}{2}$	Half life
/	per

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEWS

1.1 Introduction

Xylanases are drawing increased attention, because of their usefulness in biotechnological applications. They might also be used in the bioconversion of lignocellulosic materials to fuels and chemicals. (Grag *et al.*, 1996; Grag *et al.*, 1998; and Jeffries, 1996). *Streptomyces* Ab106.3, isolated from teak plantation area in Chiangmai University in order to use in waste utilization project, is able to produce xylanases. Study on production, purification, and characterization of xylanases produced by this microorganism may lead to further applications of the enzyme.

1.2 Xylan and Xylanases

1.2.1 Xylan

Wood is made up largely of lignocellulosic materials, cellulose hemicellulose and lignin, in various proportions (Eriksson *et al.*, 1990) as shown in Table 1.1

In general, plants contain 20-30 % of hemicellulosic materials (Kulkarni *et al.*, 1999). Hemicelluloses are non-cellulosic polysaccharides those are found in plant tissues as shown in figure 1.1, A) represent cell wall layers of tracheids; ML is middle lamella; P is primary cell wall; S₁, S₂, S₃ are layers of the secondary cell wall B) represent ultra structural of lignin, cellulose, and hemicellulose components. Figure 1.2. shows the arrangement of cellulose, hemicellulose, and lignin in plant cell wall a) transverse view, b) longitudinal view.

Xylan is the major constituent of hemicellulose and is second most abundant renewable resource with high potential for degradation to useful end products.