

รูปแบบของแอนติเจนที่สกัดได้จากพืชเลี้ยง  
ของ เชื้อปรสิตคนเนส บีสวาคัมลีโอ ที่แยกได้จากผู้ป่วย

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THE PATTERNS OF SONIC EXTRACT OF *PSEUDOMONAS PSEUDOMALLEI*  
FROM CLINICAL ISOLATES

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## พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสี่เหลี่ยมเพียงแผ่นเดียว

ศ.ดร.ลักขณา ฉันทรัชต์ดา ~ รูปแผนของแอนติเจนที่สกัดได้ด้วยคลื่นเสียงของเชื้อ *Pseudomonas pseudomallei* ที่แยกได้จากผู้ป่วย (THE PATTERNS OF SONIC EXTRACT OF *PSEUDONONAS PSEUDOMALLEI* FROM CLINICAL ISOLATES) อ. พิรัชชา รศ.พญ.สมใจ เจริญประยูร, อ.ดร.เกรียงศักดิ์ สายธนู, 122 หน้า.

วัตถุประสงค์ของการศึกษานี้ เพื่อศึกษารูปแบบน้ำหนักโมเลกุลของส่วนประกอบที่สกัดได้ด้วยคลื่นเสียงของเชื้อ *Pseudomonas pseudomallei* ที่แยกได้จากผู้ป่วย 32 สายพันธุ์โดยวิธี SDS-PAGE และหาความสัมพันธ์ระหว่างแอนติเจนของเชื้อนี้กับบัคทีเรียบางชนิดโดยวิธี immunoblot

จากผลของการศึกษาโดยวิธี SDS-PAGE เราสามารถจัดแบ่งรูปแบบโดยยี่ห้อส่วนประกอบที่มีน้ำหนักโมเลกุลดังต่อไปนี้ คือ 56.0, 27.5, 19.9, 19.6, 14.5, 14.4 และ 12.6 กิโลดาลตัน โดยจำแนกสายพันธุ์ทั้งหมดออกได้เป็น 6 Types คือ Type I ถึง Type VI ซึ่งมีจำนวน 9, 6, 1, 3, 3 และ 10 สายพันธุ์ในแต่ละ Type ตามลำดับ

ส่วนการศึกษาแอนติเจนด้วยวิธี immunoblot โดยใช้รูปแบบของแอนติเจนของเชื้อ *P. pseudomallei* NCTC 4845 เป็นหลัก สามารถแบ่งเชื้อทั้ง 32 สายพันธุ์เป็น group A, B และ C ซึ่งสายพันธุ์ใน group A จะตรงกับ Type I และ Type II ใน SDS-PAGE, group B ตรงกับ Type IV และ group C ตรงกับ Type III, V และ VI

แอนติเจนที่มีน้ำหนักโมเลกุล 16.8, 20.7, 24.1, 107.0, 115.0 และ 140.0 กิโลดาลตัน พบได้เฉพาะในเชื้อ *P. pseudomallei* ทั้ง 32 สายพันธุ์ สำหรับแอนติเจนร่วมระหว่าง *P. pseudomallei* กับบัคทีเรียชนิดอื่น ๆ คือ *P. aeruginosa* ATCC 27853, *P. cepacia* JCM 5510, *P. stutzeri* JCM 5965, *P. putida* JCM 6160, *P. maltophilia* JCM 3801, *V. cholerae* 569B, *S. typhi* NCTC 781, *E. coli*, ATCC 25922 and *S. aureus* ATCC 25923 มีประมาณ 30 ชนิด ในช่วงน้ำหนักโมเลกุลตั้งแต่ 12.0 ถึง 102.0 กิโลดาลตัน ซึ่งอาจจะอธิบายถึงปฏิกิริยาข้ามกลุ่มที่เกิดขึ้นโดยวิธีการตรวจทางน้ำเหลืองอื่น ๆ

ผลของการศึกษานี้เป็นข้อมูลเบื้องต้นในการหา common specific epitope ของเชื้อนี้เพื่อนำไปประยุกต์ใช้ในการวินิจฉัยและการผลิตวัคซีน นอกจากนี้ยังเป็นข้อมูลในการศึกษาวิทยาศาสตร์ขั้นพื้นฐานและการศึกษาทางด้าน taxonomy, epidemiology และ serology ของเชื้อนี้ต่อไป

ภาควิชา สหเวชศาสตร์จุลชีววิทยาทางการแพทย์.....  
สาขาวิชา .....จุลชีววิทยาทางการแพทย์.....  
ปีการศึกษา .....2531.....

ลายมือชื่อนิสิต .....  
ลายมือชื่ออาจารย์ที่ปรึกษา .....  
Somjai Puiyayong

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสี่เหลี่ยมเพียงแผ่นเดียว

SUDALUCK CHANTARACHADA : THE PATTERNS OF SONIC EXTRACT OF  
PSEUDOMONAS PSEUDOMALLEI FROM CLINICAL ISOLATES.  
THESIS ADVISOR : ASSO. PRO. SOMJAI REINPRAYOON, M.D., INST. KRIENGSA  
SAITANU, 122 pp.

The purposes of this study were to examine the molecular weight pattern of sonic extract of clinical isolated *P. pseudomallei* by SDS-PAGE and also possible antigenic relationship between this organism to other bacteria by an immunoblot assay.

The sonic extracts of 32 clinically isolated strains of *P. pseudomallei* were subjected to the SDS-PAGE technique, and were able to differentiate into six types. In addition to the intensity of the bands, the presence of bands at molecular weights of 56.0, 27.5, 19.9, 19.6, 14.5, 14.4 and 12.6 Kd were used as the criteria for this typing. The 32 strains could be typed as follows: 9 strains were in Type I; 6 strains were in Type II; 1 strain was in Type III; 3 strains were in Type IV; 3 strains were in Type V and 10 strains were in Type VI. A significant correlation between the anatomic sites of infection, geographic locations, and SDS-PAGE types were not found.

These clinically isolated strains were further studied by the immunoblot technique using, hyperimmune antiserum against *P. pseudomallei* NCTC 4845. The visible antigenic bands allowed the classification of our 32 isolates into three groups: A, B and C. The correlation between the SDS-PAGE typing and the immunoblot grouping was noted. SDS-PAGE Type I and II were within Immunoblot group A, the Type IV was with in group B. and the Type III, V and VI were in group C.

In addition, other organisms such as *P. aeruginosa* ATCC 27853, *P. cepacia* JCM 5510, *P. stutzeri* JCM 5969, *P. putida* JCM 6160, *P. multophila* JCM 3801, *V. cholerae* 569B, *S. typhi* NCTC 781, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 showed antigenic bands ranging from 12.0 to 140.0 Kd which were commonly found in all of the *P. pseudomallei* isolates.

Interestingly antigenic bands at the molecular weight of 16.8, 20.7, 24.1, 107.0, 115.0 and 140.0 which were in common to all strains of *P. pseudomallei* were absent from the bacterial strains named above.

Results obtained from this study may be useful as basic knowledge in the further investigation for common specific epitope of *P. pseudomallei* in the application for production of diagnostic kit as well as vaccine production. In addition they can be use for study of basic sciences, taxonomy, epidemiology, and serology of this organisms.

ภาควิชา สหสาขาวิชาจุลชีววิทยาทางการแพทย์.....  
สาขาวิชา.....จุลชีววิทยาทางการแพทย์.....  
ปีการศึกษา..... 2531.....

ลายมือชื่อนิสิต.....  
ลายมือชื่ออาจารย์ที่ปรึกษา.....  
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To my husband and daughters  
for their unlimited love and understanding through out my education.

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## ABBREVIATIONS

ATCC	=	American Type Culture Collection (Rockville, Md.)
BHIB	=	brain heart infusion broth
BSA	=	bovine serum albumin
°C	=	degree celsius
CDC	=	Centers for Disease Control (Atlanta)
CHPp	=	clinical isolation of <i>Pseudomonas pseudomallei</i> from hospital in Northern part of Thailand
cm	=	centimeter
conc	=	concentration
CPp	=	clinical isolation of <i>Pseudomonas pseudomallei</i> from Chulalongkorn hospital
DDW	=	deionized distilled water
DW	=	distilled water
<i>E. coli</i>	=	<i>Escherichia coli</i>
ed	=	editor
EDTA	=	ethylenediamine tetra acetic acid
EIA	=	enzyme immuno assay
et al	=	et alii
etc	=	et cetera
FeCl <sub>3</sub>	=	ferric chloride
Fig	=	figure
g	=	gravity
gm	=	gram
HCl	=	hydrochloric acid

$H_2O_2$	=	hydrogen peroxide
$HNO_3$	=	nitric acid
hr	=	hour
IFA	=	indirect immunofluorescent assay
Ig	=	immunoglobulin
IHA	=	indirect haemagglutination
JCM	=	Japanese Culture Microbiology
KCl	=	potassium chloride
KCN	=	potassium cyanide
Kg	=	kilogram
$KH_2PO_4$	=	potassium phosphate monobasic
$K_2HPO_4$	=	potassium phosphate dibasic
KOH	=	potassium hydroxide
KPp	=	clinical isolation of <i>Pseudomonas pseudomallei</i> from hospital in Southern part of Thailand
L	=	litre
M	=	Molarity
mA	=	milli ampre
mg	=	milligram
$MgSO_4 \cdot 7H_2O$	=	magnesium sulphate hepta hydrate
min	=	minute
mm	=	millilitre
MR	=	methyl red, methyl red test
M.W.	=	Molecular Weight
N	=	Normality
$Na_2CO_3$	=	sodium carbonate
$Na_2HPO_4$	=	sodium phosphate dibasic

NaCl	=	sodium chloride
NaOH	=	sodium hydroxide
NC	=	nitrocellulose
NCTC	=	National Collection of Type Cultures (London)
NFM	=	non fat dry milk
ng	=	nanogram
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	=	ammonium sulfate
No	=	number
NSS	=	normal saline solution
OF	=	oxidation-fermentation
ONPG	=	o-nitrophenyl-beta-D-galactopyranoside (test)
<i>P.</i>	=	<i>Pseudomonas</i>
PB	=	phosphate buffer
pb ac	=	lead acetate
PBS	=	phosphate buffer saline
PBS/T	=	Tween 20 in phosphate buffer saline
<i>S. aureus</i>	=	<i>Staphylococcus aureus</i>
SDS-PAGE	=	sodium dodecyl sulfate polyacrylamide gel electrophoresis
soln	=	solution
SRBC	=	sheep red blood cell
SS	=	salmonella shigella agar
<i>S. typhi</i>	=	<i>Salmonella typhi</i>
TEMED	=	N,N,N,N,-tetramethylenediamine
Trizma	=	Tris (hydroxy methyl) aminomethane
TSI	=	triple sugar iron ager
µm	=	micrometre

<i>V. cholerae</i>	=	<i>Vibrio cholerae</i>
VP	=	Voges Proskauer
V/V	=	volume by volume
W/V	=	weight by volume



## Objectives

1. To evaluate the patterns of sonic extract of *P. pseudomallei* from clinical isolated strains by using SDS-PAGE method.

2. To study the relationship of various antigens between strains of *P. pseudomallei* and some related organisms that have shown cross serological reactivity, microagglutination and/or indirect haemagglutination test by using immunoblot technique.