

ISOLATION, IDENTIFICATION AND CHARACTERIZATION
OF A BACTERIAL STRAIN AND MONOSACCHARIDE PRODUCED
FROM BACTERIA CULTURES USED IN MALAY
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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF BACTERIA
STRAIN AND MONOSACCHARIDE PRODUCED BY BACTERIA CULTURES
USED IN MALAY – TRADITIONAL MEDICINE

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FAKULTI SAINS DAN TEKNOLOGI
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2006

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STRAIN AND MONOSACCHARIDE PRODUCED BY BACTERIA CULTURES
USED IN MALAY – TRADITIONAL MEDICINE

By

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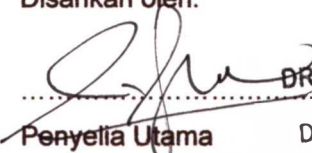


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PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF BACTERIA STRAIN AND MONOSACCHARIDE PRODUCED BY BACTERIA CULTURES USED IN MALAY-TRADITIONAL MEDICINE** oleh **TEVAN RAMANATHAN**, no. matrik: **UK8412** telah diperiksa dan semua pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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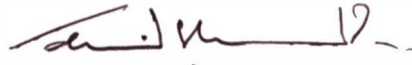

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LIST OF ABBREVIATION

ATP	-	Adenosine triphosphate
AAB	-	Acetic acid bacteria
bp	-	Base pair
CPS	-	Capsular Polysaccharides
CO ₂	-	Carbon dioxide
CH ₂ O	-	Aldehyde
C:N	-	Carbon ratio Nitrogen
CaCO ₃	-	Calcium carbonate
DNA	-	Deoxyribonucleic acid
DOS	-	Disk operating system
DTP	-	Dihydroneopterin triphosphate pyrophosphatase
TDP	-	Thiamine triphosphatase
EPS	-	Exopolysaccharides
GalU	-	UDP-glucose pyrophosphorylase
g	-	Gram
g/l	-	Gram per Litter
HoPS	-	Homopolysaccharides
HePS	-	Heteropolysaccharides
HCl	-	Acid hydrochloric
H ₂ S	-	Hydrogen sulphide
H ₂ O	-	Water
H ₂ O ₂	-	Hydrogen dioxide,
H ₂ S	-	Hydrogen sulphide

h	-	Hour
ID	-	Identity
kcal	-	Kilocalorie
kb	-	kilo base
K ₂ HPO ₄	-	Dipotassium hydrogen orthophosphate
LPS	-	Lipopolysaccharides
LAB	-	Lactic acid bacteria
ml	-	Milliliter
min	-	Minute
M	-	Molar
mm	-	Millimeter
Mg	-	mille gram
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
nm	-	Nano meter
NAD (P) ⁺	-	Nicotinamide adenine dinucleotide phosphate
O ₂	-	Oxygen
O/F	-	Oxidation/Fermentation
PTS	-	Phosphotransferase system
PEP	-	Phosphoenolpyruvet
PQQ	-	Pyrrroloquinolinquinone
PC	-	Paper chromatography
PIBWIn	-	Probiotic Identification of Bacteria for Windows
R _f	-	Retention factors
RfbA	-	dTDP-glucose pyrophosphorylase

rpm	-	rotation per minute
rRNA	-	Ribosomal RNA
S	-	Sulphur
TFA	-	Trifluoroacetic acid
UDP	-	Uridine diphosphate
UTI	-	Urinary tract infection
v/v	-	volume/volume
WPS	-	Cell wall polysaccharide
α -PGM	-	α -phosphoglucomutase
μm	-	Micrometer
α	-	Alpha
β	-	Beta
$^{\circ}\text{C}$	-	Degree Centigrade
δ	-	Delta
ϵ	-	Epsilon
ΔG	-	Free enthalpy
γ	-	Gamma
μl	-	Micro litter
%	-	Percentage

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ABSTRACT

An experiment was conducted to isolate and to identify the polysaccharide producing bacteria in Malay-traditional medicine. The medicine broth was spread in nutrient agar in order to obtain bacterial culture. Obtained bacterial cultures were characterized via series of morphological and biochemical test. A total of 14 isolates were obtained and all was characterized until genus level. All the isolated bacteria were characterized as *Bacillus* sp. A total of 10 isolates were further characterized until the suspected species level by using PIBWIn software. Isolate 01, 02 and 09 were suspected as *Bacillus subtilis ssp. subtilis*; Isolate 03, 04, 06, and 07 were suspected as *Bacillus pumilis* and isolate 08 and 10 were suspected as *Bacillus coagulans* and *Bacillus lincheniformis* respectively. Five strains were found to produce exo-polysaccharide. Species of monosaccharide present in exo-polysaccharide were purified and identified using paper chromatography method. The sugar was separated by using ethyl acetate: acetic acid: formic acid: water (18:3:1:4, v/v) as a top solvent for 18hr. Lactose, raffinose, maltose, trehalose, galactose, glucose, mannose, arabinose, xylose and rhamnose are the species of monosaccharides that have presence in exo-polysaccharide of isolated bacteria.

ABSTRAK

Suatu eksperimen telah dijalankan untuk menghasilkan polisakarida daripada bakteria yang diperolehi daripada ubat tradisi melayu. Larutan perubatan telah disebarikan dalam agar nutrisi untuk mendapatkan kultur bakteria. Kultur bakteria yang diperolehi telah dicirikan melalui satu siri ujian morfologi dan biokimia. Sejumlah 14 pencilan telah diperolehi dan dikenalpasti sehingga ke peringkat genus. Semua bakteria dicirikan sebagai genus *Bacillus* sp. Sejumlah 10 pencilan dicirikan dengan lebih lanjut sehingga tahap spesies menggunakan perisian PIBWin. Pencilan 01, 02, dan 09 dijangka sebagai *Bacillus subtilis* ssp. *subtilis*; pencilan 03, 04, 06, and 07 pula dijangka sebagai *Bacillus pumilis* dan pencilan 08 dan 10, masing-masing dijangka sebagai *Bacillus coagulans* dan *Bacillus licheniformis*. Lima strain didapati menghasilkan ekso-polisakarida. Spesis monosakarida yang terdapat dalam ekso-polisakarida dikenalpastikan dengan menggunakan kaedah kromatografi kertas. Gula yang terdapat dalam eksopolisakarida tersebut dipisahkan dengan menggunakan etil asetat: asetik asid: asid formik: air (18:3:1:4, v/v). Laktosa, rafinosa, maltosa, trihalosa, galaktosa, glukosa, mannosarabinosa, zilosa dan ramnosa adalah spesis monosakarida yang hadir dalam eksopolisakarida bakteria yang telah dipencil.