

**MICROBIAL COMMUNITY FROM MANGROVE
RHIZOSPHERE MECHANISTIC STUDY AND PLANT
PROBIOTIC POTENTIAL IN DUCKWEED**

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UNIVERSITI SAINS ISLAM MALAYSIA

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AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own unless specified and duly acknowledged by quotation.

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In the name of Allah, Most Precious Most Merciful

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ABSTRAK

Baja kimia yang digunakan untuk membekalkan nutrien kepada tumbuhan dapat meningkatkan produktiviti pertanian dengan cepat yang mana ia adalah penting untuk mencapai permintaan makanan dari penduduk dunia yang semakin meningkat. Walau bagaimanapun, baja kimia boleh memudaratkan, dengan memberikan kesan negatif terhadap ekologi alam sekitar dan kesihatan manusia. Oleh itu, bajabio telah dianggap sebagai pengganti terbaik untuk baja kimia untuk meningkatkan pertumbuhan tumbuhan perumah. Dalam kajian ini, mikrob berkaitan bakau yang dikumpul daripada tanah telah dianalisis dan digunakan untuk menghasilkan bajabio. Objektif pertama kajian ini adalah untuk mengenal pasti pelbagai spesies mikrob dan fungsi gen daripada tiga sampel tanah yang dikumpul di kawasan bakau sungai air tawar di Sungai Lukut Negeri Sembilan, Malaysia dengan menggunakan Penjujukan Seluruh Genom metagenomik (mWGS). Objektif kedua adalah untuk mengenal pasti spesies bakteria yang dihidupkan pada media pemilihan iaitu bakteria pengikat nitrogen, bakteria pelarut kalium dan bakteria pelarut fosfat dengan menggunakan PCR dan penjujukan gen 16s rRNA. Objektif terakhir adalah untuk menentukan spesies bakteria yang terpilih yang boleh memberi kesan kepada tumbuhan kiambang itik dari segi kadar pertumbuhan dan kandungan protein. Keputusan daripada analisis mWGS menunjukkan taburan bakteria yang berbeza daripada genus *Bradyrhizobium*, *Methyloceanibacter* dan *Desulfobacterales* telah dikesan dalam sampel tanah yang dikumpul dari tiga lokasi. Untuk teknik bergantung kepada kultur, spesies bakteria yang hidup pada media khusus telah dikenal pasti sebagai *Acinetobacter radioresistens*, *Brachybacterium paraconglomeratum* dan *Enterobacter cloacae* yang dikenali sebagai bakteria pengikat nitrogen, *Klebsiella quasipneumoniae*, *Bacillus tropicus* dan *Paenibacillus pasadenensis* yang disaring sebagai bakteria pelarut kalium, dan *Bacillus cereus* dan *Bacillus thuringiensis* yang disaring sebagai bakteria pelarut fosfat. Seterusnya, tiga set bajabio yang merangkumi Set A yang mengandungi *A. radioresistens*, *K. quasipneumoniae* dan *B. cereus*, Set B yang mengandungi *B. paraconglomeratum*, *B. cereus* dan *B. tropicus*, dan Set C yang mengandungi *E. cloacae*, *P. pasadenensis* dan *B. thuringiensis* dengan sebanyak $\times 10^6$ cfu/g telah diformulasi dengan mencampurkan dalam setiap set spesies bakteria dengan kompos komersial. Hasil kajian menunjukkan Set C merupakan bajabio terbaik berbanding dengan Set A dan Set B kerana ia boleh meningkatkan kandungan nitrogen, fosforus dan kalium dalam tanah serta meningkatkan pertumbuhan dan protein tumbuhan kiambang itik. Ini menunjukkan bahawa bajabio yang diformulasikan dengan bakteria berkaitan bakau boleh meningkatkan pertumbuhan kiambang itik serta kandungan proteinnya.

Kata kunci: Tanah bakau, Probiotik tumbuhan, 16s rRNA, Nutrien tanah, Kiambang itik

ABSTRACT

Chemical fertilizers have been utilized to provide nutrients to plants and resulted in rapid increase of agricultural productivity, which is important to achieve the growing world population's demand for food. However, chemical fertilizers can be harmful, with negative effects on the environmental ecology and human health. Therefore, biofertilizers are considered as the best substitute for chemical fertilizers for enhancing the growth of host plants. In this study, mangrove-associated microbes collected from soils were analyzed and used to develop biofertilizer. The first objective of this study was to identify a wide range of microbial species and function of genes from three soil samples collected at the freshwater riverine mangrove at Sungai Lukut, Negeri Sembilan, Malaysia by using metagenomic Whole Genome Shotgun Sequencing (mWGS). The second objective was to isolate and identify bacterial species grown on selective media which are nitrogen fixing bacteria, potassium solubilizing bacteria and phosphorus solubilizing bacteria by using PCR and sequencing of the 16s rRNA gene. The last objective was to determine the selected bacterial species that can affect duckweed plants in terms of growth rate and protein contents. Results from mWGS analysis show different distribution of bacteria of the genera *Bradyrhizobium*, *Methyloceanibacter* and *Desulfobactaceae* were detected in soil samples collected from the three locations. For culture-dependending technique, bacterial species that grew on selective media were identified as *Acinetobacter radioresistens*, *Brachybacterium paraconglomeratum* and *Enterobacter cloacae* which are screened as nitrogen-fixing bacteria, *Klebsiella quasipneumoniae*, *Bacillus tropicus*, and *Paenibacillus pasadenensis* screened as potassium solubilizing bacteria, and *Bacillus cereus* and *Bacillus thuringiensis* screened as phosphate solubilizing bacteria. Next, three sets of biofertilizers that include Set A containing *A. radioresistens*, *K. quasipneumonia* and *B. cereus*, Set B that contains *B. paraconglomeratum*, *B. cereus* and *B. tropicus*, and Set C containing *E. cloacae*, *P. pasadenensis* and *B. thuringiensis* with 10^6 cfu/g were formulated by mixing each set of bacterial species with commercial compost. The results showed that Set C is the best biofertilizer set compared to Set A and Set B as it can increase the content of nitrogen, phosphorus and potassium in the soil and increase the growth and protein of the duckweed plants. This indicates that biofertilizers formulated with mangrove-associated bacteria could enhance the growth of duckweed as well as its protein content.

Keywords: Mangrove soil, Plant probiotic, 16s rRNA, Soil nutrients, Duckweed

الملخص

وحقق في نهاية المطاف تلبية طلب سكان العالم. ومع ذلك ، يمكن أن تكون الأسمدة الكيماوية ضارة، مع آثار تم استخدام الأسمدة الكيماوية لتزويد النباتات بالمغذيات، مما أدى إلى نتائج سريعة وتحقيق تلبية الطلب العالمي في نهاية المطاف. ومع ذلك، يمكن أن تكون الأسمدة الكيماوية ضارة، مع آثار سلبية على الوسط البيئي وصحة الإنسان. لذلك، تعتبر الأسمدة الحيوية أفضل بديل للأسمدة الكيماوية لتعزيز نمو النباتات المضيفة. في هذه الدراسة، تم تحليل الميكروبات المرتبطة بأشجار المنغروف التي تم جمعها من التربة واستخدامها لتطوير الأسمدة الحيوية. كان الهدف الأول من هذه الدراسة هو تحديد مجموعة واسعة من الأنواع الميكروبية وملفها الأيضي من ثلاث عينات من التربة التي تم جمعها في غابات المنغروف النهريّة في المياه العذبة في سونغاي لوكوت نيجيري سيمبلان، ماليزيا باستخدام تسلسل أجزاء الجينوم الكامل الميتاجينومي. كان الهدف الثاني هو تحديد الأنواع البكتيرية التي تزرع على وسائط انتقائية تشمل أجار ألكساندرو وأجار بيكوفسكايا وأجار جنسن باستخدام تفاعل البوليميراز المتسلسل (PCR) و تسلسل الرنا الرياسي 16S. كان الهدف الأخير هو تقييم التفاعل بين الأنواع البكتيرية المختارة ونبات عشب البط من حيث معدل النمو ومحتويات البروتين. تظهر نتائج تحليل تسلسل أجزاء الجينوم الكامل (mWGS) توزيعًا مختلفًا لبكتيريا الأجناس براديزيوموم و ميثيلوشيانيباكتور وديسولفوباسيا في عينات التربة التي تم جمعها من المواقع الثلاثة. بالنسبة للتقنية المعتمدة على الثقافة، تم تحديد الأنواع البكتيرية التي نمت على وسائط انتقائية على أنها أسينتور مقاوم الأشعة و براتشيباكتيريوم باراكوجلوميراتوم و انتيروباكتير كلوكاي المعروفة باسم البكتيريا المثبتة للنيتروجين و كليسيلا كواسيتيومونيا و باسيللوس تروبيكوس و بيانيباسيللوس باسادينينسيس المعروفة باسم البكتيريا المدايية للبتواسيوم و باسيللوس سيريروس و باسيللوس ثيورينجينيسيس المعروفة باسم البكتيريا المدايية للفوسفات. بعد ذلك، تم صياغة ثلاث مجموعات من الأسمدة الحيوية التي تشمل المجموعة أ التي تحتوي على أسينتورباكتور مقاوم الأشعة و كليسيلا كواسيتيومونيا و باسيللوس سيريروس ، المجموعة ب التي تحتوي على براتشيباكتيريوم باراكوجلوميراتوم و باسيللوس سيريروس و باسيللوس تروبيكوس و المجموعة ج التي تحتوي على انتيروباكتير كلوكاي و بيانيباسيللوس باسادينينسيس و باسيللوس ثيورينجينيسيس مع 10^6 cfu/g عن طريق خلط كل مجموعة من الأنواع البكتيرية مع السماد التجاري. أظهرت النتائج أن المجموعة ج هي أفضل سماد حيوي مقارنة بالمجموعة أ والمجموعة ب لأنها يمكن أن تزيد من محتوى النيتروجين والفوسفور والبتواسيوم في التربة وتزيد من نمو وبروتين نباتات عشب البط. يشير هذا إلى أن الأسمدة الحيوية المصنوعة من البكتيريا المرتبطة بغابات المنغروف يمكن أن تعزز نمو عشب البط بالإضافة إلى محتواه من البروتين ويمكن أن تحل محل استخدام الأسمدة الكيماوية التي تضر بالبيئة.

الكلمات المفتاحية: تربة المنغروف، بروبيوتيك النبات، تسلسل الرنا الرياسي 16S، مغذيات التربة، عشب البط.

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LIST OF ABBREVIATIONS

%	percent
×g	revolution per minute
AA	auxiliary activities
ACC	1-aminocyclopropane-1-carboxylate
ACL	ATP-dependent citrate lyase
Al	aluminium
Al ³⁺	aluminium ion
ANOVA	analysis of variance
APS	adenosine-5'-phosphosulfate
As	arsenic
ATP	adenosine triphosphate
BLAST	basic local alignment search tool
bp	base pair
BPDO	biphenyl dioxygenase
Br	bromine
C	carbon
°C	degree celsius
Ca	calcium
Ca ²⁺	calcium ion
Ca ₃ (PO ₄) ₂	calcium phosphate
CAZy	carbohydrate active enzymes
CBM	carbohydrate-binding modules
CCB	centre for chemical biology
CCL	citryl-CoA lyase
CCS	citryl-CoA synthetase
CDF	chlorinated dibenzofurans
CD-HIT	cluster database at high identity
CE	carbohydrate esterases
Ce	cesium
CFU	colony forming unit
CH ₄	methane
Cl	chloride
cm	centimetre
CO	carbon monoxide
CO ₂	carbon dioxide
Cr	chromium
Cu	copper
DD	dibenzo-p-dioxins
DF	dibenzofurans
DNA	deoxyribonucleic acid
E	east
eggNOG	non-supervised orthologous groups
eV	electronvolt

FDH	formate dehydrogenase
Fe	iron
Fe ³⁺	iron ion
g	gram
GH	glycoside hydrolase
GM	genetically modified
GT	glycosyl transferase
HA	hydroxyatrazine
HOHPDA	2-hydroxy-6-oxo-6-(2'-hydroxyphenyl)-hexa-2,4-dienoic acid
HOPD	2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate
H ₂ PO ₄ ⁴⁺	hydrogen phosphate
H ₂ S	sulfide
H ₂ SO ₄	sulphuric acid
IAA	indole-3-acetic acid
IFFAH	institute of fatwa and halal
IDH	isocitrate dehydrogenase
ISR	induced systemic resistance
K	potassium
KEGG	kyoto encyclopedia of genes and genomes
KSB	potassium-solubilizing bacteria
kV	kilovolt
m	metre
MAG	metagenome-assembles genomes
MAMP	microbe-associated molecular patterns
Mbp	mega base pair
MDH	methanol dehydrogenase
Mg	magnesium
Mg ²⁺	magnesium ion
mg kg ⁻¹	milligram per kilogram
mL	millilitre
μL	microlitre
mm	millimetre
MMO	methane monooxygenase
Mn	manganese
MOB	methane oxidizing bacteria
MoFe	molybdenum-iron
mWGS	metagenomic whole genome sequencing
n	number of sample
N	nitrogen
N ₂	nitrogen gas
Na	sodium
NaCl	sodium chloride
NCBI	national centre for biotechnology institute
NFB	nitrogen-fixing bacteria
ng	nanogram
NGS	next generation sequencing

NH ₃	ammonia
NH ₄	ammonium
nm	nanometre
NO ₂	nitrogen dioxide
NO ₃ ⁻	nitrate
OAA	oxaloacetate
OD	optical density
ORF	open reading frame
OTUs	operational taxonomic units
P	phosphorus
PCR	polymerase chain reaction
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
PDH	pyruvate dehydrogenase
PE	paired-end
PCB	polychlorinated biphenyls
PGP	plant growth promotion
PGPB	plant growth-promoting bacteria
PGPM	plant growth-promoting microorganisms
PGPR	plant growth-promoting rhizobacteria
PL	polysaccharide lyase
pMMO	particulate methane monooxygenase
PO ₄ ⁻	phosphate ion
PSB	phosphate-solubilizing bacteria
PSM	phosphate-solubilizing microorganisms
qPCR	quantitative polymerase chain reaction
RA	relative abundance
Rb	rubidium
rRNA	ribosomal ribonucleic acid
rpm	rotation per minute
S	sulphur
SI	solubilization index
si	silicon
sMMO	soluble methane monooxygenase
SOM	soil organic matter
sp	species
Sr	strontium
SRM	sulfate-reducing microorganisms
TCA	tricarboxylic acid cycle
Ti	titanium
TPC	total plate count
USIM	universiti sains islam malaysia
USM	universiti sains malaysia
UV	ultraviolet
UV-VIS	ultra-violet visible
VOC	volatile organic compounds

XRF	x-ray fluorescence
W	watt
Zn	zinc
Zr	zirconium

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