MULTI-OMICS AND TAXONOMIC ANALYSES OF EMPTY FRUIT BUNCH ADAPTED MANGROVE MICROBIAL COMMUNITIES WITH LIGNOCELLULOLYTIC ABILITIES

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ABSTRACT

Current demand for energy drives the rapid progress of second-generation biofuel development. Use of lignocellulosic biomass, such as oil palm empty fruit bunch (EFB) in second-generation biofuels production resolved the limitation of first-generation biofuels which compete with food source. Lignocellulosic pre-treatment and saccharification are two crucial steps in second-generation biofuel production. These steps require synergistic action of lignocellulolytic enzymes. The use of large volume of freshwater in biofuel industry is a major concern as it creates competition between biofuel industry and human consumption. Seawater, which cover 96.5% of the biosphere could be an alternative to freshwater in biological pre-treatment and saccharification of lignocellulosic biomass. Therefore, the discovery of novel salt-tolerant microorganisms and their halophilic enzymes is an important aspect of lignocellulosic waste deconstruction using seawater. In this study, halophilic microbial community was collected from mangrove soil at Tanjung Piai National Park, Johor. Their ability to degrade lignocellulose was explored using culture independent and culture dependent approaches. The mangrove soil was used as inoculum and incubated with EFB in artificial seawater medium for 10 weeks. Total DNA, RNA and proteins were extracted (culture independent). 16S rRNA and 18S rRNA gene fragments were amplified from total DNA and composition of microbial community was analyzed based on amplicon metagenome sequencing. Taxonomic analysis showed that phyla *Proteobacteria* and *Bacteroidetes* were predominant prokaryotic population. Metatranscriptomic analysis revealed a total of 9,953 open reading frames (ORFs) related to lignocellulose degradation: 3,867 glycosyl hydrolases (GHs), 2,485 carbohydrate binding modules (CBMs), 2,156 carbohydrate esterases (CEs), 947 auxiliary activities (AAs) and 498 polysaccharide lyases (PLs). The highly expressed enzyme families were GH74, CE1, GH5, AA2, GH43, CE3, GH3, CE15, GH10 and GH6. Metaproteomic analysis identified a total of 87 lignocellulolytic enzymes in bound fraction of EFB and culture supernatant. Synergistic action of different lignocellulolytic enzymes from diverse microbial origin was observed with mostly affiliated to phyla *Proteobacteria* and *Bacteroidetes*. In addition, bacteria from the mangrove microbial community were isolated and their lignocellulolytic abilities were assessed (culture dependent). Two halophilic bacteria from the phylum *Bacteroidetes*, namely *Meridianimaribacter* sp. CL38 and *Robertkochia* sp. CL23 were selected for genomic analyses. A total of 30 and 89 lignocellulolytic enzymes were encoded in the genomes of strain CL38 and CL23, respectively. Furthermore, both strains demonstrated their abilities to degrade EFB. Genomic analyses of these two strains are the first genomic information from their respective genera. Due to the low similarity of 16S rRNA gene with closely related member, strain CL23 was further taxonomically characterized via polyphasic approach. Based on phenotypic, chemotaxonomic and genomic evidences, the strain CL23 is proposed as a new species with the name *Robertkochia solimangrovi* sp. nov. Multi-omics and taxonomic analyses in this study identified new halophilic microorganisms from mangrove with a wide array of new lignocellulolytic enzymes that are able to degrade EFB. These enzymes could be further investigated for development of enzyme cocktails which will be useful for seawater based lignocellulosic biorefining.

ABSTRAK

Tuntutan tenaga semasa telah mendorong kemajuan pesat pembangunan bahan bakar bio generasi kedua. Penggunaan biojisim lignoselulosa seperti tandan sawit kosong (TSK) dalam penghasilan bahan bakar bio generasi kedua telah menangani keterbatasan bahan bakar bio generasi pertama iaitu persaingan sumber makanan. Pra-rawatan dan sakarifikasi lignoselulosa adalah dua langkah penting dalam penghasilan bahan bakar bio generasi kedua. Langkah ini melibatkan tindakan sinergi pelbagai enzim lignoselulolitik. Penggunaan isipadu yang besar air tawar dalam industri bahan bakar bio adalah kebimbangan utama kerana ia menjadi persaingan antara kegunaan industri ini dan manusia. Air laut, yang merangkumi 96.5% biosfera boleh dijadikan alternatif menggantikan air tawar dalam pra-rawatan biologi dan sakarifikasi biojisim lignoselulosa. Oleh itu, penemuan mikroorganisma baru bertoleransi garam dan enzim halofilik adalah penting untuk mengungkai sisa lignoselulosa menggunakan air laut. Dalam kajian ini, komuniti mikrob halofilik telah didapatkan dari tanah hutan bakau Taman Negara Tanjung Piai, Johor. Keupayaan mikrob untuk menguraikan sisa lignoselulosa telah diterokai melalui kaedah bebas kultur dan kaedah bergantung kultur. Tanah hutan bakau telah digunakan sebagai inokulum dan dieram dengan TSK dalam air laut tiruan selama 10 minggu. DNA, RNA dan protin telah dipencilkan (kaedah bebas kultur). Cerbisan gen 16S rRNA dan 18S rRNA telah diamplifkasi daripada DNA jumlah dan komposisi komuniti mikrob telah dianalisa berdasarkan penjujukan metagenom amplikon. Analisis taksonomi menunjukkan bahawa filum *Proteobakteria* dan *Bakteroidetes* adalah populasi prokariot dominan. Analisis metatranskriptom mendedahkan sejumlah 9,953 rangka bacaan terbuka (ORF) berkaitan dengan degradasi lignoselulosa: 3,867 hidrolase glikosida (GH), 2,485 modul pengikatan karbohidrat (CBM), 2,156 esterase karbohidrat (CE), 947 aktiviti auksiliari (AA) dan 498 lyase polisakarida (PL). Famili enzim yang banyak terungkap adalah GH74, CE1, GH5, AA2, GH43, CE3, GH3, CE15, GH10 dan GH6. Analisis metaproteomik telah mengenal pasti 87 enzim lignoselulolitik di fraksi terikat TSK dan kultur supernatan. Tindakan sinergistik pelbagai enzim lignoselulolitik dapat diperhatikan dengan kebanyakannya berasal daripada filum *Proteobakteria* dan *Bakteroidetes*. Di samping ini, bakteria dari komuniti mikrob tanah hutan bakau telah dipencilkan dan kebolehan bakteria untuk menguraikan lignoselulosa telah dinilai (kaedah bergantung kultur). Dua bakteria halofilik dari filum *Bakteroidetes*, iaitu *Meridianimaribacter* sp. CL38 dan *Robertkochia* sp. CL23 telah dipilih untuk analisa genomik. Sebanyak 30 dan 89 enzim lignoselulolitik dikodkan masing-masing dalam genom strain CL38 dan CL23. Kedua-dua strain juga menunjukkan keupayaan untuk mengurai TSK. Analisis genomik kedua-dua strain ini merupakan maklumat genomik pertama dilaporkan bagi genus masing-masing. Disebabkan persamaan yang rendah pada gen 16S rRNA strain CL23 berbanding dengan spesies terdekat, strain ini telah dipilih untuk pencirian taksonomi melalui kaedah polifasik. Berdasarkan bukti fenotip, kemotaksonomi, dan genotip, strain CL23 telah dicadangkan sebagai spesies baharu dengan nama *Robertkochia solimangrovi* sp. nov. Analisa multi-omik dan taksonomi dalam kajian ini mengenalpasti mikroorganisma halofilik yang baru dari tanah hutan bakau dengan pelbagai enzim lignoselulolitik baru yang dapat menguraikan TSK. Enzim-enzim tersebut boleh dikaji dengan lebih lanjut untuk membangunkan koktel enzim yang akan berguna dalam bio-penulenan lignoselulosa berasaskan air laut.

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LIST OF ABBREVIATIONS

A	-	Adenine
AA	-	Auxiliary activity
AAI	-	Average amino acid identity
ABC	-	ATP binding cassette
ABTS	-	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
Ac	-	Acetyl group
ACE	-	Abundance-based coverage
AL	-	Aminolipids
ANI	-	Average nucleotide identity
ANIb	-	Average nucleotide identity based on BLAST
ANIm	-	Average nucleotide identity based on MUMmer
API	-	Analytical Profile Index
ATP	-	Adenosine triphosphate
atpD	-	ATP synthase
BCCM	-	Belgian Co-ordinated Collections of Micro-organisms
BF	-	Bound fraction
BLASTp	-	Protein-protein Basic Local Alignment Search Tool
bp	-	Base pair
BSA	-	Bovine serum albumin
C	-	Cytosine
C_2-OH	-	Bond between carbon and hydroxide
Ca.	-	Candidate
$CaCl_2$	-	Calcium chloride
CAZy	-	Carbohydrate Active Enzymes database
CAZymes	-	Carbohydrate Active Enzymes
CBM	-	Carbohydrate binding module
C-C	-	Carbon to carbon bond
cDNA	-	Complementary deoxyribonucleic acid
CE	-	Carbohydrate esterase
CLSI	-	Clinical and Laboratory Standards Institute

CMC	-	Carboxymethyl cellulose
C-O	-	Carbon to oxygen bond
CO_2	-	Carbon dioxide
COG	-	Cluster of Orthologous Group
csv	-	Comma-separated values
CTAB	-	Cetyltrimethylammonium bromide
D	-	Dextrorotatory
DDH	-	DNA-DNA hybridization
DEPC	-	Diethyl Pyrocarbonate
DHA	-	Keto-3-deoxy-D-lyxo-heptulosaric acid
DMK	-	Desmethylmenaquinones
DNA	-	Deoxyribonucleic acid
DNS	-	3,5-dinitrosalicylic acid
dNTP	-	Deoxynucleoside triphosphate
DOE-JGI	-	U.S. Department of Energy Joint Genome Institute
DSMZ	-	German Collection of Microorganisms and Cell Cultures
DTT	-	Dithiothreitol
DyP	-	Dye decolourizing peroxidase
E	-	East
e^-	-	Electron
EDTA	-	Ethylenediaminetetraacetic acid
EFB	-	Empty fruit bunch
emPAI	-	Exponentially modified protein abundance index
ESI-MS	-	Electrospray ionization mass spectrometry
EST	-	Expressed sequence tag
et al.	-	Et alia
E-value	-	Expect value
EZ-link-sulfo-NHS-SS-biotin	-	Succinimidyl 2-(biotinamido)-ethyl-1,3' -dithiopropionate
Fer	-	Ferulic acid
FPKM	-	Fragments per kilobase of transcript per million mapped reads
G	-	Guanine

G+C	-	Guanine and cytosine
GGDC	-	Genome-to-Genome Distance Calculator
GH	-	Glycosyl hydrolase
GLC	-	Gas liquid chromatography
GOLD	-	Genome Online Database
gyrB	-	DNA gyrase subunit B
h	-	Hour
H	-	Adenine or Cytosine or Thymine
H^+	-	Hydrogen ion
H_2O	-	Water
H_2O_2	-	Hydrogen peroxide
H_2S	-	Hydrogen sulphide
HCl	-	Hydrochloric acid
HG	-	Homogalacturonan
HPLC	-	High Performance Liquid Chromatography
ICSP	-	International Committee on Systematics of Prokaryotes
IMG	-	Integrated Microbial Genomes
INO	-	Inoculum
JCM	-	Japan Collection of Microorganisms
K^+	-	Potassium ion
KCl	-	Potassium chloride
KCTC	-	Korean Collection for Type Cultures
KDO	-	α-3-deoxy-d-manno-octulosonic acid (KDO)
KEGG	-	Kyoto Encyclopedia of Genes and Genomes
L	-	Laevorotatory
L	-	Lipid
L. gen. n.	-	Genus
L. neut. n.	-	Neuter gender
LC-MS/MS	-	Liquid chromatography tandem mass spectrometry
LDA	-	Lignin degrading auxiliary enzymes
LDS	-	Lithium dodecyl sulfate
LO	-	Lignin auxiliary enzymes
LPMO	-	Lytic polysaccharide monooxygenase

M	-	Adenine or Cytosine
MA	-	Marine agar
maxEE	-	Maximum number of expected errors
MB	-	Marine broth
Mbp	-	Million base pair
MDS	-	Multidimensional scaling
Me	-	Methyl group
MEGA	-	Molecular Evolutionary Genetics Analysis
MEGAN	-	MEtaGenome ANalyzer
MGAP	-	Microbial Genome Annotation Pipeline
$MgCl_2$	-	Magnesium chloride
$MgSO_4 \cdot 7H_2O$	-	Magnesium sulfate heptahydrate
min	-	Minute
MIDI	-	Microbial Identification System
MK	-	Menaquinones
ML	-	Maximum Likelihood
MLSA	-	Multilocus sequence analysis
Mn^{2+}	-	Manganese (II) ion
Mn^{3+}	-	Manganese (III) ion
$MnSO_4 \cdot 4H_2O$	-	Manganese (II) sulfate tetrahydrate
MP	-	Maximum-parsimony
mRNA	-	Messenger ribonucleic acid
MS/MS	-	Tandem mass spectrometry
mutL	-	DNA mismatch repair protein
MWCO	-	Molecular weight cut-off
N	-	North
Na^+	-	Sodium ion
NaCl	-	Sodium chloride
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NaOH	-	Sodium hydroxide
NCBI	-	National Center for Biotechnology Information
ncRNA	-	non-coding ribonucleic acid
NJ	-	Neighbour-joining

NO_3	-	Nitrate
nov.	-	Novel
O_2	-	Oxygen
ONPG	-	Ortho-nitrophenyl-β-galactoside
ORF	-	Open reading frame
OrthoANIu	-	Average nucleotide identity based on USEARCH
OTU	-	Operational taxonomic unit
Pcou	-	ρ-coumaric acid
PCR	-	Polymerase chain reaction
PE	-	Phosphatidylethanolamine
PEG 6000	-	Poly(ethylene glycol) 6000
PERMANOVA	-	Permutational multivariate analysis of variance
PES	-	Polyethersulfone
PGAP	-	Prokaryotic Genome Annotation Pipeline
PL	-	Polysaccharide lyase
pNP-Ara	-	p-nitrophenyl-α-L-arabinofuranoside
pNP-βM	-	p-nitrophenyl-β-D-mannopyranoside
pNPG	-	p-nitrophenyl-β-D-galactopyranoside
pNPGa	-	p-nitrophenyl-α-D-galactopyranoside
pNPX	-	p-nitrophenyl-β-D-xylopyranoside
POCP	-	Percentage of conserved protein
Pte Ltd.	-	Private limited
QIIME	-	Quantitative Insights Into Microbial Ecology
R	-	Guanine or Adenine
REALPHY	-	Reference sequence Alignment based Phylogeny builder
recA	-	ATP-dependent DNA repair protein
RG-I	-	Rhamnogalacturonan I
RG-II	-	Rhamnogalacturonan II
RIN	-	RNA integrity number
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
rpm	-	Revolutions per minute
rpoB	-	RNA polymerase β-subunit

rRNA	-	Ribosomal ribonucleic acid
S	-	Guanine or Cytosine
SDS	-	Sodium dodecyl sulfate
SEM	-	Scanning electron microscopy
SNT	-	Supernatant
sp.	-	Species (singular)
spp.	-	Species (plural)
SRA	-	Sequence Read Archive
T	-	Thymine
T9SS	-	Type 9 secretion system
TAE	-	Tris-acetate-EDTA
TLC	-	Thin Layer Chromatography
TR	-	Trace
Tris	-	Tris(hydroxymethyl)aminomethane
tRNA	-	Transfer ribonucleic acid
UPLC	-	Ultra Performance Liquid Chromatography
UV	-	Ultra violet
W	-	Adenine or Thymine
WebMGA	-	Web services for metagenomic analysis
XGA	-	Xylogalacturonan
Y	-	Cytosine or Thymine

LIST OF SYMBOLS

$A_{260/280}$	-	Absorbance ratio at 260 nm and 280 nm
A_{610}	-	Absorbance at 610 nm
A_{651}	-	Absorbance at 651 nm
α	-	Alpha
~	-	Approximately
β	-	Beta
°	-	Degree
°C	-	Degree Celsius
g	-	Gravity
kDa	-	Kilo Dalton
kV	-	Kilo volt
Ɛ	-	Molar absorption coefficient
O	-	Ortho
p	-	Para
®	-	Registered trademark
×	-	Times
™	-	Trademark
T	-	Type strain
U/ml	-	Units per volume
v/v	-	Volume per volume
w/v	-	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Over the last century, almost three-fold of increase in human population created a significant burden in energy resources (Prasad et al., 2019). A total of 7.6 billion of world human population in 2018 consumed an estimated 89 billion barrels of petroleum per day (Kumari and Singh, 2018). Currently, fossil fuel including petroleum and oil remains as the major contributor to meet the 80% of energy demand in the world (Raud et al., 2014; Raud et al., 2019). Nevertheless, the excessive dependence on non-renewable fossil fuel has caused detrimental effects to the environment such as global warming, loss of precious biodiversity, emission of greenhouse gases and rising of sea level (Binod et al., 2019; Gaurav et al., 2017; Robak and Balcerek, 2018). More importantly, fossil fuel is estimated to be depleted completely by next 45 years (Arifin et al., 2014).

Harnessing biofuel from various biomass resources is an important alternative to replace fossil fuel as it provides several advantages such as sustainability and eco-friendly (Bhatia et al., 2017; Raud et al., 2019). Up to this point, United States and Brazil are two chief intercontinental producers of first-generation biofuel (~87% total world production) which derived from food crops such as corn and sugarcane (Gupta and Verma, 2015; Lopes et al., 2016; Prasad et al., 2019). In details, a total of 15800 and 7060 million gallons of first-generation biofuels were produced by United States and Brazil in 2017 respectively (Liu et al., 2019a; Prasad et al., 2019).

Since corn and sugarcane are utilized as foods and feeds, these crops are not the best raw materials for first-generation biofuel production. This leads to food competition and may worsen the starvation issue in some third world countries (Banerjee et al., 2010; Owusu and Asumadu-Sarkodie, 2016; Ramos et al., 2016; Raud et al., 2019). To resolve this issue, the utilization of non-edible plant biomass as feedstocks are favorable (Kumari and Singh, 2018; Marriott et al., 2016; Shafawati and Siddiquee, 2013). The lignocellulosic biomass comprises of cellulose, hemicellulose, lignin and pectin is the most abundant form of fixed carbon on Earth (10^9 tons/annum) and its breakdown is a critical component for second-generation biofuel production (Batista-García et al., 2016). Oil palm empty fruit bunch (EFB) is one of the promising lignocellulosic biomasses. It is the major solid waste generated during the palm oil production process in the palm oil mill (Loh, 2017). The valorization of this abundant waste is highly encouraged in countries like Malaysia and Indonesia as they are the chief producers internationally (Aditiya et al., 2016; Ahmad et al., 2019).

The conversion of lignocellulosic biomass into second-generation biofuels such as bioethanol and biobutanol primarily requires four steps: pre-treatment of lignocellulosic biomass, hydrolysis/saccharification, fermentation of sugar monomers from lignocellulosic biomass and recovery of biofuel as final product (Gaurav et al., 2017; Gupta and Verma, 2015; Liu et al., 2019a). The biological pre-treatment and enzymatic hydrolysis by using microorganisms (bacteria and fungi) offered benefits such as less energy input, cost saving and environmental friendly as compared to other methods (Bhatia et al., 2017; Derman et al., 2018; Prasad et al., 2019).

The functional diversity and flexibility of bacteria make them as the good candidates for biological pre-treatment and saccharification as compared to fungi (Obeng et al., 2017). Many bacteria were able to decompose plant biomass into carbon-containing sugars by secreting lignocellulolytic enzymes, including cellulases, hemicellulases, ligninases and pectinases (de Gonzalo et al., 2016; Juturu and Wu, 2012; 2014a; Malgas et al., 2015). These enzymes have been further classified, based on structure in the Carbohydrate-Active Enzyme database (CAZy), into glycosyl hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs) and auxiliary activities (AAs) (Lombard et al., 2014). Common well-known bacteria such as *Bacillus* spp., *Brevibacillus* spp., *Cellulomonas* spp., *Streptomyces* spp. and *Pseudomonas* spp. have been widely studied in terms of their biomass degrading abilities for biorefining applications (Juturu and Wu, 2014a; Kamsani et al., 2016; Sharma et al., 2019).

In a typical bioprocessing model, large volume of freshwater is used to culture microorganism for lignocellulosic biomass degradation (Chen and Fu, 2016). It was calculated that 1.9–5.9 m^3 of freshwater were consumed to produce 1 m^3 of biofuel (Fang et al., 2015). This creates an unnecessary competition of freshwater between human consumption and biorefinery (Vörösmarty et al., 2010). While only 3.5% of the Earth water is freshwater which consists of ice caps, glaciers, groundwater and accessible surface freshwater (Nandakumar et al., 2019). This issue is worsened by unresolved water pollutions due to human activities. To have a better solution, seawater could be considered as an alternative for lignocellulose biomass pre-treatment and saccharification as seawater covers 96.5% of the biosphere (Dalmaso et al., 2015; Dhondy et al., 2019). Due to the reason that seawater contains salt such as NaCl, the search for new halophilic microorganisms and its lignocellulolytic enzymes are important.

Mangrove environment is one of the areas that reside with halophilic microorganisms. The plant biomass degrading microorganisms living in this area play an important role in recycling the organic carbon in the soil (Castro et al., 2018; Kathiresan, 2019; Lin et al., 2019; Wang et al., 2019). Thus, mangrove area serves as potential source for mining of microorganisms and their enzymes related to lignocellulose degradation.

To have a thorough understanding on halophilic microorganisms in mangrove environment and their salt tolerant lignocellulolytic enzymes, both culture independent and dependent approaches are necessary (Guo et al., 2018; López-Mondéjar et al., 2019). Culture independent approach includes amplicon metagenomics, metatranscriptomics and metaproteomics (Guo et al., 2018; López-Mondéjar et al., 2019). The profile of lignocellulosic degrading microbial population at community level requires the utilization of amplicon metagenome sequencing of gene markers such as 16S rRNA and 18S rRNA to reveal their identity (Christensen et al., 2018; McAllister et al., 2018; Schöler et al., 2017). While the metatranscriptomic and metaproteomic studies are centered on functionality of lignocellulolytic genes in terms of expressed mRNA and proteins formed by microbial community respectively (Guo et al., 2018; López-Mondéjar et al., 2019). In terms of culture dependent approach, genomic analysis and polyphasic characterization are widely utilized to reveal the new culturable halophilic bacteria with lignocellulolytic ability (López-Mondéjar et al., 2019; Raina et al., 2019). The identification of new culturable bacteria involves polyphasic characterization such as genotypic, phenotypic and chemotaxonomic analyses in order to propose new bacteria with valid taxon name (Raina et al., 2019). The genomic analysis on selected culturable strains could elucidate the lignocellulolytic genes encoded in the genomes (Berlemont and Martiny, 2015). Collectively, both culture independent and culture dependent approaches are coupled with each other to comprehensively decipher the new halophilic microorganisms with lignocellulolytic enzymes production.

1.2 Problem statement

Large volume of freshwater used in pre-treatment and saccharification of lignocellulosic biomass for second-generation biofuel production have been a major concern due to limited access of freshwater on Earth. Furthermore, this also created freshwater competition between biorefinery industry and human consumption. The utilization of seawater could be a potential for freshwater replacement in pre-treatment and saccharification of lignocellulosic biomass as seawater is abundant (96.5% of biosphere). Thus, the search for new lignocellulolytic microorganisms and its enzymes from halophilic source is necessary. The halophilic microorganisms in the mangrove area have been participated in plant biomass degradation for organic carbon recycling. So far, limited studies were performed to elucidate the ability of mangrove microorganisms for lignocellulose degradation. Therefore, in this study, research was conducted by using culture independent and dependent approaches to reveal the potential of mangrove microorganisms in decomposing lignocellulosic biomass.

1.3 Objectives of study

There are four objectives in this research in which objective 1 and 2 are related to culture independent approach, while objective 3 and 4 are related to culture dependent approach:

1. To profile the empty fruit bunch adapted mangrove microbial community that participated in lignocellulose decomposition by using amplicon metagenome analysis.
2. To mine the lignocellulolytic enzymes produced by empty fruit bunch adapted mangrove microbial community through metatranscriptomic and metaproteomic approaches.
3. To isolate and analyze the genomes of culturable bacteria with lignocellulose decomposing ability from empty fruit bunch adapted mangrove soil samples.
4. To propose a new bacterial species with lignocellulolytic ability via polyphasic taxonomy approach.

1.4 Scope of study

This study hypothesized that the potential of mangrove microorganisms (halophilic source) for oil palm EFB (lignocellulosic biomass) degradation by using culture independent (amplicon metagenomics, metatranscriptomics and metaproteomics) and culture dependent (polyphasic characterization and genomics) approaches could be elucidated.

To test the hypothesis, for culture independent part, total DNA, RNA and proteins were directly extracted from EFB-adapted mangrove soil samples. The 16S rRNA and 18S rRNA gene fragments that amplified from total DNA extracted were subjected to amplicon metagenome sequencing. The identity of EFB-adapted prokaryotic and eukaryotic communities were profiled. While the extracted total RNA and proteins were purified and subjected to metatranscriptomic and metaproteomic analyses. The potential new salt tolerant lignocellulolytic enzymes were mined and identified.

For culture dependent part, culturable bacteria were isolated and screened with lignocellulolytic enzymes production and the isolates were identified via 16S rRNA gene analysis. Two culturable bacteria, namely *Meridianimaribacter* sp. CL38 and *Robertkochia* sp. CL23 without any -omics study and no applications on lignocellulose degradation reported at the time of study were selected for further investigations. The genomes of both bacterial strains were sequenced and analyzed. As low 16S rRNA gene similarity shared between strain CL23 and the only species of *Robertkochia* genus (*Robertkochia marina*), strain CL23 was characterized by using polyphasic approach including phenotypic, chemotaxonomic and genotypic aspects to determine the taxonomy position of strain CL23. This strain was proposed as a new bacterial species with validated name.

1.5 Significance of study

By employing culture independent and culture dependent approaches, the exploration of new mangrove microorganisms that were able to produce salt-tolerant lignocellulolytic enzymes for EFB decomposition provides following significance:

1. The identity of mangrove microbial community that are able to deconstruct oil palm EFB was profiled. Many bacteria from the mangrove soil were taxonomically less-well defined and their application on lignocellulose decomposition were not established. *Meridianimaribacter* sp. CL38 and *Robertkochia* sp. CL23 are two such bacteria that were isolated from mangrove soil. An insight on potential new bacteria for lignocellulose degradation was gained.
2. A set of novel salt-tolerant lignocellulolytic enzymes were mined through multi-omics analyses (metatranscriptomics, metaproteomics and genomics). This imparts a prospective new halophilic source for enzyme cocktail development in order to be utilized in lignocellulose pre-treatment and saccharification.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Lam, M. Q.**, Oates, N. C., Thevarajoo, S., Tokiman, L., Goh, K. M., McQueen-Mason, S. J., Bruce, N. C., and Chong, C. S. (2020). Genomic analysis of a lignocellulose degrading strain from the underexplored genus *Meridianimaribacter*. *Genomics*. 112(1), 952-960. doi: https://doi.org/10.1016/j.ygeno.2019.06.011 **(Q2, IF: 3.16)**

2. **Lam, M. Q.**, Vodovnik, M., Zorec, M., Chen, S. J., Goh, K. M., Yahya, A., Md. Salleh, M., Ibrahim, Z., Tokiman, L., McQueen-Mason, S. J., Bruce, N. C., and Chong, C. S. (2020). *Robertkochia solimangrovi* sp. nov., isolated from mangrove soil, and emended description of the genus *Robertkochia*. *International Journal of Systematic and Evolutionary Microbiology*. 70(3), 1769-1776. doi: https://doi.org/10.1099/ijsem.0.003970 **(Q3, IF: 2.166)**

3. **Lam, M. Q.**, Oates, N. C., Goh, K. M., McQueen-Mason, S. J., Bruce, N. C., and Chong, C. S. (2020). Elucidating the lignocellulolytic capability of a new halophilic bacterium *Robertkochia solimangrovi* via thorough genomic analysis. *Science of the Total Environment*. **(draft manuscript completed, Q1, IF: 5.589)**

4. **Lam, M. Q.**, Chen, S. J., Goh, K. M., Abd Manan, F., Yahya, A., and Chong, C. S. (2020). Genome sequence of an uncharted halophilic bacterium *Robertkochia marina* with phosphate solubilizing ability. *3 Biotech*. **(draft manuscript completed, Q3, IF: 1.786)**

5. **Lam, M. Q.**, Oates, N. C., Bird, S. M., Leadbeater, D., Dowle, A. A., Tokiman, L., Goh, K. M., McQueen-Mason, S. J., Chong, C. S., and Bruce, N. C. (2020). Multi-omics analyses of lignocellulolytic microbial community from mangrove: new insights into oil palm empty fruit bunch deconstruction. *Biotechnology for Biofuels*. **(draft manuscript completed, Q1, IF: 5.452)**

Non-Indexed Conference Proceedings

1. **Lam, M. Q.**, Goh, K. M., Bruce, N. C., and Chong, C. S. (2018). Isolation, identification and genomic analyses of halophilic bacteria with lignocellulolytic abilities. *Asian Federation of Biotechnology Malaysia Chapter International Symposium*. 18-21 August. Sarawak, Malaysia, 49. **(ISBN 978-967-17271-0-2)**

2. Chong, C. S., **Lam, M. Q.**, Thevarajoo, S., Teo, S. C., Selvaratnam, C., Goh, K. M., and Bruce, N. C. (2018). Halophilic bacteria: insights into industrial applications. *Asian Federation of Biotechnology Malaysia Chapter International Symposium*, 18-21 August. Sarawak, Malaysia, 19. **(ISBN 978-967-17271-0-2)**

3. Chong, C. S., **Lam, M. Q.**, Zakaria, M. R., Abdul Karim, M. H., Chen, S. J., Goh, K. M., Tokiman, L., Md. Salleh, M., Yahya, A., and Bruce, N. C. (2019). Exploring lignocellulose degrading bacteria from mangrove environment. *Asian Federation of Biotechnology Malaysia Chapter International Symposium*. 20-23 October. Putrajaya, Malaysia, 28.