UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF MICROBIAL FUNCTIONAL DIVERSITY AND BACTERIAL COMMUNITY STRUCTURE AFFECTED BY FERTILISER, SOIL AND PLANT GROWTH STAGES IN AEROBIC RICE

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By

NOR AYSHAH ALIA BINTI ALI HASSAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2020

DEDICATION

I dedicate this thesis to my family and to all the people that contributed toward the successful completion of my PhD programme.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman : Associate Professor Halimi bin Mohd Saud, PhD
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Aerobic rice was introduced to increase water use efficiency. The rhizosphere of aerobic rice differs from that of conventional flooded rice due to their habitat differences. Recognizing the importance of microorganism to ecosystem function, bacterial community in aerobic rice rhizosphere was studied as a fundamental step towards the improvement of aerobic rice. Inorganic fertiliser could affect the microbial functional diversity and bacterial community structure of rhizosphere microorganism, which play a vital role in plant growth and soil fertility. Hence, the aim of this study was to determine the effect of fertiliser and soil type on microbial functional diversity bacterial community in aerobic rice rhizosphere. To achieve this, a total of 3 specific objectives was conducted. The first objective is to assess the effect of fertiliser rate and soil type on microbial functional diversity and bacterial community structure. The second objective is to determine the influence of fertiliser and soil type on the bacterial community composition and nitrogen cycling functional potential in the rhizosphere of aerobic rice. The third objective for this study is to evaluate the effect of plant growth stages on the microbial community in the rhizosphere of aerobic rice. Aerobic rice variety MRIA 1 was chosen for this study. A total of 4 different fertiliser rate was selected and tested with 2 different soil types. Non-fertilised, 100 kg/ha, 200 kg/ha, and 400 kg/ha of NPK compound fertiliser were applied to peat and sandy clay loam soil. The soil properties tested for this study were pH, soil moisture, cation exchange capacity, soluble phosphorus, total carbon, total nitrogen, and mechanical analysis. Plant growth characteristic recorded were plant length, numbers of tillers per hill, panicle length and straw nitrogen concentration. Microbial functional diversity was characterized by assessing the microbial activity and community level physiological profile using Biolog Ecoplate™ system. 16s amplicon sequencing was used to determined bacterial community structure, bacterial community composition, and nitrogen cycling functional potential. RDP FunGene was used to predict the functional potential of bacterial communities. Aerobic culturable microbial abundance was performed using nutrient agar, Burk’s agar, potato dextrose agar, Pikovskaya’s agar, and starch casein agar was used to quantify the abundance of bacteria, nitrogen-fixing microbes, fungi, phosphate-solubilizing microbes and actinomycetes, respectively. For the plant growth stages, samples were taken during the tillering, booting, grain filling, and mature stage. Microbial community were estimated using physiological fingerprints and microbial culturable abundance. Although peat and sandy clay loam were impacted differently when applied with different fertiliser rate, microbial functional diversity responded positively for both soils. The distinct microbial evenness between peat and sandy clay loam suggested that microbial functional diversity is dependent on soil properties. Carboxylic acid and acetic acid as well as carbohydrates were sensitive carbon sources that create dissimilar metabolic function of rhizosphere bacterial community in aerobic rice. L-arginine, L-asparagine, L-serine, α-cyclodextrin, N-acetylglucosamine and α -d-lactose were good indicators for distinguishing rhizosphere that added with fertiliser. 16s amplicon sequencing analysis demonstrated that *Alphaproteobacteria*, *Nitrospirae* and *Bacteroidetes* were identified as critical phyla when fertiliser added in peat while *Gammaproteobacteria* was the crucial phylum in sandy clay loam. *Acidobacteria-6* and *Chloracidobacteria* were positive indicator showing that excessive fertiliser was applied in both soils. The addition of all fertiliser rate tested influenced the bacterial community composition by shifting the communities. This was shown through the increase of abundance by *Candidatus Nitrososphaera* and the decrease of abundance by *Dok59*, *Olivibacter*, *Thermomonas* and *Dactylosporangium* in peat. The bacterial communities in the sandy clay loam were shifted and shown by the increase of *Clostridia* and *Solirubrobacter.* The changes in bacterial communities such as increase and decrease of type of genera were dependent on soil chemical and physical properties. Although, rhizosphere bacterial communities were different in peat and sandy clay loam, the function of the genera in the communities was the same after fertiliser was introduced. The selection of genera during the bacterial communities’ shift were dedicated for plant development but depended on soil type particularly soil properties. *Desulfovibrio* was identified as a genus that presence only in nonfertilised rhizosphere and can be used as an indicator in natural rhizosphere. *Chitinophaga*, *Desulfosporosinus*, *Geobacillus*, *Nannocystis*, *Nitrosovibrio*, and *Perlucidibaca* were the genus that solely presence in fertilised rhizosphere and can be used as an indicator in fertilised rhizosphere. Genera that solely presence in the fertilised rhizosphere were mainly contributed to nitrogen cycling. The growth stage of aerobic rice has an effect on determining the response and composition of microbial community in soil. Microbial community in rhizosphere responded according to plant effect produced during plant development which is primarily determined by soil type. Soil type particularly the soil physical and chemical properties are important factors in shaping the microbial community by directly influencing the environment of rhizosphere. This study concluded that microbial functional diversity and bacterial community in aerobic rice rhizosphere were influenced by fertiliser, soil type and growth stage. The use of fertiliser in the recommended rate (200 kg/ha of NPK compound fertiliser) is suitable in maintaining the function of bacterial community and thus positively influence the growth of aerobic rice.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN KEPELBAGAIAN BERFUNGSI MIKROORGANISMA DAN STRUKTUR KOMUNITI BAKTERIA YANG TERKESAN OLEH BAJA, TANAH DAN PERINGKAT PERTUMBUHAN PADI AEROB

Oleh

NOR AYSHAH ALIA BINTI ALI HASSAN

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Padi aerob diperkenalkan bagi meningkatkan kecekapan penggunaan air. Menyedari kepentingan mikroorganisma di dalam fungsi ekosistem, komuniti mikroorganisma di rhizosphere padi aerob dikaji sebagai langkah asas bagi menambahbaik padi aerob. Baja inorganik boleh mempengaruhi kepelbagaian berfungsi mikroorganisma dan struktur komuniti mikroorganisma di rhizosphere di mana mikroorganisma ini memainkan peranan penting kepada pertumbuhan tanaman dan kesuburan tanah. Oleh itu, kajian ini bertujuan untuk mengenalpasti kesan baja dan jenis tanah terhadap komuniti mikroorganisma di rhizosphere padi aerob. Untuk mencapai matlamat ini, sebanyak 3 objektif spesifik telah ditentukan. Objektif pertama ialah untuk menilai kesan kadar baja dan jenis tanah kepada kepelbagaian berfungsi mikroorganisma dan struktur komunitit mikroorganisma. Varieti padi aerob MRIA 1 telah dipilih bagi kajian ini. Sebanyak empat kadar baja berbeza dipilih dan diuji dengan 2 jenis tanah. Tiada baja, 100 kg/ha, 200 kg/ha, dan 400 kg/ha baja kompaun diaplikasi kepada tanah gambut dan tanah lom liat berpasir. Sifat tanah yang diuji adalah pH, kelembapan, kapasiti pertukaran kation, fosforus larut, jumlah karbon, jumlah nitrogen, dan analisis mekanikal. Kepelbagaian berfungsi mikroorganisma dicirikan dengan menilai aktiviti mikroorganisma dan profil fisiologi tahap komuniti dengan menggunakan sistem Biolog Ecoplate™. Penjujukan 16s amplikon digunakan bagi mengenalpasti struktur komuniti mikroorganisma. Kelimpahan mikroorganisma aerobik yang boleh dikultur diuji menggunakan agar nutrien, agar Burk’s, agar dekstros kentang, agar Pikovskaya’s, dan agar kanji kasein digunakan bagi mengukur kelimpahan bakteria, mikroorganisma pengikat nitrogen, kulat, mikroorganisma pelarut fosforus, dan aktinomisit, masing-masing. Kepelbagaian berfungsi mikroorganisma meningkat dengan pertambahan baja di tanah gambut dan tanah lom liat berpasir. Walaupun tanah gambut dan tanah lom liat berpasir dipengaruhi secara berbeza apabila diaplikasi dengan kadar baja yang berbeza, terdapat kesan positif bagi kedua-

dua jenis tanah ini. Terdapat beberapa spesies tertentu meningkat di dalam komuniti mikroorganisma namun terdapat juga spesies yang mengurang bagi adaptasi kepada perubahan dan ini telah mengekalkan kepelbagaian berfungsi mikroorganisma. Terdapat perubahan pada penggunaan sumber karbon selepas penambahan baja. Asid karboksilik dan asid asetik serta karbohidrat merupakan sumber karbon yang sensitif di dalam menghasilkan fungsi metabolik yang berbeza di dalam komuniti mikroorganisma rhizosphere padi aerob. L-arginine, L-asparagine, L-serine, α-cyclodextrin, N-acetylglucosamine dan α -d-lactose merupakan penunjuk yang bagus bagi membezakan rhizosphere yang ditambah dengan baja. *Alphaproteobacteria*, *Nitrospirae* and *Bacteroidetes* dikenalpasti sebagai phyla yang kritikal apabila baja ditambah di dalam tanah gambut manakala *Gammaproteobacteria* merupakan phyla penting bagi tanah lom liat berpasir. *Acidobacteria-6* dan *Chloracidobacteria* merupakan penunjuk positif yang menunjukkan baja berlebihan digunakan di dalam kedua-dua jenis tanah. Objektif kedua ialah bagi mengenalpasti pengaruh baja dan jenis tanah kepada komposisi komuniti mikroorganisma dan potensi kitaran nitrogen berfungsi di rhizosphere padi aerob. Pencirian pertumbuhan padi yang diukur adalah panjang pokok, bilangan anak pokok serumpun, panjang panikel, dan kepekatan nitrogen di jerami. Penjujukan 16s amplikon digunakan bagi mengenalpasti komposisi komuniti mikroorganisma, dan potensi kitaran nitrogen berfungsi. Potensi berfungsi komuniti mikroorganisma di ramal dengan menggunakan RDP FunGene. Komposisi komuniti mikroorganisma dipengaruhi oleh penambahan baja melalui pengalihan komuniti. Penambahan baja meningkatkan kelimpahan arkaea terutamanya *Candidatus Nitrososphaera* di tanah gambut. Penambahan baja meningkatkan kelimpahan bakteria di tanah lom liat berpasir terutamanya *Clostridia*, *Gammaproteobacteria* dan *Acidobacteria-6*. Perubahan komuniti mikroorganisma seperti peningkatan dan penurunan jenis genera bergantung kepada sifat kimia dan fizik tanah. Walaupun komuniti mikroorganisma rhizosphere adalah berbeza pada tanah yang berlainan, fungsi genera di dalam komuniti adalah sama selepas baja diperkenalkan. Pemilihan genera semasa peralihan komuniti mikroorganisma ditumpukan untuk pertumbuhan tumbuhan tetapi bergantung kepada jenis tanah terutamanya sifat tanah. *Desulfovibrio* dikenalpasti sebagai genus yang hanya hadir di rhizosphere yang tidak dibaja dan boleh digunakan sebagai penunjuk di rhizosphere semulajadi. *Chitinophaga*, *Desulfosporosinus*, *Geobacillus*, *Nannocystis*, *Nitrosovibrio*, dan *Perlucidibaca* merupakan genus yang hanya hadir di rhizosphere yang dibaja dan boleh digunakan sebagai penunjuk di rhizosphere yang dibaja. Genera yang hanya hadir di rhizosphere yang dibaja menyumbang terutamanya kepada kitaran nitrogen. Objektif ketiga bagi kajian ini ialah menilai kesan peringkat pertumbuhan padi aerob kepada komuniti mikroorganisma di rhizosphere. Bagi peringkat pertumbuhan padi aerob, sampel diambil semasa peringkat vegetatif, pembiakan, masak, dan matang. Pencirian pertumbuhan padi yang diukur adalah panjang pokok, bilangan anak pokok serumpun, panjang panikel, dan kepekatan nitrogen di jerami. Komuniti mikroorganisma di ukur menggunakan cap jari fisiologi dan kelimpahan mikroorganisma yang boleh dikultur. Komuniti mikrorganisma di rhizosphere bertindakbalas mengikut kesan pokok yang dihasilkan semasa pertumbuhan pokok yang mana ditentukan oleh jenis tanah. Jenis tanah terutamanya sifat fizikal dan kimia tanah merupakan faktor penting di dalam membentuk komuniti mikroorganisma dengan mempengaruhi secara langsung persekitaran

rhizosphere. Daripada kajian ini, dapat disimpulkan bahawa penambahan baja dan jenis tanah yang berbeza mampu memberi kesan dan mempengaruhi kepelbagaian berfungsi mikroorganisma dan komuniti mikroorganisma di rhizosphere padi aerob.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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%	Percentage
℃	Degree Celsius
ul	Microliter
um	Micrometer
$(NH_4)_2SO_4$	Ammonium sulfate
AWCD	Average Well Color Development
C/N	Carbon/Nitrogen ratio
Ca	Calcium
$Ca_3(PO_4)2$	Calcium phosphate
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
CLLP	Community Level Physiological Profile
cm	Centimetre
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
E	Shannon Evenness
EC	Electrical Conductivity
$FeSO_4$	Iron (II) Sulfate
$FeSO_4.7H_2O$	Ferrous sulfate heptahydrate
FLASH	Fast Length Adjustment of Short Reads
g	Gram

H	Shannon Diversity Index
ha	Hectare
IRRI	International Rice Research Institute
K	Potassium
K_2O	Potassium oxide
KCl	Potassium chloride
kg	Kilogram
KH_2PO_4	Monopotassium phosphate
KNO_3	Potassium nitrate
l	liter
LEfSe	Linear discriminant analysis effect size
LSD	Fisher’s Least Significance Difference
M	Moisture
Mg	Magnesium
$MgSO_4$	Magnesium sulfate
$MgSO_4.7H_2O$	Magnesium heptahydrate
min	Minutes
ml	Milliliter
mm	Millimeter
$MnSO_4$	Manganese Sulfate
N	Nitrogen
NA	Nutrient Agar
Na	Sodium
$Na_2MoO_4.2H_2O$	Sodium molybdate

NaCl	Sodium chloride
$NaSO_4$	Sodium sulfate
NF	Burk’s Agar
NGS	Next Generation Sequencing
nm	Nanometer
OD	Optical Density
OTU	Operational Taxonomy Unit
P_2O_5	Phosphorus Pentoxide
PC1	Principal Component 1
PC2	Principal Component 2
PC3	Principal Component 3
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PLFA	Phospholipid Fatty Acid Analysis
PS	Pikovskaya’s agar
PTN	Plant total nitrogen
Qiime	Quantitative insights into microbial ecology
q-PCR	Quantitative Polymerase Chain Reaction
R	Richness
RCBD	Randomized Complete Block Design
rRNA	Ribosomal Ribonucleic Acid
SCA	Starch casein Agar
SN	Soil total nitrogen
SP	Soil Phosphorus

ssu	Small subunit
TC	Total carbon
TN	Total nitrogen
TP	Total phosphorus
T-RFLP	Terminal restriction fragment length polymorphism
UPGMA	Unweighted Pair Group with Arithmetic Mean
VWC	Volumetric Water Content

CHAPTER 1

INTRODUCTION

1.1 Background

Nitrogen and phosphorus are important components in fertiliser for agricultural crops. It has been observed that the leaching of nitrogen had increased in the past 100 years because of the excessive use of chemical fertilisers (Stocker et al., 2013). The acidification of soil and nutrients differences were among the negative effects due to high amount of fertiliser in soil. Nitrogen and phosphorus in fertiliser could negatively affect the functional diversity and community structure of rhizosphere microorganisms, which play a vital role in plant growth and soil fertility (Dotaniya & Meena, 2015).

Rhizosphere is the narrow zone surrounding and effected by plant roots (Mendes et al., 2013). The rhizosphere has been regarded as a remarkable ecosystem because microorganisms inhabiting this area showed a variety of metabolic activities. Hence, rhizosphere ecosystem offers numerous services such as structuring below and above multitrophic interactions and nutrient cycling across different trophic levels and a decrease in bacterial community could potentially limit these services. Therefore, it is important to study how bacterial community in the rhizosphere responds towards environmental changes. For paddy yield and rice nutrition, microorganism can contribute significantly and thus justified the evaluation of microbial communities and their responses against agricultural practices.

Because microbes can contribute strongly to rice nutrition and production, there is much interest in surveying the microbial communities in this flooded agroecosystem and determining how agricultural practices influence the composition and structure of these communities. The rhizosphere of aerobic rice differs from that of conventional flooded rice. The oxic and anoxic conditions in normal flooded rice have different chemical requirements, while the rhizosphere of aerobic rice is similar to that of other non-flooded crops like maize. Therefore, the bacterial community in these two crops might be different due to the difference in their habitat and soil properties around the rhizosphere.

The influence of fertiliser against microbial functional diversity and bacterial community was numerously conducted. Earlier studies have identified the role of general and specific microorganisms in nutrient input in paddy fields. These include the function of microbial biomass in supplying phosphorus and nitrogen in low fertile paddy fields (Gijsman et al., 1997) and also the contribution of nitrogen-fixing microorganisms for nitrogen input. However, the capacity of these microorganisms to produce nutrient is influenced by land management for example the addition of rice straw that enhanced microbial activity (Roper et al., 1995), while nitrogen fertiliser reduces the growth of nitrogen-fixing and increase their population in phosphorus (Cassman, 1998). Thus, the growth of microorganisms with specific function can be influenced by the addition of introducing input.

For example, the addition of organic and chemical fertiliser improved microbial functional diversity (Ding et al., 2018) and increases bacterial community in paddy fields (Liu et al., 2018 and Kumar et al., 2018). Soil physicochemical properties also reported to have an influence on microbial functional diversity. The combination of inorganic fertiliser with organic material elevated microbial activity, species richness, catabolic diversity and carbon source utilization with the relation on soil organic carbon availability (Li et al., 2015). By using molecular techniques such as terminal restriction fragment polymorphism dan denaturing gradient gel electrophoresis, microorganism that involved in nitrogen cycling such as ammonia-oxidizing bacteria dan nitrogen-fixing bacteria were reported to increase when applied with fertiliser (Gu et al., 2008; Wang et al., 2009; Wartiainen et al., 2008). However, there's also contrary findings showing that chemical fertiliser negatively affected the bacterial communities in paddy fields (Guanghua et al., 2008, Yoshida et al., 2009). In the recent years, next generation sequencing has been able to identify the bacterial communities in paddy fields (Huang et al., 2019; Luo et al., 2019; Kumar et al., 2018).

Economic water insufficiency is expected to hit 22 hectares million irrigated dry-season rice in Southeast Asia by 2025 (Tuong and Bouman, 2003). Conventional lowland rice required continuous flooding and elevated water inputs. Therefore, aerobic rice was introduced with the aim to reduce water inputs and increase water use efficiency. Aerobic rice is cultivated in non-saturated and non-puddled conditions with additional irrigation and high external inputs (Bouman et al., 2005). The aerobic rice cultivation in Malaysia is around 66.3 hectares in 2014 to 2015 (Rosli, 2015). This is only 0.02% from the total rice cultivation in Malaysia. This is considered small and could be explained by the newly released local aerobic rice variety along with its planting technology. Most of microbial research on aerobic rice was on the use of beneficial microbes to improve plant growth as well as for soil enrichment (Nahi et al., 2016 and Nasarudin et al., 2018).

1.2 Problem Statement

As mentioned earlier, the bacterial community in flooded paddy and aerobic rice might be different due to the difference in their habitat and soil properties around the rhizosphere. Hence, there's a need in studying the microbial functional diversity and bacterial community in aerobic rice. Aerobic rice is a potential crop for future food production. The ability of aerobic rice to grow in non-saturated condition is suitable to address water scarcity which is beneficial for food security. A decrease in bacterial community due to inorganic disturbance such as fertiliser could potentially limit the services that they're provided in the soil and plant. Microbial functional diversity reflects genetic diversity (Carolina, 2018) and it is important to determine the analytical level of diversity to sustain ecosystem function from disturbances.

There are very few studies on the microbial functional diversity and community in aerobic rice. These studies examined the rhizosphere microbial diversity between aerobic rice growth stages (Sutradhar, 2015) and the diversity of endophytic bacteria (Vishwakarma and Dubey, 2019). High bacterial richness and diversity was identified under aerobic rice compared to flooded paddy which could benefited the aerobic rice productivity (Vishwakarma and Dubey, 2019).

To date, there is a lack of information on the effect of fertiliser and soil type on microbial functional diversity and bacterial community particularly in the local aerobic rice variety. Prior studies mentioned (Vishwakarma and Dubey, 2019) use restriction fragment length polymorphism and there is no research of bacterial community using the 16s amplicon sequencing.

It is important to know whether there are effects of fertiliser and soil type on microbial functional diversity and bacterial community structure. If there is an effect on the bacterial community structure, then how fertiliser and soil type affected the bacterial community composition and their nitrogen cycling potential. Since microorganism directly involved in ecosystem function, the addition on external input such as fertiliser may have influenced microorganism which could benefit or detriment the growth of aerobic rice. Information on microbial functional diversity and bacterial community structure could aid in the development of better aerobic rice growing strategies in drought or water scarcity area. In addition, the response of microbial community towards aerobic rice plant growth stages is important to determine whether plant physiology have an influence on the microbial community. Hence, the effect of fertiliser and soil type on bacterial community in aerobic rice rhizosphere was investigated.

1.3 Objectives

The objective of this study was to determine the effect of fertiliser and soil type on bacterial community in aerobic rice rhizosphere. The specific objective of this study is as followed:-

1. to assess the effect of fertiliser rate and soil type on microbial functional diversity and bacterial community structure in the rhizosphere of aerobic rice.

2. to determine the influence of fertiliser and soil type on the bacterial community composition and nitrogen cycling functional potential in the rhizosphere of aerobic rice.

3. to evaluate the effect of plant growth stages on the microbial community in the rhizosphere of aerobic rice.

Based on the objectives, 3 hypotheses were deduced as followed: -

1. H1 - there are fertiliser and soil type effect on microbial functional diversity and bacterial community structure.

2. H2 - there are influence of fertiliser and soil type on the bacterial community composition and nitrogen cycling functional potential in aerobic rice rhizosphere.

3. H3 - there is an effect of plant growth stages on microbial community.

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LIST OF PUBLICATIONS

Ali Hassan, N.A.A., Othman, R., & Sadi, T. (2019). The response of microbial community in aerobic rice rhizosphere that affected by various plant growth stages and soil types. *Malaysian Journal of Microbiology*, 15(4) Special Issue 2019, 178-189

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