ISOLATION AND CHARACTERIZATION OF LIGNIN MONOMER-DEGRADING BACTERIA UNDER ACIDIC CONDITION FROM TROPICAL PEATLAND IN MALAYSIA

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DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

Tropical peatlands account for one of the largest carbon storages in the form of organic matter as a result of the accumulation of plant litter and waterlogged conditions. Recent anthropogenic disturbances such as forest fire, agricultural conversion, and draining of natural tropical peatlands have caused a vast amount of carbon released into the atmosphere, whereby these changes broadly impact microbial activities. A recent study states that many phenol-degrading and lignin-degrading bacteria prefer alkaline and neutral pH conditions. The acidic environment in tropical peatlands does not prevent microorganisms from utilizing lignin and phenolic derivative from the peat. This study aims to isolate and characterize lignin monomer-degrading bacteria from tropical peatlands under acidic conditions using phenolic aldehyde as the substrate for carbon input. Evaluation of greenhouse gas (GHG) emission and analysis of the chemical composition of tropical peat soil are essential to relate the peat utilization by the isolated bacteria. The GHG emission in pristine forests, secondary forests, and oil palm plantations was monitored using the closed chamber method. Peat water chemical composition were also identified using ultrahigh performance liquid chromatography quadrupole time of flights (UPLC-QTOF). Two isolates showed the capability to utilize phenolic aldehyde based on building blocks of lignin abundant in tropical peatlands, including hydroxyphenyl, guaiacyl, and syringyl branches. Identification of these isolates by 16S rRNA gene showed that strain S38 is similar to *Stenotrophomonas* sp. while strain S46 is similar to *Burkholderia* sp. Further characterization of these isolate showed their ability to degrade 4-hydroxybenzaldehyde and vanillin into phenolic acids within 24 hours of incubation and 7 days for syringaldehyde respectively. The findings indicated an increase in GHG emissions during the dry season due to the peat being exposed to oxidation. This is due to the peat soil composition containing a vast amount of lignin and phenolic derivative. In conclusion, the isolated strains show the ability to degrade lignin monomers into phenolic acid and withstand the acidic environment of tropical peatland, which is useful in the lignin industry.

ABSTRAK

Tanah gambut tropika menyimpan kadar karbon yang tinggi dalam bentuk pepejal organik atas faktor kekerapan pengumpulan sisa tumbuhan yang tinggi dan keadaan tanah yang sentiasa ditenggelami air. Gangguan antropogenik baru-baru ini seperti pembakaran hutan, penukaran tanah kepada pertanian, dan penyaliran air tanah gambut tropika semula jadi menyebabkan sejumlah besar karbon dibebaskan ke atmosfera, di mana perubahan ini memberi kesan secara meluas kepada aktiviti mikrob. Satu kajian baru-baru ini menunjukkan bahawa banyak bakteria pengurai fenol dan pengurai lignin lebih suka keadaan pH alkali dan neutral. Persekitaran berasid di tanah gambut tropika tidak menghalang mikroorganisma daripada menggunakan lignin dan terbitan fenolik daripada gambut. Kajian ini bertujuan untuk mengisolasi dan melaksanakan pencirian bakteria yang menguraikan monomer molekul lignin daripada tanah gambut tropika dalam keadaan berasid menggunakan aldehid fenolik sebagai substrat untuk input karbon. Penilaian pelepasan gas rumah hijau (GHG) dan analisis komposisi kimia tanah gambut tropika adalah penting untuk mengetahui hubung kait dengan penggunaan gambut oleh bakteria yang telah di isolasi tersebut. Pelepasan GHG di hutan asli, hutan sekunder, dan ladang kelapa sawit dipantau menggunakan kaedah ruang tertutup. Komposisi kimia air gambut juga telah dikenal pasti menggunakan instrumen spektrometri jisim kromatografi kuadrupol masa penerbangan prestasi tinggi (UPLC-QTOF). Dua isolat bakteria menunjukkan keupayaan untuk menggunakan aldehid fenolik berdasarkan blok binaan lignin yang banyak terdapat di tanah gambut tropika, termasuk cabang hidroksifenil, guaiasil dan syringil. Pengenalpastian isolat oleh gen 16S rRNA menunjukkan bahawa isolat S38 adalah serupa dengan *Stenotrophomonas* sp. manakala isolat S46 serupa dengan *Burkholderia* sp. Pencirian selanjutnya bagi isolat ini menunjukkan keupayaannya untuk menukarkan 4-hydroksibenzaldehid dan vanillin kepada asid fenolik dalam masa 24 jam pengeraman dan 7 hari untuk syringaldehid. Kesimpulannya, pelepasan GHG meningkat pada musim kemarau kerana tanah gambut terdedah kepada pengoksidaan. Komposisi tanah gambut mengandungi sejumlah besar lignin dan terbitan fenolik. Dua isolat bakteria menunjukkan keupayaan untuk merendahkan monomer lignin kepada asid fenolik dan menahan persekitaran berasid tanah gambut tropika, yang berguna dalam industri lignin.

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LIST OF ABBREVIATIONS

GC	-	Gas Chromatogram
GHG	-	Greenhouse gas
HPLC	-	High Performance Liquid Chromatogram
MS	-	Mass Spectrometry
NIST	-	National Institute of Standard and Technology
NPSF	-	Natural Peat Swamp Forest
OPP	-	Oil Palm Plantation
OD	-	Optical Density
PSF	-	Peat Swamp Forest
QTOF	-	Quadrupole Time of Flight
RMFR	-	Raja Musa Forest Reserve
RPSF	-	Recovery Peat Swamp Forest
UPLC	-	Ultrahigh Performance Liquid Chromatogram
WT	-	Water Table

LIST OF SYMBOLS

g	-	Gravitational force
U	-	Initial concentration
f	-	Final concentration
m/z	-	Mass per charge ratio

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Climate change has posed severe issues in recent decades; one of the main concerns is the global warming problem due to the increasing amount of greenhouse gases (GHG) released to the atmosphere, mainly by human activity and economic development. The major contributor to GHG is carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which absorb the heat radiation from the earth's surface and disrupt global temperature, which leads to global warming (Sabir et al., 2020). 50.44-0.74% of current GHG emissions are from peatlands due to land degradation, mainly by human activity such as agricultural conversion, logging, drainage, and burning the area (Cooper et al., 2020). Two-thirds are from south-east Asia (SEA), which is the equivalent total of 0.81 to 2.57 Gigatonnes of CO_2 released into the air (Kane et al., 2016). GHG emission conversion of peat swamp forest (PSF) in SEA contributes around 16.6% to 27.9% of total national GHG emission from Malaysia and Indonesia (Cooper et al., 2020).

In Malaysia, most tropical peatland has undergone forest conversion in agriculture, mainly for oil palm plantation. The easiest way to clean up the tropical peat swamp forest is by burning the area since this method is economically low in cost. Forest clearing by burning the peatlands is one of the world's critical threats to climate change. The effect of burning the forest causes the formation of suspended solid particles that causes air pollution known as haze which annually happens in the SEA. Tropical peatland is the vital carbon storage where it contains a large amount of organic matter from the accumulation of litter, debris, and dead plant material.

Tropical peatlands are believed to have a vast amount of microbial diversity responsible for most oxidation peat substrate present in that area. Recent changes in the environment can alter the behaviour of microorganisms in responding to the decomposition of tropical peatlands. Aerobic microbes release CO_2 by utilizing the organic compound from tropical peatlands, while N_2O gas is released in the agricultural fertilizer area containing nitrogen. CH_4 is released during the anaerobic condition of the peatland, where the water table is high, affecting anaerobic microbe to decompose peat substrate that lacks oxygen gas. Lowering water tables results in the peat substrate being exposed to oxidation and further decomposing by the aerobic microbe, increasing CO_2 and N_2O gases in the air. Monitoring of GHG from the sample site has been done periodically to support the evidence of microbial activity in tropical peatlands. Preliminary experimentation, such as the close chamber method, was applied to analyze the CO_2 emission from the peat soil. Peat substrate's decomposition process is enlightened by the chemical identified in the peat with the characterization of the microbial process to degrade the chemical involved.

The bacterial community's degradation of lignin monomer in tropical peatlands has limited reports regarding the process and mechanisms involved. *Burkholderia* sp. is known to produce the enzyme ligninase, isolated from tropical forests in Pahang, Malaysia (Roslan et al., 2015). Ong et al. (2016) also stated that the specific influence on the level of phenolic compounds was not inspected yet, which raises the question of how the disturbance of PSF relates to microbial activity. As previously mentioned, the waterlogged condition hugely preserved the lignin-related component from aerobic degradation, causing the increase of phenolic compound concentration due to the lack of decomposition by bacteria. Disturbance of peat exposed the preserved material to be oxidized, which caused the increase of microbial activity to degrade phenolic compounds rich in tropical peatlands.

Phyla *Proteobacteria* are well known to have aromatic and lignin degradation, including the genus *Pseudomonas, Pandoraea,* and *Enterobacter* (Manter et al., 2010; Wang et al., 2016; Kumar et al., 2018). Such genus has an essential enzyme that facilitates lignin degradation from various soil sources. Tropical peat soil is rich in organic matter, promoting the lignin-degrading activity regardless of the acidic and highly toxic phenolic compound present in the tropical peatlands. These unique environments bring out novelty in lignin degradation by bacteria since the optimal condition for the breakdown of lignin occurs dominantly in alkaline pH. Characterization of metabolite produced is required to observe lignin-degrading ability from the substrate introduced to the bacteria. Major reports show that lignin degradation occurs at *ortho* and *meta* cleavage pathways which is part of aromatic degradation by bacteria. Therefore, it is possible to determine different pathways from lignin degrading bacteria with different pH as the enzyme produced is expected to be unique in tropical peatlands. Some bacteria also have the exceptional ability to assimilate enzymes produced by fungi, thus helping further degrade the opening of the ring cleavage.

This evidence shows that the current trends for lignin-degrading bacteria are also observed in another habitat. Since there are also a few successful isolated strains from the tropical peatlands, it is safe to postulate that there are changes in microbial process of the degradation by the bacteria in factor of anthropogenic disturbance which cause bloom in oxidation lignin materials stored at the waterlogged tropical peatlands. The mechanism and metabolite production of the lignin-monomer in this acidic environment is expected to show interesting outcomes that are essential for lignin degradation in the low pH.

1.2 Problem Statement

Recent research on tropical peatland in Malaysia shows there is high emission of GHG, including methane (CH_4) and carbon dioxide (CO_2), into the atmosphere due to the human activity from logging, forest fire, drainage, and agricultural conversion. These activities lead to peat substrate exposure mainly from organic material abundant in phenolic derivatives, which is essential in carbon sink and storage function. The oxidation of peat further utilized by aerobic microorganisms leads to the depletion of peat. However, there is no clear evidence on which species of bacteria are responsible for the degradation of peat composition in tropical peatland. The initial hypothesis shows that the higher composition of peat is made up of phenolic derivatives because of the high rate of plant litter that consists of 76% lignin. Therefore, it can be postulated that lignin monomer-degrading bacteria are responsible for utilizing the phenolic substrate in the tropical peatlands.

While methanotroph and nitrogen fixation microorganisms such as fungi, archaea, and bacteria responsible for the production of CH_4 and N_2O gases were reported from tropical peatland, and their pathway of degradation is extensively known. Phenol degrading bacteria from tropical peatland lack specific information on how the microorganism utilized the scarce nutrient, limited by organic carbon and high acidity. Biodegradation of phenolic derivatives usually occurs in neutral and alkaline conditions. At the same time, high acidity in peatland may show a unique pathway in phenolic biodegradation in the formation of CO_2 emission as exposed peat substrate remains in low pH regardless of the reduction in water level that influences acidity of the soil.

1.3 Research Objectives

The objectives of the research are:

(a) To evaluate GHG emission from aerobic bacteria and analyze the chemical composition in tropical peatland

(b) To isolate lignin-monomer degrading bacteria from acidic tropical peatland

(c) To characterize isolated lignin-monomer degrading bacteria under the acidic and aerobic conditions from tropical peatland.

1.4 Scope of Study

GHG is evaluated based on CO_2, CH_4, and N_2O gases species related to the impact of human disturbance on the tropical peatland and microbial activity, showing evidence of aerobic and anaerobic respiration from the microbial community in tropical peat soil. This information is essential to show that significant microbial processes happen in the soil and are limited to the natural forest, recovery forest, and oil palm plantation. The observation did not cover the physical emission, such as forest fires that produce massive CO_2. The results also do not necessarily co-relate the direct production of GHG by degradation. Instead, to enlighten the idea that different peat soil properties affected the microbial activity in tropical peatlands.

The organic compound identification was limited to the phenolic derivative compounds, which contain a benzene ring attached to the hydroxyl group. This chemical structure is abundant in tropical peatland since the peat substrate was made up of the accumulation of plant debris that consists high percentage of lignin from their plant cell wall.

Isolation of bacteria is limited to aerobic and acidophilic bacteria that can sustain high acidity in the environment. The isolated bacteria are selected based on their higher degradation towards phenolic derivatives identified from tropical peatland. Utilization of the phenolic aldehyde by the isolated strain is expected to have a higher rate of degradation since the genera of that bacteria are known to degrade aromatic compounds. Although an acidic environment may hinder the decomposition process, it is possible to sustain this limitation. The metabolite identification is expected to have a different pathway and unique degradation mechanisms in acidic conditions compared with the well-known reported alkaline condition of lignin-monomer degradation.

1.5 Significance of Study

Evaluating greenhouse gases from the soil in tropical peatland provides a clear picture of the effect of land conversion of pristine forests into oil palm plantations and other anthropogenic disturbances with the microbial process. The emission of CO_2 shows aerobic activity, while CH_4 shows anaerobic activity in the tropical peat soil. The physical and chemical properties of peat soil information show a relationship between microbe's activity with biogeochemical changes in tropical peatlands. Isolation of lignin monomer-degrading bacteria shows a few species that have higher tendencies to degrade lignin monomer in tropical peatlands. Metabolite analysis provides a significant idea on a new metabolic pathway of lignin monomer degradation at tropical peatland in acidic conditions as another mechanism commonly occur in alkaline and neutral condition. This study created extensive information regarding GHG emission from peatland, the composition of tropical peatland, degradation of lignin monomer by bacteria in acidic pH, and their role in tropical peatlands since there is limited research on this subject area, especially in this area Malaysia. This research also follows Sustainable Development Goals (SDGs) on Climate Action and Life on Land that promotes the preservation of Tropical Peatland supporting the carbon sink and storage function, manage sustainably of forest and unique microorganisms.

1.6 Research Gap

(a) Isolation of lignin monomer-degrading bacteria provides vast information about the specific function of bacteria that can degrade phenolic derivatives and lignin monomers in acidic conditions.

(b) The chemical compound composition in tropical peatland helps identify carbon species that are more prone to degradation after peat exposure by anthropogenic disturbance.

(c) Pathway of biodegradation peat substrate in tropical peatland is not yet confirmed either it possesses the same process in temperate peatland.

(d) GHG-related peat only covers the chemical and physical factors contributing to the emission, while less study focuses on the aftereffect of anthropogenic disturbance on bacteria, which further decomposes the carbon sink in tropical peatlands.

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