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ISOLATION OF BACTERIA FROM THE ACIDIC PEAT SWAMP FOREST SOIL AND THEIR LIGNIN DEGRADATION POTENTIAL

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Abstract

The tropical peat swamp forest in Malaysia has reduced significantly due to increasing pressure for development and demand for agricultural land. Pekan peat swamp forest is part of the 200,000 hectares of peat swamp forest located in Pahang, Peninsular Malaysia. While more extensive studies were done on flora and fauna, the study on microbial diversity in this habitat is very limited. The highly acidic environment, low concentrations of nutrients and anoxic condition of the peat are among challenges that hampered the cultivation of microorganism from this environment. In this study two types of agar-based medium, M1 minimal medium (M1) and peat water medium (PW) supplemented with glucose, methanol and lignin were used to isolate bacteria from the peat sediment. In comparison to M1, the use of PW has resulted with higher number of isolates with different morphologies. The PW mainly contains the acidic peat water that was collected from the sampling location. Based on the growth on medium supplemented with lignin, selected isolates were identified using 16s rDNA sequencing. At least three of the isolates showed sequence similarity to *Burkholderia* sp., which is one of the common species, studied on their ligninase-producing abilities. The results from this study serve as the preliminary data for further work on growth characteristics and enzymatic potential of isolates from acidic peat swamp soil.

Keywords: Peat swamp forest, acidic environment, bacteria, 16s rDNA analysis, lignin degradation

1.0 INTRODUCTION

Tropical peat swamp forests are unique ecosystems that are found in tropical lowland, primarily in Southeast Asia where they are concentrated on the island of Borneo and Sumatra as well as Peninsular Malaysia. The tropical peat swamp forests of the Indo-Malayan region differ dramatically from north temperate and boreal peat lands which are dominated by Sphagnum spp., grasses, sedges and shrubs [1], [2]. Interestingly, northern and tropical acidic peatlands appear to display similar patterns of bacterial that was dominated by members of the phyla Acidobacteria and Proteobacteria [3], [2], [4], [5]. The peat consists mainly of slightly or partially decomposed trunks, branches and roots of trees within a matrix almost structureless organic material [6]. The rain-fed, perched water table of this forest is close to or above the peat surface throughout the year and fluctuates with intensity and frequency of the rainfall [7].

Despite the extreme conditions of low pH (2.9–4), low nutrients and anaerobic, unstable, spongy substrate of peat that may be 20 m deep or more, peat swamp forest are habitats for flora and fauna with a high proportion of endemic species of undiscovered potential for medicinal and other important human uses [1], [8]. There was about 1.5 million hectares of peat swamp forest in Malaysia, of which 200,000 is in the state of Pahang [1], [9]. Peat swamp forests are currently threatened either legally or illegally with logging (e.g. for high quality timber such as ramin, *Gonystylus bancanus*), drainage, agricultural conversion (mostly to oil palm, as well as rice, rubber, coconut and pineapple), fire, fragmentation of habitats, hunting and collecting, tin

mining and reclamation for residential centres and industries [8]. It is generally assumed that the characteristic of the peat swamp forest, which has high acidity, low concentrations of nutrient and frequent waterlogged conditions are unfavorable for microbial activities that lead to the accumulation of organic matter as peat [10], [1]. However recent studies had shown that tropical Malaysian peat swamp (North Selangor peat swamp forest) has biodiversity of bacteria that decrease with the increase of peat depth [11]. In fact the studies of leaf litter decomposition in North Selangor Peat Swamp Forest, [12] revealed microbial flora from the peat were responsible to leaf litter decomposition.

It is well known that lignin is one of the most abundant components in plant litter of tropical peat swamp forest. Since the study to understand the role of microorganisms towards plant litter decomposition in the acidic peat swamp forest is still very limited, this paper described the effort to isolate and identify bacteria from Pekan peat swamp forest soil that might have potential in lignin degradation.

2.0 EXPERIMENTAL

2.1 Samples Collection

Soil samples were collected from Compartment 74 in the Peat Swamp Forest, Pekan Forest Reserve, Pahang, Malaysia (03°26'05.4" N, 103°22'40.4" E). The samples were collected at a range of 0 to 30 cm depth and labelled separately based on the site environment and soil condition. The pH and temperature of the samples were recorded as shown in Table 1. Peat water was collected at the peat swamp waterlogged area by using HydroLab Quanta at a depth of 0 to 100 cm. All samples were transported to laboratory and stored at 4 °C for later use.

2.2 Cultivation Medium

The bacteria were cultivated on two types of solid media which were M1 Minimal Medium (M1) and Peat-water Medium (PW). M1 was prepared according to [13] with some modifications. Each 1 liter batch contained; 0.25 g of potassium nitrate (KNO_3); 0.1 g of monopotassium phosphate (KH_2PO_4); 0.1 g magnesium sulphate ($MgSO_4$); 0.1 g yeast extract; 0.005 g of sodium molybdate (Na_2MoO_4); 0.02 g of calcium chloride ($CaCl_2.2H_2O$) and 3.0% w/v agar powder. Different carbon sources; glucose (0.1% w/v), methanol (0.1% w/v) and lignin (0.25% w/v) were supplemented separately to investigate the best carbon source promoting optimum growth of soil bacteria. The pH of the medium was adjusted to pH 4 by using concentrated hydrochloric acid. Peat-water medium (PW) was prepared by using filtered peat-water, supplemented with yeast extract (0.2% w/v), peptone (0.2% w/v), glucose (0.1% w/v) and agar (1.5% w/v) agar. All media were sterilised at 121 °C for 20 minutes at 15 psi in the autoclave. Glucose solution was sterilised using syringe filter 0.22 µm before being added into the sterilized medium.

2.3 Isolation of Pure Culture and Morphology Observation

One gram of soil sample was serially diluted with 100 mL 0.85% saline water and 0.1 mL of each dilution was spread onto the cultivation medium. The inverted solid media were then incubated at 30 °C for 4 to 21 days and observed regularly for any bacterial colony growth. Bacterial colonies were then subculture on fresh plates several times to obtain pure culture. Pure culture isolates were observed and differentiated based on their colony morphologies. All data were recorded accordingly and the pure colonies with distinctive morphological characteristics were subjected to gram staining.

2.3 16S rDNA Analysis

DNA extractions were performed using Vivantis GF-1 Nucleic Acid Extraction Kit according to the manufacturer's instructions. To amplify the 16S rRNA gene from the isolates, PCR was performed using a reaction mixture (50 µL) containing a final concentration of 0.5 U Taq DNA Polymerase, 1x Taq buffer, 200 µM deoxynucleotide solution mix, 2 mM $MgCl_2$ and 0.75 µM bacterial 16S rRNA gene primers, Bac8F and 1492R. Thermal cycling was performed with a (Bio-Rad) thermal cycler using the following parameters: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation, annealing, and extension at 95°C for 1 min, 55°C for 1 min and 72°C for 1 min min, respectively, plus a final extension step at 72°C for 5 min. The purified PCR products were sent off for sequencing at the First BASE Laboratories Sdn. Bhd.

The 16S ribosomal DNA sequences were analysed in National Center Biotechnology Information (NCBI) public database by using nucleotide blast (BLASTn) tool. The entries of organisms with high percentage similarity and identity were recorded from the NCBI website. The phylogenetic tree was reconstructed using MEGA 6 software [14].

3.0 RESULTS AND DISCUSSION

3.1 Peat Water as Growth Medium

PW that was prepared using acidic peat water with pH 3.34 (Table 1), has shown to be the suitable medium to isolate bacteria from the peat soil. The peat water that was collected from the sampling area was included in the growth medium to mimic the natural condition of the peat swamp. We were

able to isolate at least 29 bacteria from acidic soil of Pekan Peat Swamp Forest using PW medium. The colour of the peat water used in the medium are dark brown which due to the high levels of humus, humic acids and tannins [15]. The dark brownish peat water with pH around 3, had low electrical conductivity and low concentration of ions K, Ca, Mg, NO_3-N and PO_4-P [7], [15].

Table 1 Physical parameters of the peat water in Pekan Peat Swamp Forest

Parameter	Description
Temperature	27.5°C
pH	3.34
Dissolved oxygen	2.97 mg/L
Turbidity	17.9

The use of dilute (1:10–1:100) and acidic (pH 4.0–5.5) media demonstrated high potential of success in isolation of bacteria from poorly studied phyla [3], [16] compared to the use of common nutrient media that have a near-neutral pH and a salt content of 1–3 gL^{-1} [4].

3.2 Growth on Lignin Medium

Amongst the 29 colonies, after 7 days of incubation at 30°C, only four isolates which were AR3, AR8, AR10 and AR13 have the ability to grow on M1 supplemented with lignin as shown in Figure 1. All four are Gram-negative bacteria, showed varied morphologies and ranged from small and medium size. While AR8 and AR13 are translucent white colonies, isolates AR3 and AR10 have yellow and pink pigment.

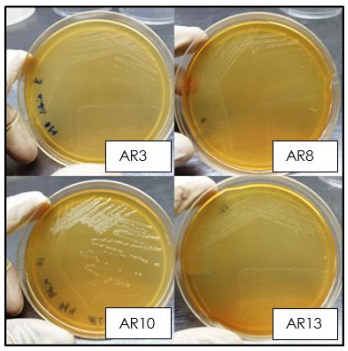

Figure 1 Growth of bacteria on Minimal M1 media with 0.1% w/v alkali lignin as sole carbon and energy source after 7 days incubation at 30°C

3.3 16S rDNA Analysis

Isolate AR3, AR8, AR10 and AR13 were further identified based on their 16s rRNA profiles. Based on the BLAST results, isolate AR3, AR8 and AR13 had 99% identity to *Burkholderia contaminans* strain J2956 (NR_104978.1), *Burkholderia rinojensis* strain A396 (NR_118637.1) and *Burkholderia multivorans* strain LMG 13010 (NR_114523.1) respectively. Another isolate, AR10, had 98% identity to *Serratia marcescens* WW4 strain WW4 (NR_102509.1). In relation to the results, all four isolates were further designated with the respective genuses which were *Burkholderia* sp. AR3, *Burkholderia* sp. AR8, *Burkholderia* sp. AR13 and *Serratia* sp. AR10. The phylogenetic tree for all four isolates and their highest matches in the BLAST results were reconstructed using MEGA 5 as in Figure 2.

Burkholderia species are among the most common species which have been recently studied on their ligninase-producing abilities. In the attempt to discover possible relationships between lignocellulose degradation activity and phylogeny of aerobic microbes in wet tropical forest, it was found that *Burkholderia* was among the most dominant taxa in soil which contributes to the rapid plant litter degradation [17]. This is in line with molecular identification of the three isolates AR3, AR8 and AR13 from this study that was closely related to *Burkholderia* sp. The soil sampled from Pekan peat swamp was obviously rich of decomposing plant litter. According to Bugg *et al*. [18], *Burkholderia multivorans* and *Burkholderia cepacia* contains 3,4-protocatechuate dioxygenase (3,4-PCD, ortho-cleavage) gene which indicates their part in lignin-degrading pathway. Another research on bacterial lignin peroxidase discovered that the *Serratia marcescens* were able degrade 44 to 49% lignin whilst giving out 60% to 75% colour reduction on a study of bio bleaching of paper-pulp mill effluent [19].

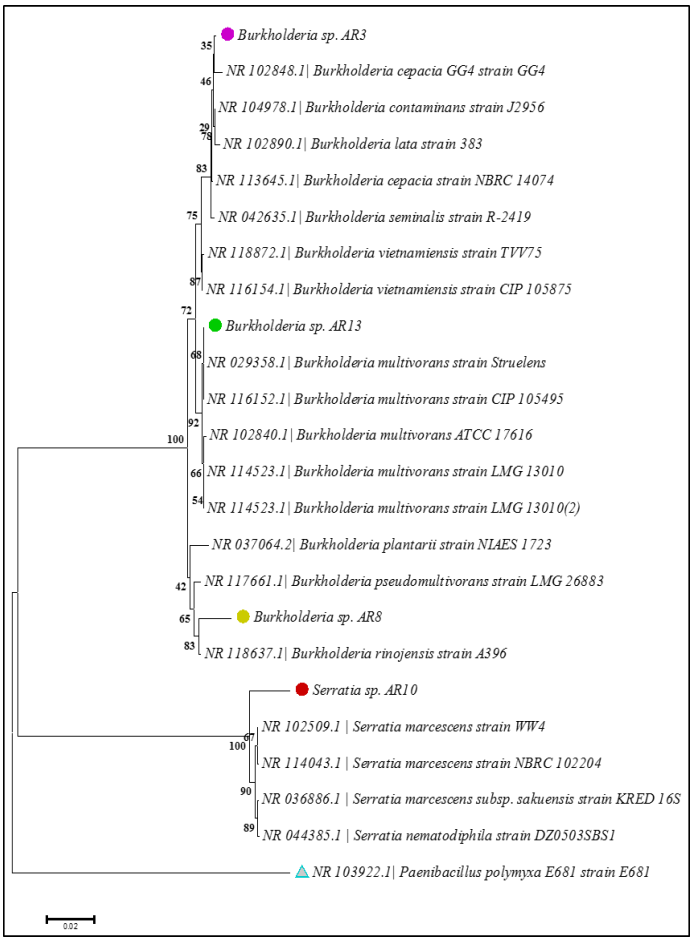

Figure 2 Phylogenetic tree of *Burkholderia* sp. AR3, *Burkholderia* sp. AR8, *Burkholderia* sp. AR13 and *Serratia* sp. AR10 that were isolated from the peat soil and their closely related sequences obtained from NCBI database

4.0 CONCLUSION

The ability of *Burkholderia* sp. AR3, *Burkholderia* sp. AR8, *Burkholderia* sp. AR13 and *Serratia* sp. AR10 to grow on medium supplemented with lignin indicated the potential of AR3, AR8, AR13 and AR10 for further studies on lignin degradation. These potential should be further confirmed through more specific ligninase enzyme assay such as using veratryl alcohol or 2, 6-dimethoxy-phenol as substrate.

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