SHORT COMMUNICATION

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SHORT COMMUNICATION: CULTURABLE BACTERIA POPULATION IN DIFFERENT OIL PALM PEAT SOIL OPERATIONAL ZONES

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ABSTRACT

The bacterial community in an ecosystem can contribute to crucial soil ecosystem functions. Different soil variations in a single oil palm tree may lead to the diversity of bacterial populations. Hence, this study aimed to isolate the culturable bacteria from different operational zones in oil palm peat soil. A total of four operational zones, namely Frond Pile (FP), Harvesting Path (HP), Weeded Circle (WC), and Inter Palm Row (IPR) at Sungai Miang, Pekan, Pahang, Malaysia were selected as the sampling plots. The soil samples were cultivated in selective mineral media. The bacterial isolates were obtained by three times repeated series of plating on solid media and grown at 30°C for four days. Results of the 16S ribosomal ribonucleic acid (16S rRNA) discovery revealed a diverse group of bacteria in groups of Proteobacteria, which were detected in a higher percentage relative to other classes of bacteria. The γ-proteobacteria was the prevalent class of Proteobacteria followed by α-proteobacteria and β-proteobacteria. The highest γ-proteobacteria population was obtained in IPR followed by HP, WC and FP. These diverse Proteobacteria communities at the aforementioned operational zones harboured significant roles in the peat soil ecosystems, which need to be further analysed in future research.

Keywords: culturable bacteria, oil palm, operational zones, peat soil.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is planted in an equilateral triangular design with a spacing of 8.50-10.25 m (Corley and Tinker, 2003). There is significant variability in soil properties on the scale of individual palms, caused by plant features and management practices (Anamulai *et al*., 2019; Tinker, 1960). Oil palm is becoming an increasingly important crop in the tropics (Cramb and Curry, 2012), and accurate evaluation of soil properties under oil palm is crucial to the industry's productivity and sustainability. Consideration of tree-scale variability is needed for soil condition monitoring, fertiliser application recommendations, and calculations of water, carbon, and nutrient stock (Nelson *et al*., 2014; 2019).

Soil biodiversity plays a major role in the functioning of the ecosystem, which helps to maintain soil sustainability (Delgado-Baquerizo *et al*., 2020). However, information on the ecosystem function in different oil palm operational zones is still lacking. According to Carron *et al*. (2015), the zones around the palms contain varying amounts of soil fauna and nutrients. The samples collected

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in any particular management zone do not describe anything about processes in other zones, which may be important for the palm. As a result of typical management regimes, direct linkage of the operational zones with the soil microbes in an oil palm plantation, which is crucial for many critical ecosystem functions, including nutrient cycling, carbon sequestration and plant nutrient uptake (Schröder *et al*., 2016), requires further investigation.

This study involves the cultivation and comparison of the bacterial diversity in the peat soil of an oil palm plantation in Pekan, Pahang, Malaysia. The studied area was the four operational zones, which consists of the frond pile (FP), harvesting path (HP), weeding circle (WC), and inter palm row (IPR).

METHODOLOGY

Soil Sampling

A sampling of soil for methanotrophic bacteria diversity study at fertilised oil palm plantation, Pekan, Pahang, Malaysia, was conducted from four operational zones consisting of FP, HP, WC and IPR (*Figure 1*) in August 2017. Sampling was done at the respective GPS coordinates of 3°26'09.748" N, 103°23'23.555" E, 3°26'10.794" N, 103°23'17.438" E, 3°26'18.062" N, 103°23'19.458" E and 3°26'17.328" N, 103°23'28.560" E at a depth of 0-30 cm. Soil sampling was done in triplicates using a 5 cm internal diameter auger, carefully kept in the ice box, and stored at -80°C for further analysis.

Enrichment Culture

The basal medium, nitrate mineral salts (Whittenbury *et al*., 1970), was used for bacterial consortium enrichment. 1 L of the basal medium was sterilised by autoclaving at 121°C for 15 min. Then, a total of 1 g of the soil sample was inoculated into a serum flask filled with a 20% capacity of the basal medium. Methane gas was continuously supplied at 10% (v/v). The culture was stirred at 150 rpm in a shaker incubator and incubated at 30°C for three days.

Isolation of Soil Bacteria

Nitrate mineral salt agar, nutrient agar, soil enriched medium, nitrogen-deficient medium, Pikovskaya medium, Aleksandrov medium, anaerobic medium and actinomycetes medium were used for bacterial isolation using the spread plate method. Isolated colonies were subcultured repeatedly to obtain a single type of isolated bacterial colony. The culture was incubated at 30°C for three days.

Polymerase Chain Reaction (PCR) Amplification of 16S Ribosomal Ribonucleic Acid (16S rRNA) and Purification

Isolates from plate cultures were added into 100 µL of sterilised distilled water and boiled for 10 min at 100°C to lyse the cells and subsequently amplified using PCR. The primer set used was forward (f) primer, 341f (5'-cct-acg-gga-ggc-agc-ag-3') and reverse (r) primer 907r (5'-ccc-cgt-caa-

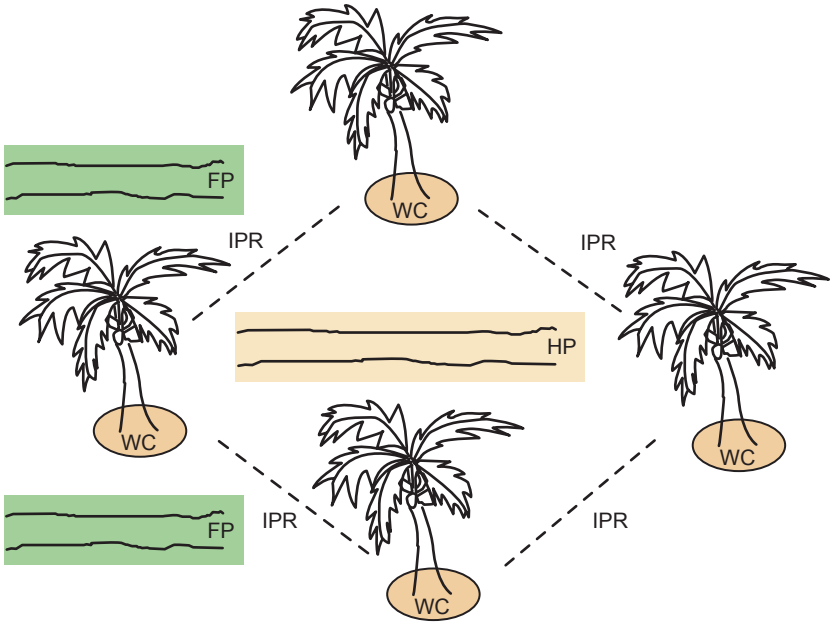

Figure 1. Typical operational zones of a mature oil palm plantation. The pattern is repeated throughout the plantation. Operational zones are the frond pile (FP), where pruned fronds are placed, the weeded circle (WC), which is kept bare to facilitate harvesting, the harvest path (HP), upon which fruit is removed from the plantation and workers access for other management practices, and the inter palm row (IPR), is the space between palms.

ttc-att-tga-gtt-t-3′) using the PCR program (Muyzer *et al*., 1993). The PCR was performed in 25 µL of reaction volume with a thermocycler (gradient) containing a succession of 10 pmol of each primer, 100 mM dNTPs, 1X PCR buffer, 50 mM Mg_2Cl, 0.3% BSA, and 2.5 units of Taq polymerase. The PCR started with initial denaturation at 94°C for 2 min, 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s, final extension at 72°C for 2 min, and held at 10°C. Eluted DNA from excised agarose gel was purified using QIAquick gel extraction kits (QIAGEN, Inc., Valencia, CA, USA) according to the kit's protocol.

Sequencing Analysis

The purified PCR products were sent to First Base Laboratories for sequencing. The nucleotide sequences were read using the software ChromasPro (www.technelysium.com.au/ChromasPro.html) and analysed using an online sequence database available at the National Center for Biotechnology Information (NCBI). A sequence similarity search was conducted using the nucleotide-nucleotide basic logic alignment search tool (BLASTn) in the NCBI GenBank database to identify the nearest relatives of the partially sequenced 16S rRNA genes of excised bands.

Statistical and Phylogenetic Analysis

Alpha diversity and principal component analysis (PCA) matrices scatter biplot were performed based on the identified isolated bacteria to compare the bacterial diversity in the soil samples among the four aforementioned operational zones using the PAST software program (Hammer *et al*., 2001).

The nucleotide sequences determined in this study were aligned, and neighbour-joining trees were constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 (Kumar *et al*., 2016). Neighbour-joining phylogenetic trees were constructed based on the 16S rRNA gene by using the Kimura two-parameter substitution model evaluated by 1000 bootstrap samplings of the data, and nodes with bootstrap values were indicated.

RESULTS AND DISCUSSION

Based on the data of total microbes, the Dominance (D) and Berger Parker indices data in *Table 1* showed that the Proteobacteria dominated the soil community. This is due to the highest value of Proteobacteria classes compared to other bacterial classes. Among them, β-proteobacteria was the most dominant, followed by α-proteobacteria and γ-proteobacteria. In comparison, the Shannon (H) index (*Table 1*) shows that bacilli was the most diverse among other bacterial classes.

The relative abundance of the identified bacterial classes was plotted in *Figure 2* and the accession number is included in *Table 2*. The Proteobacteria was the most prevalent phylum amongst the prokaryotic population. Firmicutes was the second most frequent, followed by Actinobacteria. The phylum Bacteroidetes for class Sphingobacteria could only be found in the HP area and was also the only site, which did not harbour the ß-proteobacteria. The γ-proteobacteria was found in the highest percentage and could be found within all sites with the highest occurrence in IPR (52%), followed by HP (32%), WC (29%), and FP (10%). The second prevalent class was α-proteobacteria, which mostly appeared in FP (43%), followed by HP (32%), WC (10%), and IPR (5%). ß-proteobacteria could be found mostly in WC (14%), IPR (10%), and FP (5%). Bacterial class of bacilli can also be found in peat soil with the percentage occurrence of FP (38%), WC, and IPR both (24%), and HP (21%). Whilst, the percentage of occurrences in class Actinobacteria were WC (24%), HP (11%), IPR (10%), and FP (5%).

PCA was used to correlate the bacterial classes with the operational zones (*Figure 3*). The differences between the bacterial communities can be seen clearly in the distribution of the bacterial classes. The α-proteobacteria and bacilli were generally clustered together in the FP. Both classes are essential in FP, mainly involved in decomposition and as an additional carbon source (Hirano *et al*., 2009). The α-proteobacteria was also clustered in HP along with γ-proteobacteria. Most of the known methanotrophs belong to α-proteobacteria and γ-proteobacteria (Semrau *et al*., 2010). This is an

TABLE 1. BACTERIAL BIODIVERSITY INDICES FOR 16S rRNA GENE LIBRARIES REPRESENTING PEAT SOIL SAMPLE PEKAN, PAHANG, MALAYSIA

Diversity indices	Bacterial class				
	Actinobacteria	Bacilli	Proteobacteria		
			Alpha	Beta	Gamma
Dominance_D	0.3288	0.2653	0.3701	0.3817	0.3086
Berger-Parker	0.4800	0.3551	0.4778	0.4828	0.4228
Shannon_H	1.238	1.358	1.125	1.022	1.259

TABLE 2. SUBMITTED NCBI BLASTn BACTERIAL NAME AND ACCESSION NUMBER

Description	Accession
Sphingomonas zeae	KX682019.1
Sphingomonas paucimobilis	LN867216.1
Sphingomonas zeae strain	KX682019.1
Methylobacterium radiotolerans	KT592238.1
Stenotrophomonas sp.	KY084474.1
Methylobacterium radiotolerans	KT923692.1
Pseudarthrobacter defluvii	KY882049.1
Stenotrophomonas sp.	KY084474.1
Sphingomonas zeae	KX682019.1
Methylobacterium radiotolerans	KF777382.1
Luteibacter jiangsuensis	KY029044.0
Stenotrophomonas maltophilia	JN592614.1
Methylobacterium radiotolerans	KF777382.1
Paenibacillus barengoltzii	KP704353.1
Arthrobacter defluvii	FN908791.1
Stenotrophomonas maltophilia	JN592614.1
Stenotrophomonas sp.	KY084474.1
Luteibacter jiangsuensis	KY029044.1
Luteibacter jiangsuensis	KY029044.1
Staphylococcus sp.	KF777547.1
Bacillus koreensis	KT986105.1
Staphylococcus epidermidis	KR809425.1
Bacillus subtilis	JQ246902.1
Bacillus subtilis	KX453903.1
Staphylococcus sp.	KY865751.1
Bacillus subtilis	KX453903.1
Arthrobacter chlorophenolicus	GU326384.1
Moraxellaceae bacterium	KF777626.1
Micrococcus luteus	LN884071.1
Arthrobacter sp.	KY476117.1
Pseudarthrobacter defluvii	KY882049.1
Pandoraea thiooxydans	CP014839.1
Moraxellaceae bacterium	KF777626.1
Moraxella osloensis	LT718623.1
Arthrobacter sp.	KY476117.1
Brevibacillus fluminis	KF958491.1
Bacillus altitudinis	KY820045.1
Brevibacillus fluminis	NR_116293.1
Staphylococcus hominis	KP780178.1
Spirometra erinaceieuropaei	LN020105.1
Bacillus altitudinis	KY820045.1
Brevibacillus sp.	KU578096.1

Description	Accession
Brevibacillus fluminis	NR_116293.1
Serratia sp.	KY848325.1
Bacillus thuringiensis	KU180424.1
Bacillus cereus	KY029074.1
Pseudarthrobacter equi	LT629779.1
Bacillus sp.	JX532715.1
Pseudomonas stutzeri	KF318832.1
Bacillus megaterium	FJ944677.1
Brevibacillus panacihumi	KU921113.1
Bacillus flexus	KX853169.1
Mesorhizobium soli	NR_145552.1
Luteibacter jiangsuensis	KY029044.1
Burkholderia sp.	JQ316420.1
Dyella yeojuensis	FN796854.1
Bacillus amyloliquefaciens	AB735985.1
Serratia marcescens	KU522248.1
Serratia sp.	KY848325.1
Rhizobium sp.	KU097063.1
Methylobacterium radiotolerans	KY882119.
Sphingomonas sp.	FR872453.1
Ochrobactrum sp.	HQ652578.1
Luteibacter jiangsuensis	KY029044.1
Luteibacter jiangsuensis	KY029044.1
Dyella yeojuensis	FN796854.1
Methylobacterium oryzae	AY683046.1
Methylobacterium radiotolerans	KF777382.1
Ralstonia sp.	KF777622.1
Dyella japonica	AM268334.1
Methylobacterium radiotolerans	KF777382.1
Luteibacter jiangsuensis	KY029044.1
Amycolatopsis sp.	KP232907.1
Streptomyces diastaticus	KY458979.1
Burkholderia soli	KP687356.1
Luteibacter jiangsuensis	KY029044.1
Brevundimonas aurantiaca	KC429645.1
Methylobacterium oryzae	AY683046.1
Pedobacter cryoconitis	KC788066.1
Massilia aurea	LT718650.1
Oxalobacteraceae bacterium	KM274103.1
Pseudomonas luteola	KX301304.1
Dyella japonica	AM268334.1

exciting discovery since both bacterial classes were also reported to co-dominate the active methane-oxidising communities in an acidic boreal peat bog (Esson *et al*., 2016). The soil structure in HP usually has a higher bulk density to facilitate the movement of labour and equipment on the plantation (Melling and Henson, 2011). Actinobacteria and ß-proteobacteria were both clustered together in WC, whereas γ-proteobacteria was only clustered in IPR. WC-clustered actinobacteria can play critical roles in various plant growth-promoting attributes, such as phosphorus solubilisation, potassium and zinc, and biological nitrogen fixation (Yadav and Yadav, 2019). Both HP and IPR showed the prevalent cluster of γ-proteobacteria. Further research needs to be conducted to unravel the role of γ-proteobacteria in methane mitigation in these two operational zones and oil palm plantations subsequently.

Phylogenetic tree indicated the presence of 56 bacterial species isolated from peat soil is shown in *Figure 4*. This phylogenetic tree was constructed based on similar nucleotide sequences using BLASTn, Kimura two-parameter algorithm, and the neighbour-joining method. The major phyla of Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes are shown and clustered. The abundance presence of bacterial genus under the class of α-proteobacteria and γ-proteobacteria would be related to previous studies on their contribution to the global nutrient cycle. According to Aislabie and Deslippe (2013), the important genus isolated for α-proteobacteria was among the heterotroph and methanotrophs. The genera include *Methylobacterium*, *Mesorhizobium*, *Rhizobium*, and *Sphingomonas*. *Methylobacterium* plays a major role as a soil methane oxidiser. Both *Mesorhizobium* and *Rhizobium* have dual functions as nitrogen fixers and form a symbiotic relationship with legumes. In contrast, *Sphingomonas* can degrade toxic compounds like pentachlorophenol and polyaromatic hydrocarbons. The *Pseudomonas* genus in γ-proteobacteria was implicated in oil degradation studies. Under aerobic conditions, isolated *Pseudomonas* genes and enzymes can degrade alkanes, monoaromatics, naphthalene, and phenanthrene as a sole carbon source (Martirani-Von Abercron *et al*., 2017).

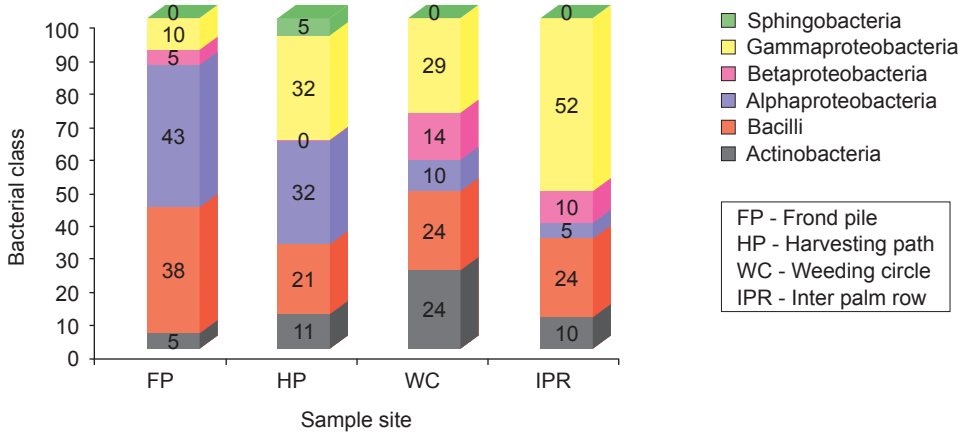

Figure 2. Percentage of bacterial class via 16S rRNA gene identification.

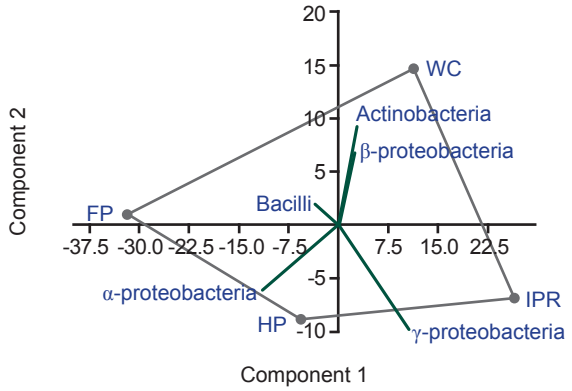

Figure 3. Principal Component Analysis (PCA) of bacterial classes isolated from oil palm peat soil at Pekan, Pahang, Malaysia.

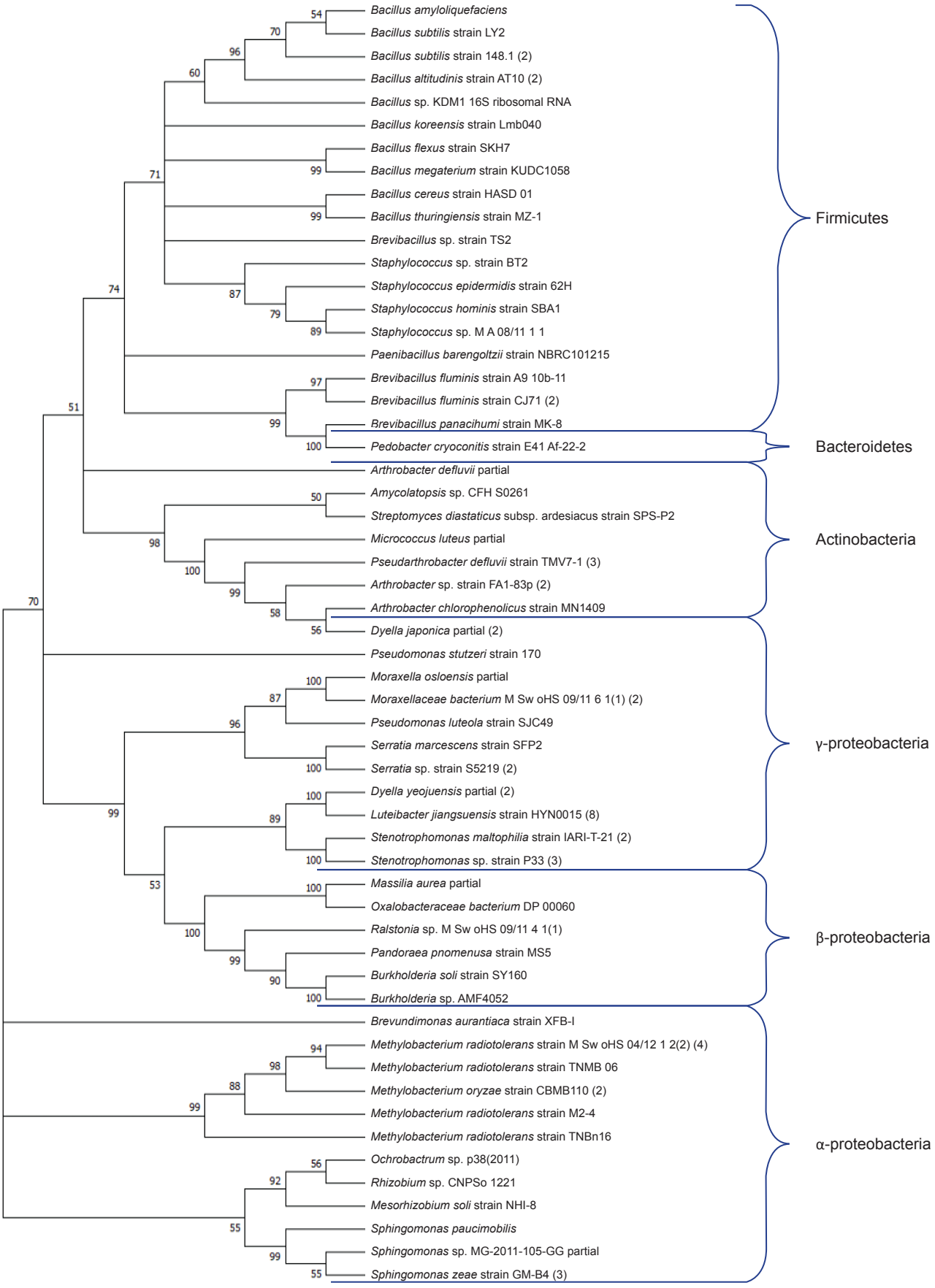

Figure 4. Phylogenetic tree derived from microbes isolated from peat soil at Pekan, Pahang, Malaysia. The tree was constructed based on similar nucleotide sequences using BLASTn, Kimura two-parameter algorithm and the neighbour-joining method. Bootstrap values (expressed as percentage of 1000 replications) are reported at each node. The scale bar indicates 0.1 substitutions per nucleotide positions.

CONCLUSION

Phylum Proteobacteria dominated the peat soil in Pekan, Pahang, Malaysia. Proteobacteria, comprised of α-proteobacteria, ß-proteobacteria, and γ-proteobacteria, were the most dominant bacterial class in the FP, HP, WC, and IPR. The Proteobacteria community that inhabits the aforementioned operational zones is the crucial indicator of nutrient cycling, carbon sequestration and plant nutrient uptake for sustainable soil in oil palm plantations.

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