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MOLECULAR APPROACHES IN ASSESSING THE SOIL MICROBIAL DIVERSITY OF MALAYSIAN OIL PALM PLANTATIONS: A REVIEW

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ABSTRACT

Soil microorganisms are vital to the overall function, stability and sustainability of the ecosystem. Microbial communities are key bio-indicators for sustainable agro-ecosystem management and for evaluating oil palm soil health in Malaysia. The development of effective methods for studying the diversity, distribution and behaviour of soil microorganisms is essential for a broader understanding of soil quality and fertility. In the past, culture-dependent, Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal-Restriction Fragment Length Polymorphism (T-RFLP) were used to assess soil microbial community, abundance and diversity in the oil palm soils. However, these methods have significant shortcomings and limitations in microbial ecology monitoring. In recent years, high-throughput next-generation sequencing has dramatically increased the resolution and detectable spectrum of microbial from environmental samples, and has played a significant role in microbial ecology studies. Next-generation sequencing using 454-pyrosequencing, Ion Torrent, Illumina and PacBio platforms are rapid and flexible. These sequencing platforms are powerful metagenomic tools for analysing microbial communities, including their structure, metabolic potential, diversity and their impact on ecosystem functions. In this review, the major development of molecular approaches used in the characterisation of soil microbial community dynamics and their functions in Malaysian oil palm plantations are also discussed.

Keywords: culture-dependent, culture-independent, microbial diversity, oil palm plantation.

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INTRODUCTION

Soil is one of the main reservoirs of biodiversity which consists of complex and diverse species of living organisms (Bardgett and Van Der Putten, 2014). Soil supports a large fraction of below-ground organisms ranging from macrofauna (earthworms, woodlice, ants, termites, beetles, arachnids), mesofauna (mites, springtails, enchytraeids) to microscopic organisms such as protozoa, bacteria, fungi and archaea (Food and Agriculture Organization, 2020). Among these organisms, soil microorganisms play a key role in regulating ecosystem functions and services such as biogeochemical cycles, organic matter decomposition, plant productivity and greenhouse gas emissions (GHG) (Bardgett and Van Der Putten, 2014; Delgado-Baquerizo *et al.*, 2016). These organisms regulate climate change through the emission of atmospheric GHGs, such as carbon dioxide, methane and nitrous oxide. Their roles are particularly crucial in agricultural ecosystems to enable sustainable farming, food security and climatic impacts (Singh *et al.*, 2010).

The year 2017 was a historical milestone for the Malaysian oil palm industry as it marks 100th year celebration of commercial oil palm planting in this country. Oil palm (*Elaeis guineensis* Jacq.) cultivation occupies over 5.67 million hectares of land by 2022 and impacted the country's economy in the last 100 years (Parveez *et al.*, 2023). In 2022, Malaysia became the major exporter of palm oil, as reflected by the 24.77 million tonnes of the global oils and fats exported, while contributing to 57.9% of the total

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palm oil trade (Parveez *et al.*, 2023). Maintaining oil palm plantations through regular harvesting, pruning, fertilisation and pest management have resulted in significant alterations to the environment and microclimate (Dhandapani *et al.*, 2020). Various studies have suggested that plantation agriculture would affect the microbial composition in soils of tropical regions (Lee-Cruz *et al.*, 2013; McGuire *et al.*, 2015).

According to Food and Agriculture Organization (2020), a gram of soil contains up to one billion bacterial cells, and 200 m of fungal hyphae belonging to thousands of taxa. Therefore, it is crucial to identify and characterise these diverse microorganisms with appropriate methods and tools. Traditionally, methods such as isolation, inoculation, and cultivation of pure cultures from soils were used many years ago (Dobrovol'skaya *et al.*, 2009). Through these methods, soil microbes can be distinguished based on colony morphology characterisation, which would provide a solution for microbial identification. However, there are microbes' group or taxa that are unable to grow under laboratory conditions and selective media, which would not have been accounted for in the diversity (Stewart, 2012). Thus, these methods will only be able to identify a minor proportion of the total microbial community and do not reflect the whole microbial diversity in soil (Dobrovol'skaya *et al.*, 2009).

The use of molecular approaches has been applied to quantify the diversity and identity of soil microbial communities. Application of genes such as 16S rDNA (bacteria, archaea), 18S rDNA (eukaryotes) and ITS (fungi) were employed in Terminal-Restriction Fragment Length Polymorphisms (T-RFLP), Temperature Gradient Gel Electrophoresis (TGGE) and Denaturing Gradient Gel Electrophoresis (DGGE) (Prosser, 2002). These PCR-based genetic fingerprinting techniques provide microbial profiling based on the variation of targeted genes amplified from the soil (Anderson and Cairney, 2004). These techniques are reproducible and effective at characterising the dynamics of microbial diversity, including uncultivated microbes (Ayob and Kusai, 2021; Gryta and Frąc, 2020). Nevertheless, these methods have limitations as they are very laborious, expensive with low throughput (Nkongolo and Narendrula-Kotha, 2020).

The recent development of molecular tool has facilitated in measuring of soil microbial community and diversity. The most useful approach involves sequencing ribosomal genes which are highly conserved and can reveal community structure across the taxa such as prokaryotes (bacteria, archaea) and eukaryote (fungi) (Nkongolo and Narendrula-Kotha, 2020). One of the promising approaches to studying complex soil microbial communities is using next-generation sequencing (NGS). The revolution of NGS technologies is demonstrated through a variety of platforms such as 454 pyrosequencing, Ion Torrent, Illumina MiSeq, HiSeq and NovaSeq, PacBio and Oxford Nanopore MinION (Nilsson *et al.*, 2019). These platforms provide comprehensive information in detecting microbial communities and their functional ecology over large spatial and temporal scales. Advancements in molecular methods has allowed for a higher resolution assessment of microbial compositions (Dom *et al.*, 2021).

Furthermore, the analysis of diversity can be conducted by considering factors that influence the biodiversity of soil microbial communities and its impact on oil palm management practices, and predicting ecosystem responses to a changing environment (Ayob and Kusai, 2021; Tripathi *et al.*, 2012). There have been several studies that investigated the effects of land use change on the microbial community in the Malaysian oil palm plantation ecosystem using traditional and advance molecular methods, as listed in *Table 1*. In this article, we briefly describe the major development in molecular methodological strategies to better assess and understand the distribution and characterisation of soil microbial diversity in Malaysian oil palm plantation ecosystems. *Figure 1* briefly demonstrates both culture-dependent and -independent approaches for identifying soil microbial communities. In particular, we also identify the potential function of each microbial species in oil palm plantations using molecular approaches, as summarised in *Table 2*.

TABLE 1. MOLECULAR APPROACHES USED TO IDENTIFY AND CHARACTERISE SOIL MICROBIAL COMMUNITIES IN MALAYSIAN OIL PALM PLANTATIONS

Method	Microorganism	Locality	Age of oil palm	References
454-pyrosequencing (Roche)	Bacteria	Peninsular Malaysia	ND	Tripathi *et al.* (2012)
Illumina HiSeq	Bacteria	Sabah	20 to 30-yr-old	Lee-Cruz *et al.* (2013)
454-pyrosequencing (Roche)	Fungi	Sabah	20 to 30-yr-old	Kerfahi *et al.* (2014)
Illumina MiSeq	Fungi	Negeri Sembilan	25-yr-old	McGuire *et al.* (2015)
Culture-dependent	Fungi	Sarawak	7 and 11-yr-old	Ahmad Ali *et al.* (2016)

TABLE 1. MOLECULAR APPROACHES USED TO IDENTIFY AND CHARACTERISE THE SOIL MICROBIAL COMMUNITIES IN MALAYSIAN OIL PALM PLANTATIONS (continued)

Method	Microorganism	Locality	Age of oil palm	References
Illumina HiSeq	Bacteria, eukaryotes, archaea, and functional gene	Sabah	20 to 30-yr-old	Tripathi *et al.* (2016)
Illumina MiSeq	Bacteria	Negeri Sembilan	25-yr-old	Wood *et al.* (2017)
Culture-dependent	Fungi	Sarawak	3.5-yr-old	Ayob *et al.* (2018)
Culture-dependent	Fungi	Sarawak	7.5 and 11.5-yr-old	Kusai *et al.* (2018)
Denaturing Gradient Gel Electrophoresis (DGGE)	Bacteria	Sarawak	7 -yr-old	Maidin *et al.* (2018)
454-pyrosequencing (Roche)	Eukaryotes	Sarawak	7 and 11-yr-old	Ahmad Ali *et al.* (2018)
Illumina MiSeq	Bacteria	Sabah	10-yr-old	Tin *et al.* (2018)
Denaturing Gradient Gel Electrophoresis (DGGE)	Bacteria	Sarawak	1.5 – 5.5-yr-old	Ahamed Bakeri *et al.* (2019)
Culture-dependent	Bacteria	Sarawak	7.5-yr-old	Kusai and Ayob, 2020
Illumina MiSeq	Fungi	Sabah	7-yr-old	Robinson *et al.* (2020)
Illumina HiSeq	Bacteria	Kelantan	ND	Rupert *et al.* (2020)
Culture-dependent; Denaturing Gradient Gel Electrophoresis (DGGE)	Bacteria	Sarawak	12-yr-old	Ayob and Kusai, 2021
Illumina MiSeq	Bacteria, eukaryotes, fungi	Sabah	2, 7 and 12-yr-old	Drewer *et al.* (2021)
Culture-dependent; Denaturing Gradient Gel Electrophoresis (DGGE); Illumina MiSeq	Bacteria	Negeri Sembilan	25-yr-old	Salamat *et al.* (2021)
Culture-dependent; Denaturing Gradient Gel Electrophoresis (DGGE)	Fungi	Sarawak	3 to 4-yr-old	Wong *et al.* (2021)

Note: ND - Not determined; yr - year.

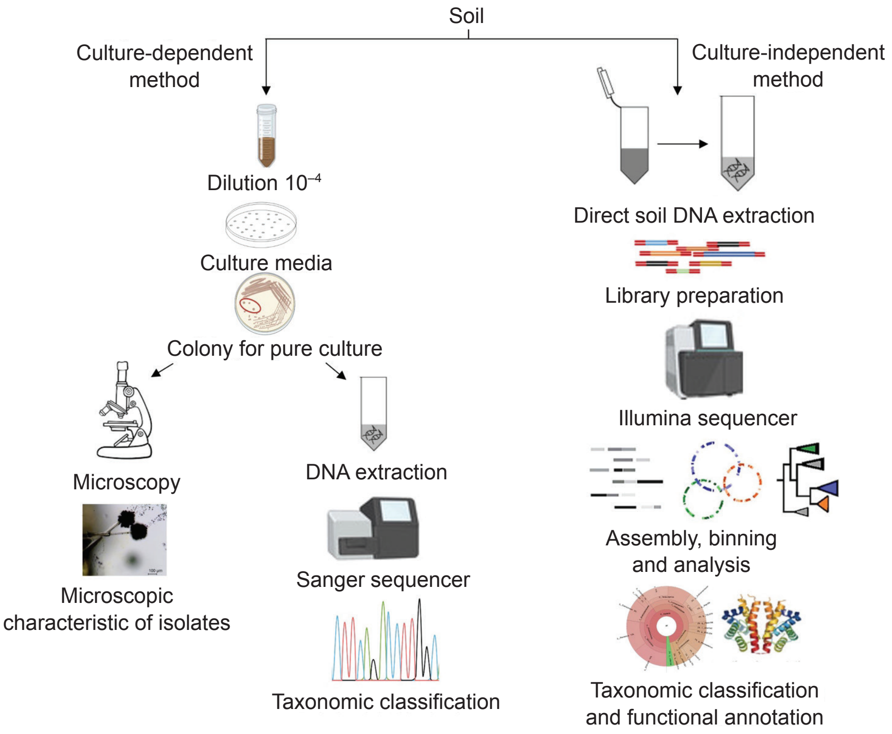

Figure 1. Schematic diagram of culture-dependent and culture-independent methods for identifying soil microbial communities.

TABLE 2. SUMMARY OF POTENTIAL FUNCTIONS OF EACH SPECIES IDENTIFIED IN OIL PALM PLANTATIONS USING MOLECULAR APPROACHES

Method	Phylum	Order/ Family/ Species	Function/ role	References
Culture-dependent	**Actinobacteria**	*Micrococcus* sp.	i. Degrades lignocellulose ii. Plant-growth promoting bacteria	Ayob and Kusai, 2021
		Arthrobacter sp.	i. Ability to remove atrazine herbicide in soil ii. Remediation of atrazine contaminated soil	Kusai and Ayob, 2020
	Firmicutes	*Bacillus cereus*	i. Nitrogen-fixing bacteria	Kusai and Ayob, 2020
		Bacillus subtilis	i. Produce cellulose-degrading enzymes to hydrolyse cellulose of plant litter	Kusai and Ayob, 2020
	Proteobacteria	*Burkholderia* sp.	i. Involve in the decaying process of the plant litter ii. Ability to breakdown lignin	Ayob and Kusai, 2021; Kusai and Ayob, 2020
		Burkholderia cepacia	i. Biocontrol agent to inhibit *Ganoderma boninense*, the causal agent of basal stem rot of oil palm ii. Improve plant growth	Kusai and Ayob, 2020
	Ascomycota	*Aspergillus niger*	i. Enhance phosphate solubilisation process in agricultural soils ii. Increase fertility of soil and oil palm health	Kusai *et al.* (2018)
		Penicillium chrysogenum	i. Ability to degrade wood in soils	Kusai *et al.* (2018)
		Penicillium sp.	i. Produce cellulases for degradation of plant biomass	Kusai *et al.* (2018)
		Trichoderma atroviride	i. Produce laccases for degradation of lignocellulosic materials	Kusai *et al.* (2018)
		Trichoderma harzianum	i. Possess biocontrol properties against basal stem rot disease of oil palm ii. Incorporate in biofertiliser to reduce disease incidence in oil palm plantation	Kusai *et al.* (2018)
		Talaromyces purpurogenus	i. Involve in phosphate solubilisation in soil	Kusai *et al.* (2018)
		Trichoderma koningii	i. Involve in nitrous oxide (N_2O) production	Kusai *et al.* (2018)
Denaturing Gradient Gel Electrophoresis (DGGE), 454-pyrosequencing, Illumina Sequencing	**Actinobacteria**	-	i. Breakdown macromolecules such as cellulose, lignin, chitin and starch by producing extracellular enzyme	Maidin *et al.* (2018)
		Actinomycetales, Solirubrobacterales	i. Potential biomarker in responses to land disturbance	Ahamed Bakeri *et al.*, 2019; Lee-Cruz *et al.*, 2013; Salamat *et al.*, 2021
		Streptomyces spp.	i. Synthesise antibiotics in the presence of plant pathogens ii. Mediating species interactions in soil	Ahamed Bakeri *et al.* (2019)
		Sinomonas humi	i. Amylolytic bacteria ii. Utilise carbon sources	Tin *et al.* (2018)
	Acidobacteria	-	i. Decompose lignocellulosic plant biomass	Ahamed Bakeri *et al.* (2019)
		Frankiales, Pseudonocardiales	Nitrogen fixing bacteria	Ahamed Bakeri *et al.* (2019)
	Bacteroidetes	-	i. Utilise available organic matter under agriculture soils ii. Cellulose degradation	Ahamed Bakeri *et al.* (2019)

TABLE 2. SUMMARY OF POTENTIAL FUNCTIONS OF EACH SPECIES IDENTIFIED IN OIL PALM PLANTATIONS USING MOLECULAR APPROACHES (continued)

Method	Phylum	Order/ Family/ Species	Function/ role	References
	Bacteroidetes	*Chryseobacterium*	i. Plant-growth promoting bacteria	Ayob and Kusai, 2021
	Chloroflexi	-	i. Involve in nitrogen cycle such as nitrogen fixation and nitrite oxidation	Ahamed Bakeri *et al.* (2019)
	Cyanobacteria	-	i. Plant-growth promoting bacteria ii. Utilise nitrogen through nitrogen fixation process iii. Increase phosphorus availability iv. Photosynthetic bacteria	Ahamed Bakeri *et al.* (2019)
	Firmicutes	*Paenibacillus polymyxa, Paenibacillus jamilae, Paenibacillus peoriae*	i. Involve in nitrogen-fixation process ii. Biocontrol agent	Maidin *et al.* (2018)
	Nitrospirae	Nitrospirales	i. Involve in nitrification activity in soils	Ahamed Bakeri *et al.*, 2019; Maidin *et al.*, 2018; Tripathi *et al.*, 2016
	Proteobacteria	*Rhodoplanes*	i. Plant growth promoting bacteria	Maidin *et al.* (2018)
		Rhizobium sp.	i. Plant growth promoting bacteria ii. Nitrogen-fixing bacteria	Maidin *et al.* (2018)
		α-Proteobacteria	i. Degrade inorganic compounds ii. Involve in nitrogen-fixation process	Ahamed Bakeri *et al.* (2019)
		β-Proteobacteria; *Burkholderia*	i. Involve in nitrogen-fixation process	Ahamed Bakeri *et al.* (2019)
		β-Proteobacteria *Nitrosomonadales*	i. Ammonia-oxidising bacteria	Ahamed Bakeri *et al.* (2019)
		γ-Proteobacteria	i. Decompose soil carbon substrate ii. Increase supply of labile carbon substrates to soil	Ahamed Bakeri *et al.* (2019)
		δ-Proteobacteria	i. Bacterial indicator of land-use change	Ahamed Bakeri *et al.* (2019)
	Ascomycota	*Polyporales*	i. Saprophytic fungi growing on fallen woods and grasses	Wong *et al.* (2021)
		Euratioles, Hypocreales, Sordariales	i. Involve in denitrification process in soil	Wong *et al.* (2021)
		Xylariales	i. Wood inhabiting saprotrophs	Wong *et al.* (2021)
		Sebacinales	i. Endophytic fungi ii. Enhance plant growth and disease resistance	Wong *et al.* (2021)
	Basidomycota	*Agaricales*	i. Degrades plant-derived cellulose ii. Lignicolous fungi	Kerfahi *et al.* (2014)
		Sporidiobolales	i. Saprophytic and mycorrhizal fungi	Kerfahi *et al.* (2014)
	Glomeromycota	-	i. Arbuscular mycorrhizal fungi ii. Involve in nutrient cycling iii. Involve in soil aggregation	Ahmad Ali *et al.* (2018)

CULTURE-DEPENDENT APPROACH

Traditional culture-dependent methods are highly determined by on the medium used for isolation, incubation condition and microscopic colonial morphology to identify the microbes (Jackson *et al.*, 2013). Although these methods are easy, fast and reliable for identifying the most prevalent culturable microbial taxa, they have a number of drawbacks that impede a proper microbial diversity assessment. Processing cultures can be time-consuming and laborious when dealing with a large number of isolates and during these processes, the risk of culture contamination is always high (Jeewon and Hyde, 2007). Moreover, this method provides limited information on microbial communities because more than 99% soil microorganisms remain 'unculturable' in the laboratory as they require specific growth conditions. As a result, a large proportion of microbial communities remains unexplored, and organisms such as Acidobacteria are underrepresented in isolated cultures (Ayob and Kusai, 2021; Liu *et al*., 2006) when compared to the molecular methods. The molecular methods that describe communities dominated by predominantly uncultivated microorganisms' groups that are not detected by culture, provide a more rapid result, less time-consuming, and been shown to have great flexibility in terms of gene target and target region (Montalvo *et al*., 2014; Rudkjøbing *et al*., 2016). A further drawback to the culture-dependent method when predicting fungal community is that some slow-growing species may be overlooked, while the abundance of species with high sporulating rates may be over-estimated (*Table 3*) (Ayob *et al*., 2018; Jeewon and Hyde, 2007). Combining culture-dependent and molecular methods, such as PCR-based amplification of rDNA genes and Sanger sequencing, would provide reliable results of identification and would resolve the taxonomic relationship among taxa compared to morphological characterisation alone (Ayob *et al*., 2018; Kusai *et al*., 2018). These techniques have resulted in isolation of only the most common and abundant fungi such as asexual ascomycetes *Aspergillus* (*Figures 2a-c*), *Penicillium* (*Figures 2d-2f*), and *Trichoderma* (*Figures 2g-2i*) (Ayob *et al*., 2018; Kusai *et al*., 2018) as compared to using the culture-independent technique. For microscopic characterisation, *Aspergillus* sp. had pale brown radiate conidial heads (*Figure 3a*) while the conidia were globose and found in clusters (*Figures 3b*-*3c*). *Penicillium* sp. produced branch conidiospores (*Figure 3d*) and conidia formed in globose shaped clusters (*Figures 3e*-*3f*). Meanwhile, *Trichoderma* sp. produced branched conidiophores that grew from the main hyphae while stout phialides were formed from the branch (*Figure 3g*) with alobose shaped phialospores (*Figures 3h*-*3i*). Most of the isolated fungi phyla are regarded as cellulolytic fungi that are able to produce cellulases for degradation of biomass (Vaishnav *et al*., 2018). Filamentous fungi such as *Aspergillus niger* (*Figure 2a*) can enhance phosphate solubilisation in agricultural soils (Li *et al*., 2016) and are promising microorganisms to be used in oil palm plantations to increase fertility of the soil and oil palm health (Acevedo *et al*., 2014). On the other hand, *Trichoderma harzianum* (*Figure 2h*) has biocontrol properties against devastating oil palm disease; basal stem rot caused by *Ganoderma boninense*, and has widely been used as a biofertiliser to reduce disease incidence in oil palm plantations (Naher *et al*., 2012; Sundram *et al*., 2008). Meanwhile, the most isolated bacteria in the oil palm plantations of Sarawak are Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Ayob and Kusai, 2021; Kusai and Ayob, 2020). Isolated bacteria *Burkholderia cepacia* (class Betaproteobacteria) has been used as a biocontrol agent to reduce incidene of basal stem rot in oil palm by 42%. Members belonging to Firmicutes (class Bacilli), such as *Bacillus cereus* and *B. subtilis,* were commonly isolated from oil palm planted in peat soils (Kusai and Ayob, 2020). *Bacillus cereus* plays an important role as a nitrogen-fixing bacteria (Karagöz *et al*., 2012), while *B. subtilis* is a cellulolytic bacteria which helps in the hydrolysis process of cellulosoic material of plant litter (Kim *et al*., 2012). Direct microscopic counts of microbes in soil samples, recoverable colony forming units, and sequencing of 16S rDNA, 18S rDNA and ITS genes are used to characterise and better understand the nature of the microbial community composition from oil palm plantation ecosystems associated with agricultural practices and environmental conditions.

CULTURE-INDEPENDENT APPROACHES

Denaturing Gradient Gel Electrophoresis (DGGE)

Recent studies involving identification and characterisation of microbial communities in environmental samples have focused on the use of methods based on genetic diversity that do not require cultivation. This method is known as culture-independent. Theoretically, DNA sequences which differ by even one base-pair can be separated across temperature gradients by Denaturing Gradient Gel Electrophoresis (DGGE). The advantages of DGGE include reliability, reproducibility and the capacity to process multiple samples rapidly by analysing bands that migrate separately on DGGE gels (Ayob and Kusai, 2021; Fakruddin and Mannan, 2013). However, this method is often limited to the low-throughputs from Sanger sequencing, PCR biases, laborious sample handling, and only detects dominant species (*Table 3*) (Muyzer, 1999; Nkongolo and Narendrula-Kotha, 2020). A study by Miguel *et al.* (2010) suggested that this method possibly detected multiple banding patterns attributed to sequence heterogeneity between copies of 16S rDNA strains, and one band may not necessarily represent one species. Recent studies have reported that unculturable bacteria were the dominant group in the study sites, followed by Proteobacteria, Acidobacteria, Firmicutes and Actinobacteria (Ahamed Bakeri *et al*., 2019; Ayob and Kusai, 2021; Maidin *et al*., 2016). The abundance of Acidobacteria in oil palm planted on peat soil suggested that due to the acidic environments, these bacteria grow under oligotrophic conditions. This is consistent with the results obtained from the boreal forest soils (Chapman *et al*., 2017; Sun *et al*., 2014; Tsitko *et al.*, 2014). In addition to that, phylum Acidobacteria was only detected using culture-independent methods (Ayob and Kusai, 2021). The fertiliser application increased in certain bacterial communities such as Firmicutes, in a 1.5-year-old oil palm plantation and this application also increased soil pH and phosphate content (Ahamed Bakeri *et al.*, 2019). Firmicutes that thrive in oil palm soils, such as *Bacillus* and *Paenibacillus*, have been reported to be involved in the denitrification of nitrate to nitrogen in soil (Kusai and Ayob, 2020), and have antagonistic properties towards fungal plant pathogens (Maidin *et al.*, 2018). At the genus level, *Nitrospirales* (phylum Nitrospirae) was found in a 3.5-year-old oil palm plantation as nitrifiers correlated with nitrogen input compared to rainforest soil (Ahamed Bakeri *et al.*, 2019). Similarly, the PCR-DGGE profile in the study conducted by Salamat *et al.* (2021) indicated that the oil palm soil with 25 years of fertiliser application was dominated by organisms belonging to the phyla Actinobacteria, Firmicutes and Proteobacteria. The differential responses of bacterial groups may indicate changing environmental variables and reflect the shifting of ecological conditions, such as nitrogen availability and organic carbon, due to long-term fertilisation in mineral soils (Salamat *et al.*, 2021; Sun *et al.*, 2014).

Next-Generation Sequencing (NGS)

Deep sequencing and its capacity to sequence multiple samples simultaneously make metagenomic sequencing very attractive for exploring microbial species in agriculture, ecology and human health. NGS is a high-throughputs sequencing technology and can be conducted at low analytical cost to assess microbial communities at high resolution (Fierer, 2017). The revolution of NGS technologies from 'second' to 'third' generation sequencing can be seen in a variety of platforms such as 454-pyrosequencing, Ion Torrent, Illumina MiSeq, HiSeq and NovaSeq, PacBio and Oxford Nanopore MinION. These technologies allow data processing and analysing through bioinformatics pipelines more user-friendly and accessible, but still require high expertise and proficiency (Fierer *et al.*, 2021; Nilsson *et al.*, 2019). Besides that, these platforms have provided comprehensive information in detecting microbial communities and their functional ecology over large spatial and temporal scales (Nilsson *et al.*, 2019).

454-Pyrosequencing

Pyrosequencing is a sequencing method that utilises a four-enzyme technology to monitor DNA synthesis via bioluminescence detection (Ahmadian *et al.*, 2006). Although discontinued in 2016, 454-pyrosequencing was the first 'next' generation sequencing system and utilised a novel approach, namely, the detection of pyrophosphate, a by-product of nucleotide incorporation (Saleem, 2020; Slatko *et al.*, 2018). 454-pyrosequencing (Roche) was superior as it was high-throughput, quick, takes only 10 hr from start to finish, and generated long read lengths (up to 700 bp) with 99% accuracy after only 4 hr of run. However, the major drawbacks included high cost of reagents and consumables, and high error rates in homopolymer repeats (*Table 3*) (Liu *et al.*, 2012; Slatko *et al.*, 2018). Based on previous studies by Kerfahi *et al.* (2014) and Ahmad Ali *et al.* (2018),

TABLE 3. ADVANTAGES AND DISADVANTAGES OF MOLECULAR-BASED METHODS FOR IDENTIFYING SOIL MICROBIAL COMMUNITIES

Method	Advantages	Disadvantages
Culture-dependent	• Fast • Inexpensive • Ease in handling • Ability to further analyse colonies	• Low resolution • Unculturable microbes not detected • Unable to grow under laboratory condition • Lack of expertise and experience on taxonomy and morphological characteristics • Bias towards fast-growing species • Bias towards high sporulating rate species
Denaturing Gradient Gel Electrophoresis (DGGE)	• Direct extraction from soil • No culturing of microorganisms required • Rapid, reliable and reproducible • Large number of samples can be analysed simultaneously	• Laborious • Low throughput • Dependent on DNA extraction efficiency • PCR biases present • One band represent more than one species • Only detects dominant species
454-pyrosequencing	• Longer reads • Short run time • Does not require cloning step	• Expensive reagents and consumables • Error rate with polybase more than six
Illumina sequencing	• High yield • Short run time and scalable • All-in-once analysis in high-throughput • High potential for comparative • studies	• Low number of total reads • High instrument cost • Require expertise, high proficiency and training

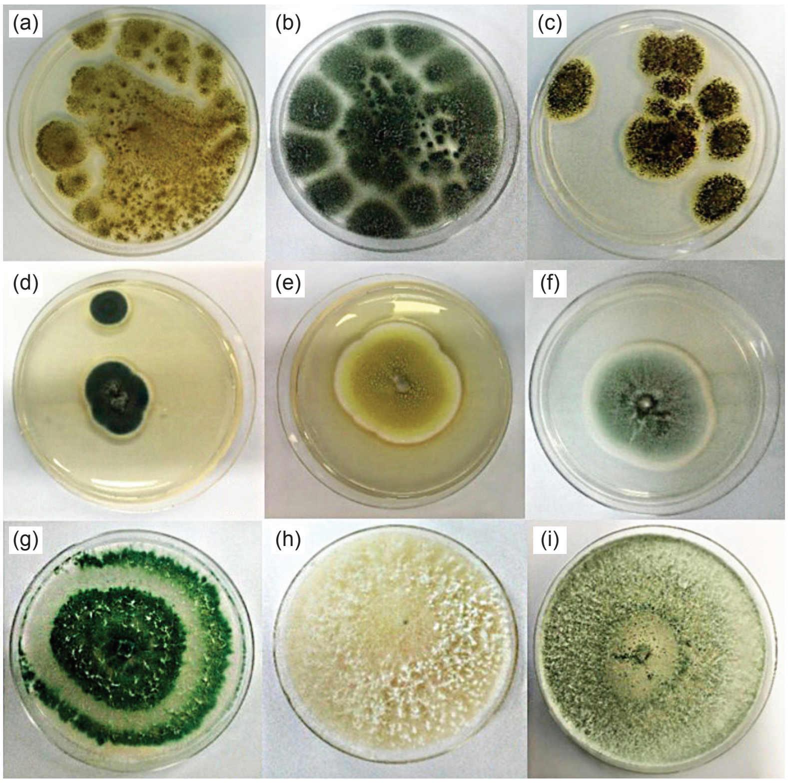

Figure 2. Colony characteristics of fungal cultures isolated from oil palm soil on plate. (a) Aspergillus niger, *(b)* Aspergillus fumigatus, *(c)* Aspergillus *sp., (d)* Penicillium freii, *(e)* Penicillium commune, *(f)* Penicillium *sp., (g)* Trichoderma koningii, *(h)* Trichoderma harzianum *and (i)* Trichoderma *sp.*

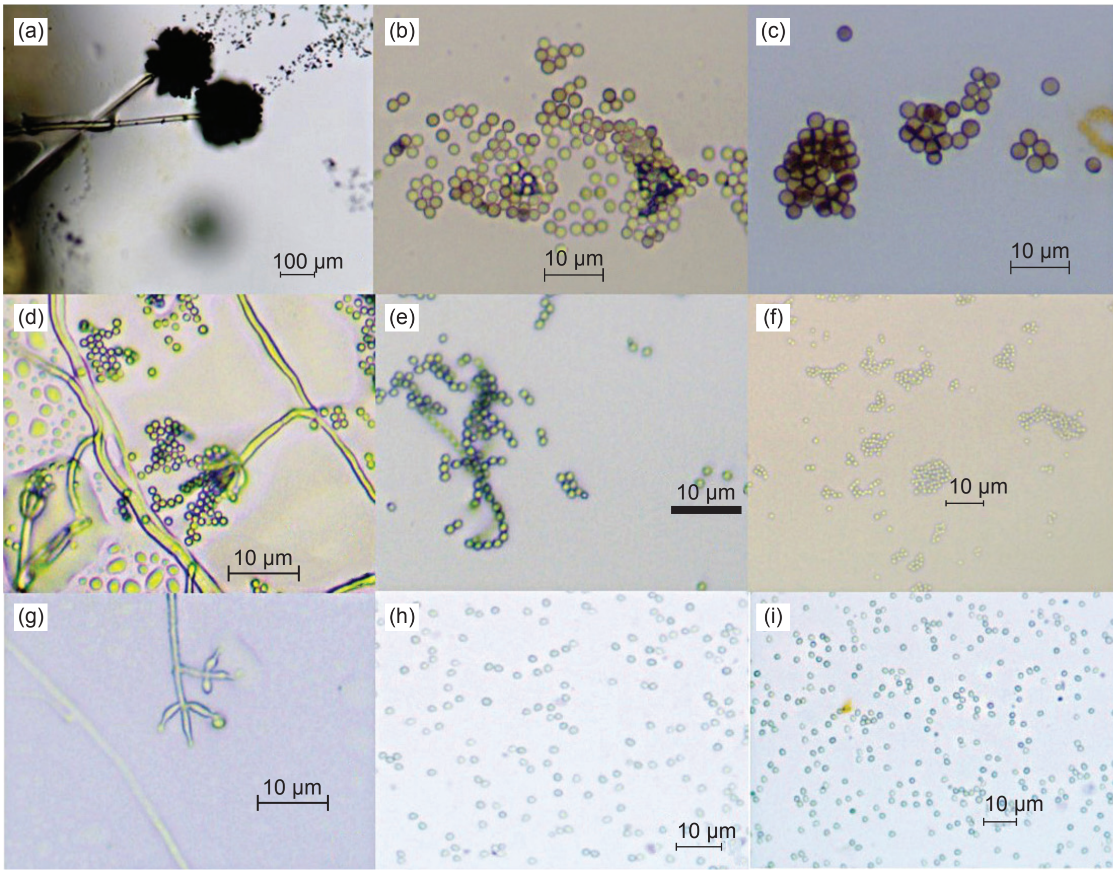

Figure 3. Microscopic characteristics of fungal isolated from oil palm soil on plate. Aspergillus *sp.: (a) Conidial head, (b, c) Conidia.* Penicillium *sp., (d) Conidiophore bearing conidiospores, (e, f) Conidia.* Trichoderma *sp., (g) Phialides, (h, i) Phialospores.*

Ascomycota and Basidiomycota dominated the oil palm plantations in Sabah and Sarawak, respectively. The impact of land use change of fungal communities (β-diversity) in Borneo mineral soils resulted in spatial homogenisation of fungal communities in oil palm plantations compared to forests (Kerfahi *et al.*, 2014). In contrast, in the same land use change and sites in Borneo, there was an increase in β-diversity of bacteria detected using Illumina HiSeq in logged over and converted to oil palm plantings (Lee-Cruz *et al.*, 2013). However, the impact of agricultural activities such as water management system resulted in physical, chemical, and biological changes in tropical peatland ecosystem. This in turn led to an increase in eukaryotic diversity in operational taxanomic unit (OTU), α-diversity and total number of phylotypes in oil palm plantations and logged forests (Ahmad Ali *et al.*, 2018). A study by Tripathi *et al.* (2012) observed that the dominant taxa in oil palm plantation were Acidobacteria, Proteobacteria, Chloroflexi and Actinobacteria. The bacterial community composition and diversity was strongly correlated with soil properties, especially soil pH, total carbon, and C/N ratio across land use types such as primary forest, logged forest, and agriculture and pasture lands (Tripathi *et al.*, 2012).

Illumina Sequencing

Illumina sequencing technology was introduced in 2006, and it generates a larger amount of data per run in a more cost-effective manner. The Illumina utilises a sequencing-by-synthesis (SBS) approach, and it differs from 454-pyrosequencing (Nkongolo and Narendrula-Kotha, 2020). However, there are still some limitations in using this technology, as described in *Table 3*. Illumina amplicon sequencing (HiSeq and MiSeq) has become recently the most popular NGS method used for analysis of microbial diversity and functions in Malaysian oil palm plantations as documented in many studies (Drewer *et al.*, 2020; Lee-Cruz *et al.*, 2013; McGuire *et al.*, 2015; Robinson *et al.*, 2020; Rupert *et al.*, 2020; Salamat *et al.*, 2021; Tin *et al.*, 2018; Tripathi *et al.*, 2016; Wood *et al.*, 2017). Previous studies reported using Illumina HiSeq and MiSeq, oil palm cultivation in a tropical forest ecosystem did not affect the bacterial diversity and ecosystem functioning (Lee-Cruz *et al.*, 2013; Tin *et al.*, 2018; Tripathi *et al.*, 2016). The predominant bacterial taxa in oil palm plantations are Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes (Rupert *et al.*, 2020; Tripathi *et al.*, 2016; Wood *et al.*, 2017). Based on the functional genes analysis using Illumina HiSeq, the most prevalent genes associated with DNA metabolism, RNA metabolism, protein metabolism, cell division and cell cycle are relatively more abundant in oil palm plantations compared to forests and logged forests (Tripathi *et al.*, 2016). Furthermore, using amplicon and shotgun metagenomic sequencing, studies on the impact of selective logging and oil palm plantation revealed that structural and functional gene composition of the total soil microbial community is strongly influenced by the conversion of forests into agricultural land (Tripathi *et al.*, 2016). This study provides a preliminary framework and serves as a new metrics to facilitate selection of secondary forest land optimal for oil palm cultivation while preserving and conserving microbial diversity based on the knowledge of its soil microbial composition and genomic potential, as illustrated in *Figure 4* (Ayob *et al.*, 2023). Using ITS amplicon sequencing, different mycelial fungal communities such as saprotrophic, mycorrhizal, and pathogenic fungi were characterised in oil palm plantations compared to selectively logged forests (Robinson *et al.*, 2020). The composition and function of certain bacterial and fungal communities in cultivated soils vary according to nutrient regimes and prevailing plant communities (Sun *et al.*, 2014).

CONCLUSION

The development of molecular tools and technologies have increased our knowledge on microbial diversity and function in oil palm plantation compared to traditional methods. Despite the challenges of the developed methods, culture-dependent methods are still employed for pure isolates and integrated taxonomy and the consolidated species concept, which uses both morphology and DNA sequence data, are now broadly accepted by modern taxonomists. With the advancement of molecular technology, NGS has flourished in the identification and characterisation of microbial communities and functional studies on DNA from environmental samples. The use of this technique provides a new way of assessing soil microbial diversity and ultimately, a complete understanding of the potential impacts of environmental processes and human activities on soil microorganisms to improve soil fertility and promote soil quality. This would help to address ecological issues related to ecosystem services in agricultural systems, especially in oil palm plantations. In addition, a clear understanding on microbial data is crucial to develop robust solutions in managing oil palm plantation for various aspects of soil health, ecosystem restoration, the use of microbial resources, and climate change. Proper oil palm management practices could be adopted and implemented by farmers and stakeholders which will allow sustainable oil palm production and conserve ecosystem functions. Future studies of soil

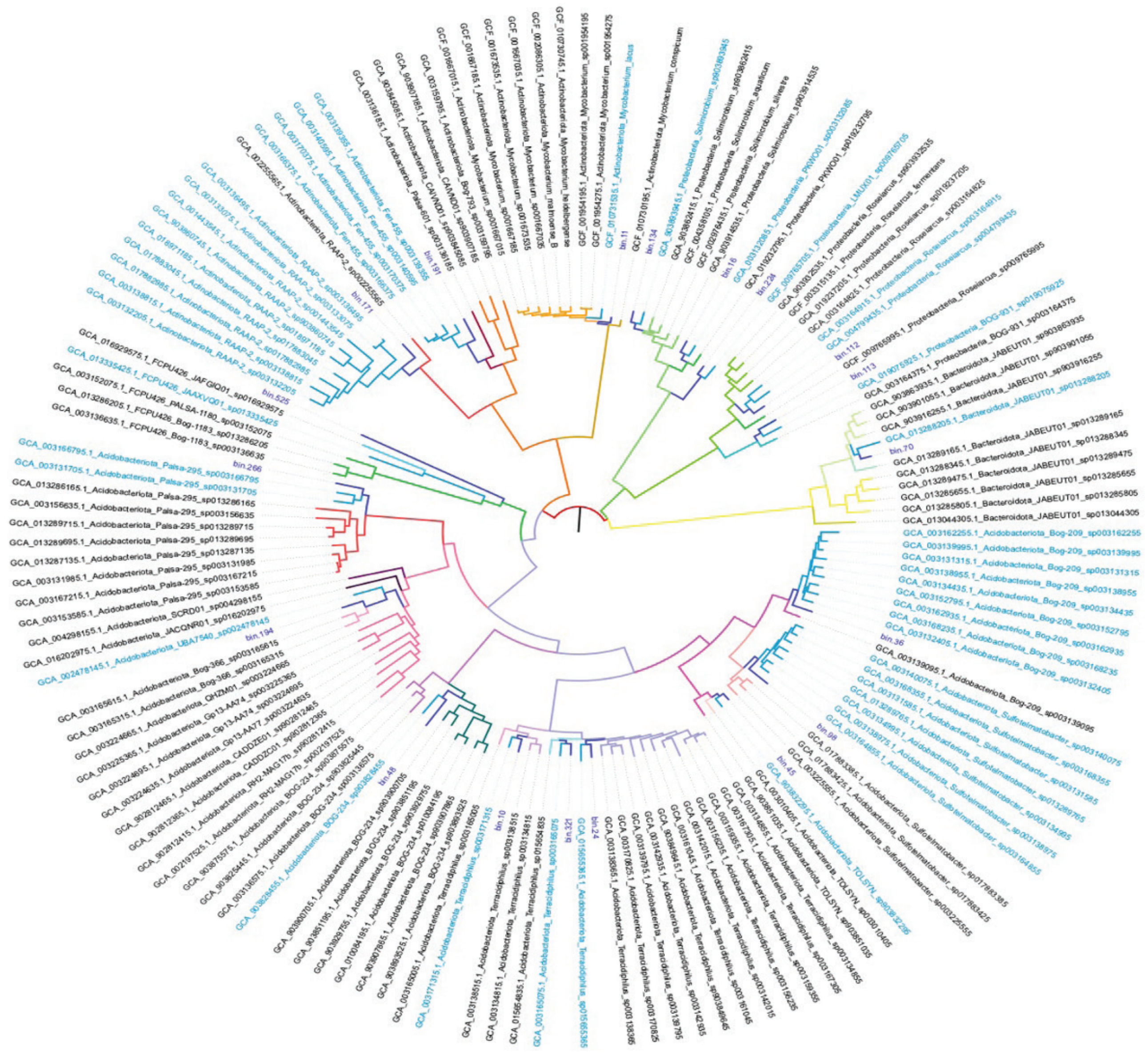

Figure 4. Maximum likelihood tree showing the phylogenomic placement of bacterial metagenome-assembled genomes (MAGs) against closely related representative genome from the Genome Taxonomy Database (GTDB) at logged forest soil using Illumina NovaSeq 6000. Branches were coloured based on the phylum and genus classification. Taxa forming immediate monophyletic group with the MAGs were coloured light blue while the MAGs were coloured blue.

microbial communities must rely on a combination of culture-dependent and culture-independent approaches. This will allow the development of an efficient monitoring tool and assessment of the long-term implications of such changes for agricultural sustainability, and as a bio-indicator to be included in the management and other decision making in the oil palm industry.

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