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Identification of culture-dependent microbes from mangroves reveals dominance of *Bacillus* including medically important species based on DNA signature

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Abstract. *Dechavez R, Calub ML, Genobata DR, Balacuit R, Jose R, Tabugo SR. 2022. Identification of culture-dependent microbes from mangroves reveals dominance of* Bacillus *including medically important species based on DNA signature. Biodiversitas 23: 5342-5350.* Mangroves are among the most crucial plant hosts in the marine environment because of their notable role in the ecosystem and their benefits. Identification of plant-associated and culture-dependent microbes is key to having a rich reservoir of bioactive substances. DNA barcoding was done based on 16S rRNA gene and ITS region. In this study, seven (7) mangrove species were identified as hosts. Results show that most bacterial isolates from plant leaves were gram-positive bacteria belonging to phylum: Firmicutes and genus *Bacillus*. Phylogenetic inference reveals a diverse non-monophyletic group with medically and economically necessary species. Among the host plants, *Sonneratia alba* harbored the most species of bacteria and fungi. These include two medically important strains of *B. cereus* and *B. thuringiensis* and host-specific bacteria like *B. altitudinis* in *S. alba*, *B. velezensis* in *Rhizophora apiculata*, *B. clausii* in *Avicennia marina* and *B. firmus* in *A. marina* were revealed. Meanwhile, some bacteria like *B. subtilis* and *B. megaterium* were found in three host species and *B. cereus* was the most abundant being recorded in four host plants. Diverse strains of fungi, *Aspergillus* sp., *A. nomiae*, *A. tubingensis* and *A. niger* were also present. This study served as baseline data for future research on diversity and host ecology for possible drug discovery.

Keywords: *Bacillus*, bacteria, diversity, fungi, mangrove

INTRODUCTION

Mangroves have been a subject of conservation studies because of many natural products with beneficial activities and commercial value derived from them (Kurniadi and Koeslulat 2020). Mangrove habitats are a prolific source of endophytic microorganisms. The roots and leaves of mangroves serve as a dwelling place for many bacteria, fungi, and algae. To present, there is no adequate evaluation of mangrove-dependent life forms. The goal of this study is to identify culture-dependent microbes that can be harbored from different mangrove hosts. Identification based on DNA short sequence tags was tested. Plants have diverse microbiomes on the surfaces (epiphytic) and inside plant tissues (endophytic). Such microbes play a vital role in plant health and benefits. The interplay between plants and microbes had been evident. Various studies show that prokaryotic endophytes both bacteria and fungi can invade plant tissues via wounds and openings by utilizing hydrolytic enzymes (Eid et al. 2021). At some point, they exhibit adaptability to undesirable conditions and may undergo genetic variations that lead to their ability to synthesize certain phytochemicals. Endophytic microorganisms can be a very rich source of biological and active natural products. They can secrete various substances such as cytokines, phytohormones, and other plant-growth-promoting compounds that can impact the growth of plants. Noteworthy, some strains help increase the absorption of nutrients by the host (Ullah et al. 2019). Various studies attest that microbial communities are dynamically shaped by factors like season, soil, daytime and hosts of the species (Vorholt 2012). Many metabolites are produced as an end-product of the interaction between hosts and microorganisms (Bui et al. 2019). Secondary metabolites include xanthones, steroids, tetralones, alkaloids, terpenoids, quinones, phenolic acids, benzopyranones and flavonoids.

In the past, there are various studies of microorganisms isolated from mangrove species. In fact, the major importance of mangrove habitats is the existence of endophytic microorganisms (bacteria and fungi). The novel compounds extracted from them can be of high economic value. However, extensive research on its diversity is noteworthy to be explored. Several metabolites secreted as well as enzymes served as novel chemicals by endophytic strains coming from mangroves. These can then be utilized for biotechnology aspects from practical to large-scale applications (Chatterjee and Abraham 2020). As per

research reports, applications are in the field of antibiotics, agrochemical agents, antioxidants, anti-parasitics, immunosuppressive and anti-cancer (Fadiji and Babalola et al. 2020). Meanwhile, in recent years numerous strains of Bacillus of medical and economic importance were reported abundant in mangroves and the genus itself was regarded as a heterogenous phylogenetic group thus, there is a need to shed light on its phylogeny and 16S rDNA is a vital standard for the taxonomy of bacteria.

Throughout the years, genome sequencing aroused interest in identifying plant microbiomes in host plants. Noteworthy is that all microbes that can be cultured in the lab (culturome) and metagenome sequencing provided more knowledge of microbiome in plants (Pang et al. 2021). The culturome is an important component of the microbiome because culture-dependent microbes may hold the key to having a rich reservoir of bioactive substances. The identification of both epiphytes and endophytes (bacteria and fungi) is essential to provide baseline data for future research on host ecology and drug discovery hence, this study. The availability and affordability of sequencing allowed researchers to investigate the deep association of host-associated microbiota. DNA barcoding using bacterial 16S rRNA gene and fungal ITS amplicons, becomes a justifiable tool for biodiversity assessment. It is a fast, accurate and standardized method for species-level identification (Lebonah et al. 2018). Most species of microbes are cryptic and thus, DNA barcoding was used in the identification process based on short sequence tags.

MATERIALS AND METHODS

Field sampling and sample collection

Remote unexplored regions in Mindanao, Philippines served as sampling sites thus, the data gathered is worthwhile. Five (5) healthy and mature leaves from different mangroves (host plants) were harvested from the three sampling sites: Lebak, Palimbang and Kalamansig, Sultan Kudarat, Southern, Mindanao (Figure 1). Specimens were placed in an ice chest and brought to the laboratory for processing. Species identification was accomplished using the Field Guide to Philippine Mangroves by J.H. Primavera.

Preparation and isolation of epiphytic microorganisms

The leaf washing technique was used for the isolation of microorganisms from the leaves of mangrove species. Leaves were washed with 10ml sterile isotonic solution 0.01% Tween80, then it was shaken for 1 min. in a sonic bath at 40 Hz. After which 200ul aliquot of leaf wash solution for each dilution (10^{-1},10^{-2} and 10^{-3}) was plated in Marine agar for bacteria and Sabouraud dextrose agar (SDA) for fungi (Sanchez-Lopez et al. 2018). Then plates were incubated for 1 week at 30°C in darkness.

Preparation and isolation of endophytic microorganisms

Leaf samples were cut into small discs using a puncher and disinfected with 70% ethanol for 30 sec., rinsed thoroughly with sterile distilled water and followed by surface sterilization with 3% sodium hypochlorite for 3 min. This was done to remove surface microorganisms and ensure that only endophytes will be processed (Deivanai et al. 2014). Sample discs were then placed in agar plates (appropriate media) inside the biosafety cabinet. Plates were then incubated for 24-48h at 32°C for microbial growth. Isolated colonies were then subcultured in appropriate media (marine agar and SDA). A total of one hundred eighteen (118) endophytic and epiphytic samples (bacteria and fungi) were tested for gram staining. Isolated fungi were further subcultured in SDA and incubated for 2 weeks. Initial morphological identification of microorganisms was done based on cell and colony characterization and representative samples were processed for molecular identification through DNA barcoding.

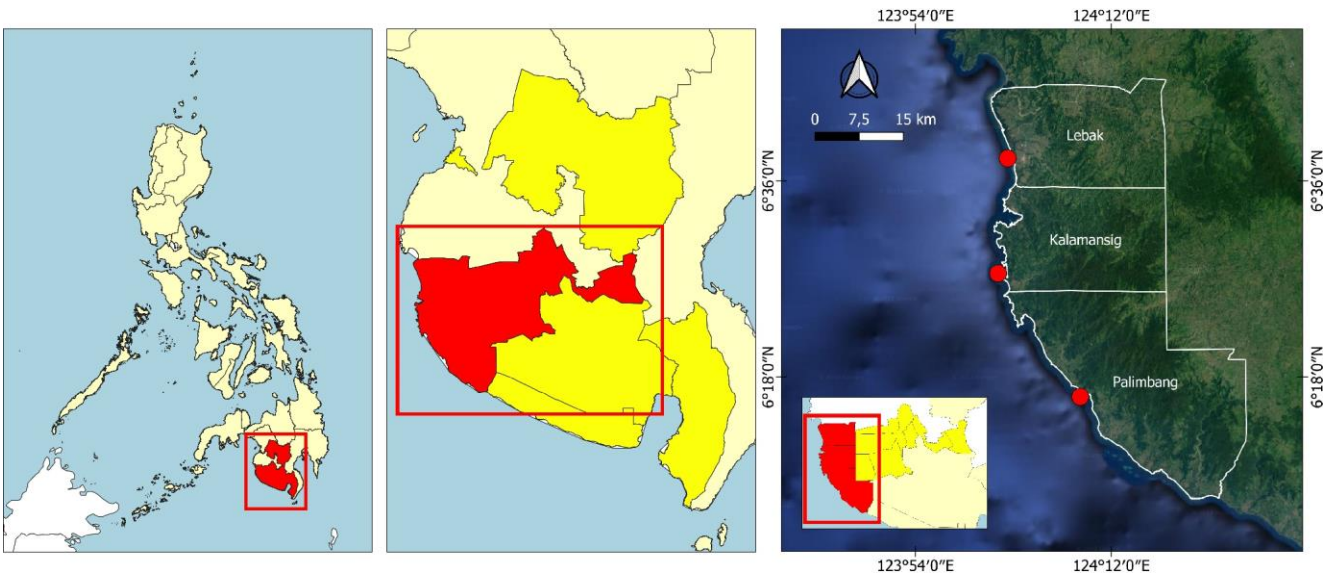

Figure 1. The three sampling sites: Lebak, Palimbang and Kalamansig, Sultan Kudarat, Southern, Mindanao, indicated in red

DNA extraction, amplification, and sequencing

Extraction of DNA of each representative bacteria via NucleoSpin Kit (Machery-Nagel, Germany) and Vivantis kit was conducted following the manufacturer's protocol. DNA integrity check was done using 1.5% agarose gel electrophoresis. Taking into consideration the 16S rRNA gene, DNA amplification was performed through the following primers: 27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′ respectively. Amplification conditions were at initial denaturation of 95°C for 3min., followed by 30 cycles of denaturation: 95°C for 30 sec.; annealing: 50.6°C for 30 sec.; extension: 72°C for 30 sec. and final extension: 72°C for 5 min.

Fungal genomic DNA was extracted using the NucleoSpin Kit (Machery-Nagel, Germany) following the manufacturer's instruction with some modifications. For the pre-lysis step, 1M Tris-HCl, 0.5M EDTA, 10% SDS, and sterilize distilled H_2O were added along with Dithiothreitol (DTT), Lyticase and Lyticase buffer. It was vortexed and incubated at 30ºC for 10min and centrifuged at 12000rpm for 12 minutes to pellet the cells. The supernatant was removed then proteinase K was added to yield a homogenous solution. It was vortexed for an hour and incubated 65ºC for 10 min. The lysis step until the elution step as indicated by the manufacturer's instructions was then followed. A set of primers based on nuclear ribosomal internal transcribed spacer (ITS) region were used for amplification process (ITS1: 5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4: 5′-TCCTCCGCTTATTGATATGC-3′) primer pairs. Initial denaturation: 94°C for 1min.; followed by 37 cycles of denaturation at 94°C for 45s.; annealing at 52°C for 45s; extension at 72°C for 1min and final extension 72°C for 5min. (Apurillo et al. 2019).

PCR amplification was done in a 50ul reaction: 25ul 2x PCR MasterMix (BIORAD, iTaq Universal SYBR Green SuperMix), 2.5ul FP; 2.5ul RP,15ul ultrapure water or sterilized distilled water; 5ul template DNA for all samples. PCR products were then assessed by gel electrophoresis in 1.5% (w/v) agarose gel in 1xTBE buffer using BlueGel system (by MiniPCR) with built-in power supply (AC 100-240V, 50-60hz). Gels were dyed with GelGreen (CA,USA) (10,000x in water). PCR products were purified using NucleoSpin Gel and PCR Clean-up (Machery-Nagel, Germany). Purified PCR products were sent to Macrogen, Korea for standard sequencing (Sanger sequencing). The sequences generated were subjected to further bioinformatics analysis.

Sequence alignment and phylogenetic analysis

The BLAST (Basic Local Alignment Search Tool) in NCBI identified the resulting sequences. This was convenient in search for matches and related sequences. Trace files were then utilized to generate consensus sequences using SnapGene software, BLAST and the DNA Subway. Alignment of sequences was made via MUSCLE in MEGA 7. Phylogenetic analysis was carried out using maximum likelihood (ML) approach in MEGA 7.

Best-fit models of evolution of nucleotide substitution of sequences based on 16S rRNA gene dataset and ITS region were determined using jModel Test 2 on XSEDE (Darriba et al. 2012) based on AIC, AICc, BIC values. Bayesian Inference under Mr.Bayes 3.2.2 on XSEDE (Ronquist and Huelsenbeck 2003) was used to reconstruct phylogenetic relationships among species (available in the CIPRES Science Gateway v.3.3 Web Portal) using the model of sequence evolution recommended by jModel Test 2 for each partition. The analyses run for 5,000 generations with Markov chains being sampled every 1000 generations. Posterior probabilities values (PP) expressed as probability percentage was calculated by Markov Chain Monte Carlo (MCMC) sampling. Bayesian posterior probability values (BIPP) over 0.95 were considered for BI trees, and trees were rooted with the outgroup. The tree obtained was then viewed and edited using FigTree 1.4.0 software (Rambaut 2009).

RESULTS AND DISCUSSION

Phylogenetic inference

Sequences were submitted to GenBank with the following accession numbers: OK335827-OK335881 (bacteria) and OM403527-OM403536 (fungi). Most bacterial isolates were gram-positive bacteria belonging to phylum: Firmicutes; family: Bacillaceae. The species based on DNA barcoding are the following: *Bacillus clausii*, *B. flexus*, *B. megaterium*, *B. firmus*, *B. cereus*, *B. thuringiensis*, *B. licheniformis*, *B. pumilus*, *B. altitudinis*, *B. subtilis* and *B. velezensis.* There was one isolate belonging to the genus *Brevibacterium* of the family Brevibacteriaceae which are common gram-positive bacteria in the soil and were isolated from the leaves of *Avicennia marina*.

Figure 2 shows the Molecular Phylogenetic analysis by Maximum Likelihood (ML) method for bacteria isolated from mangrove species, with accession numbers OK335827-OK335881, from selected areas in Mindanao, Philippines, reference sequences were used and an outgroup (MK918483.1 *E. coli*). The evolutionary history was inferred using the ML method based on the Kimura 2-parameter model. The bootstrap consensus, ML tree was inferred from 1000 replicates to represent the evolutionary history of the taxa being analyzed in MEGA 7. Noteworthy, bootstrap ML consensus tree produced three (3) clusters. Three (3) bacteria species (accession nos. OK335835, OK335852 and OK335863) did not find a match on NCBI database hence, labeled as a bacterium or uncultured bacterial isolate. However, looking at the tree, the following strains are related to Bacillus species. Many species of Bacillus are ubiquitous in nature. It includes free-living, non-parasitic species and two parasitic pathogenic species. The two medically significant Bacillus species are *B. anthracis* and *B. cereus*. According to literature, there are many proposals representing the phylogeny of the genus Bacillus covering 16S and ITS region dividing the genus into various groups. The third proposal classified Bacillus species based on its nature whether it is pathogenic, found in soil, benthic, aquatic and halophilic (Favaro et al. 2018). In this study, the generated

phylogenetic tree supports the proposal hereby, clustering bacillus into three clades. The first clade encompassed isolates from plant leaves which are Bacillus species that are also dominant in the soil, these are *B. pumilus, B. licheniformis, B. altitudinis, B. subtilis* and *B. velezensis* strains. The second clade is comprised of both potentially pathogenic and known to be pathogenic in nature bacteria like *B. cereus, B. flexus, B. megaterium, B. thuringiensis,* and one strain of *B. licheniformis.* Whereas the last group composed of halophilic bacteria *B. clausii* strains and one isolate belonging to the genus *Brevibacterium.*

Bayesian inference phylogenetic tree was constructed based on TrN+G as the best-fit evolutionary model for nucleotide substitution as determined by jModelTest2. High posterior probability values (PP) expressed as probability percentage indicate a high rate of recovery for species position in the generated non-monophyletic tree (Figure 3).

Noteworthy, is that the microbial community in the rhizosphere and endosphere of plants are dominated by four bacteria phyla: Actinobacteria, Bacteriodetes, Firmicutes, and Proteobacteria (Bulgarelli et al. 2012). Research has long regarded the genus *Bacillus* as a heterogenous phylogenetic group based on 16S rDNA sequences revealing it as not a monophyletic group with medical and economical importance. The generated ML and Bayesian tree demonstrate a non-monophyletic group with diverse Bacilli species. The presence of the complex heterogenous cluster was consistent with research findings suggesting the genus Bacillus as a phylogenetically incoherent taxon with members of the group lacking a common evolutionary history (Bhandari et al. 2013; Rodriguez-Torres et al. 2017). It is comprised of spore-forming bacteria that are both aerobic and anaerobic. There are no characteristics known to distinguish species of this genus from other similar endospore-forming genera. A similar study based on conserved signature indels (CSIs) shed light on differentiating '*Bacillus subtilis* clade' and '*Bacillus cereus* clade' respectively from all other species of the genus Bacillus (Bhandari et al. 2013). In this study, subtilis and cereus clades were observed as the largest groupings and are of medical importance.

Among the beneficial microbiota are *Bacillus velezensis*, which possess versatile traits and protect rice against diverse abiotic stresses, including heat, cold and freezing (Tiwari et al. 2017). A study showed a combination of *Bacillus pumilus, B. subtilis* and *Curtobacterium flaccumfaciens* was highly effective in enhancing resistance against different pathogens in cucumbers (Correa et al. 2014). *Bacillus clausii* is known as a probiotic that has been used to treat acute diarrhea among adults and children and as adjunctive therapy for *Helicobacter pylori* infection (Ianiro et al. 2018). *B. altitudinis* was found to have a notable influence on plant growth and could be a favorable candidate for sustainable agriculture (Zhang et al. 2021). Meanwhile, *B. firmus* has been tested as a nematode antagonist and can help induce systemic resistance in plants (Ghahremani et al. 2020). Studies on *B. megaterium* showed that it can adapt and survive in acid-stress conditions. This can have significant relevance to agriculture, food, and human health. *B. licheniformis* which is closely related to *B. subtilis* is used in the biotechnology industry to manufacture enzymes, antibiotics, biochemicals, and other consumer products. *B. thuringiensis* lives in the soil and make proteins that are toxic to insects and not toxic to humans thus used as a popular biopesticide (Radhakrishnan et al. 2017).

Moreover, bayesian tree was inferred based on F81 as the best-fit evolutionary model for nucleotide substitution as determined by jModelTest2 (Figure 4) for fungal isolates. It showed a well-supported tree with high percentage posterior probabilities (PP) or bootstrap values clustering fungi epiphytes on appropriate branches indicating a high rate of recovery of species position in the generated monophyletic tree. Samples with accession codes OM403527-OM403536 are fungi samples showing different species of *Aspergillus* plus reference sequences and an outgroup (MH279417.1 *A. pseudonomius*). Diversity seems to be inherent in this genus because of the different strains observed. It is so diverse based on protostome divergence as a scale, although both inter- and intra-specific genome structures are relatively plastic in nature. *Aspergillus* is considered among the most economically important fungal genera importance in biotechnology (enzymes, organic acids and bioactive metabolites). There are studies that implicated the monophyly of this genus. The spore-bearing structure characteristic of the genus resembled an aspergillum hence, the name *Aspergillus.* However, the morphological characteristic demonstrates variation in physiological and morphological features (Gibbons and Rokas 2013). In this study, diverse species were recorded based on fungal morphology and molecular data based on ITS region, and it included strains that belong to *Aspergillus* sp., *A. nomiae, A. tubingensis* and *A. niger*.

Fungi in mangroves are vital for growth and stress combat. They also serve as biofertilizers, phytostimulators and biopesticides to pathogens. Endophytic fungal species contain metabolites that serve as prospects for pharmaceutical and biocontrol effects. New information about fungi diversity highlights future management and applications (Pusztahelyi et al. 2015).

Plant hosts and microbiome

Mangrove species diversity is high in the Philippines recording 40 out of 65 species in the world, making it a biodiversity hotspot in Asia (Primavera 2000). In this study, mangroves serve as a rich reservoir of genus Bacilli that can possess special metabolic capabilities. The interplay between microbes and hosts can be described as a gradient form ranging from beneficial or harmless such as mutualism and commensalism to harmful parasitism (Drew et al. 2021).

Figure 2. Phylogenetic inference using Maximum Likelihood (ML) method for bacteria species isolated from mangroves from selected areas in Mindanao, Philippines; ML consensus tree inferred from sequences with 1000 bootstrap replicates; bootstrap support (only values above 50% bootstrap support are illustrated)

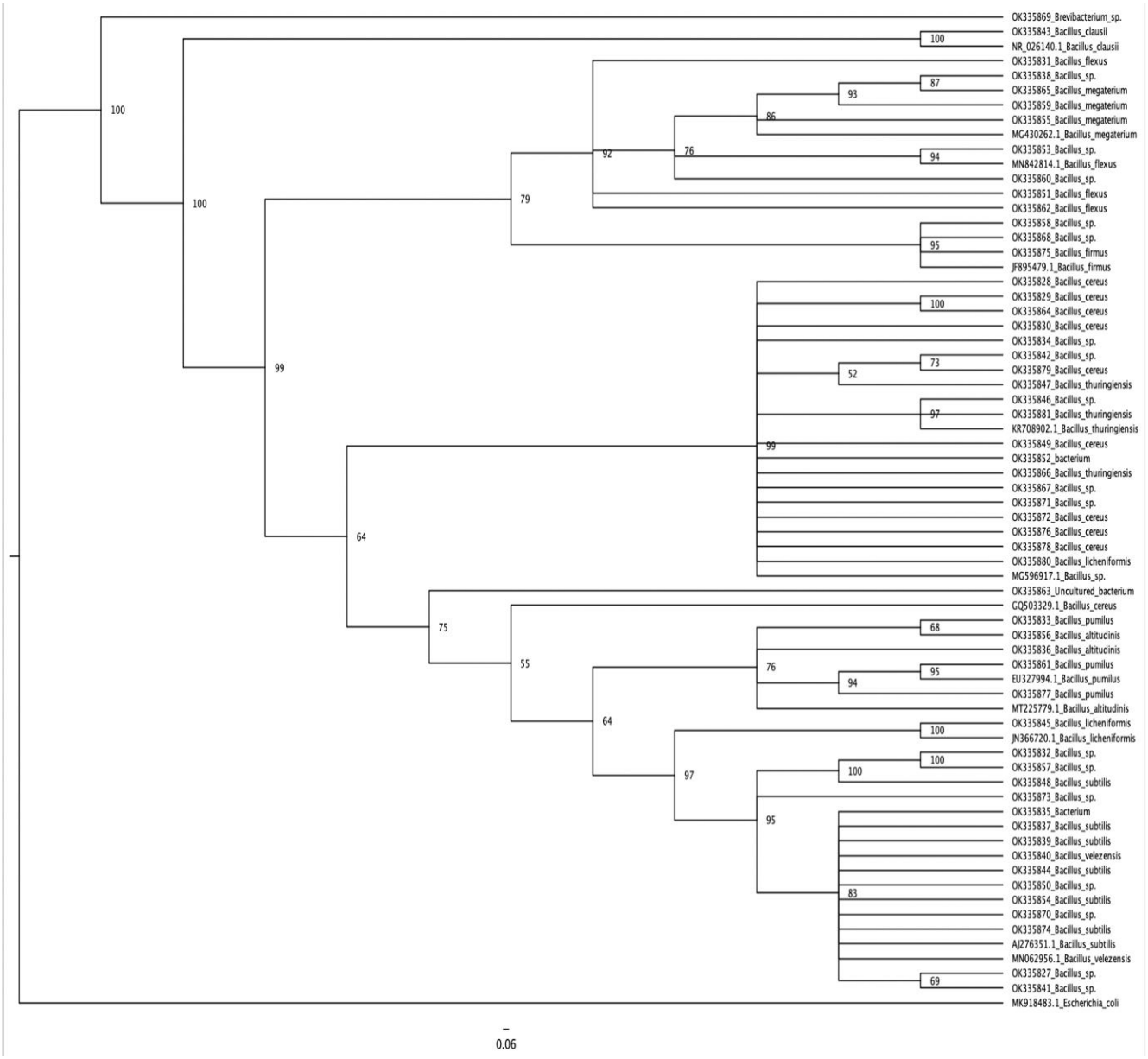

Figure 3. Phylogenetic consensus tree based on Bayesian inference analysis showing the relationship among bacterial species isolated from mangroves; Bayesian posterior probabilities are expressed as percent probabilities in branches and nodes

Environmental parameters and host species can affect its diversity and colonization efficiency. The following mangrove plants that served as hosts are *Sonneratia alba*, *Avicennia marina*, *A. alba*, *Rhizophora mucronata*, *R. apiculata*, *Xylocarpus moluccensis* and *Aegiceras corniculatum.* Among the host plants, *S. alba* harbored the most species of bacteria and fungi. These include the two medically important strains, *B. cereus* and *B. thuringiensis*. Diverse fungi were also recorded and included strains that belong to *Aspergillus* sp., *A. nomiae*, *A. tubingensis* and *A. niger*. Some bacteria species were found only on a single, specific host such as *B. altitudinis* in *S. alba*, *B. velezensis* in *R. apiculata*, *B. clausii* in *A. marina* and *B. firmus* in *A. marina*. In addition, some microbial species can utilize at least two host plants such as *B. pumilus* was isolated from both *R. apiculata* and *X. moluccensis*; *B. licheniformis* was found from both *R. mucronata* and *R. apiculata* and *B. thuringiensis* in *S. alba* and *A. alba*. Meanwhile, some bacteria like *B. subtilis* and *B. megaterium* were found in three host species and *B. cereus* was the most abundant being recorded in four host plants (Table 1).

Previous research reports important microbiota as mangrove inhabitants in Southeast Asia, including several bacteria such as *Bacillus* sp., *Staphylococcus* sp., *Serratia* sp., *Pseudomonas* sp., *Sporosarcina* sp., *Stenotrophomonas* sp. and fungi like *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Fusarium* sp. and *Cladosporium* sp. (Chatterjee and Abraham 2020). In another similar study, it identified 16 Bacillus species associated with five (5) mangrove species (Eldeen et al. 2015). These endophytes

possessed wide range of bioactivity against certain pathogens and *B. tequilensis* and *B. subtilis* to have the most active metabolites. For this study, *Bacillus* strains from subtilis and cereus clades are common and formed the largest groupings. They proved to be versatile being dominant in numerous host plants. Another study reported 12 species of mangrove fungal endophytes (MFEs) from Luzon Island, Philippines coming from 12 healthy host mangroves (Moron et al. 2018). In addition, 16 species of fungi were isolated from leaves of 4 mangrove species: *S. alba, R. mucronata, A. floridum* and *A. alba* in Visayas Island (Apurillo et al. 2019). The secretion of secondary metabolites from fungal microbiome shows potent bioactivity and several species have been linked with the mangroves *R. apiculata* and *R. mucronata*. Apart from *Rhizophora* sp. additional other plants from tropical regions have been reported with endophytic associations such as *A. marina* where several endophyte isolates have medical prospects (Khalil et al. 2021). Secondary metabolites by fungal endophytes are lignans, steroids, alkaloids, terpenoids, quinones, phenylpropanoids, phenolic acids, aliphatic metabolites, phenol, and lactones. Antimicrobial metabolites serve as potential alternatives that can hinder resistance to drugs for instance, extracts of endophytic fungi isolated from leaves, roots and barks of *S. alba* can restrict the growth of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Chatterjee and Abraham 2020). Like the host plants, endophytic and rhizosphere microorganisms yield bioactive compounds or secondary metabolites as a result of coevolution (Rhamawati 2021); thus, mangroves can be a rich reservoir of potential bioactive substances.

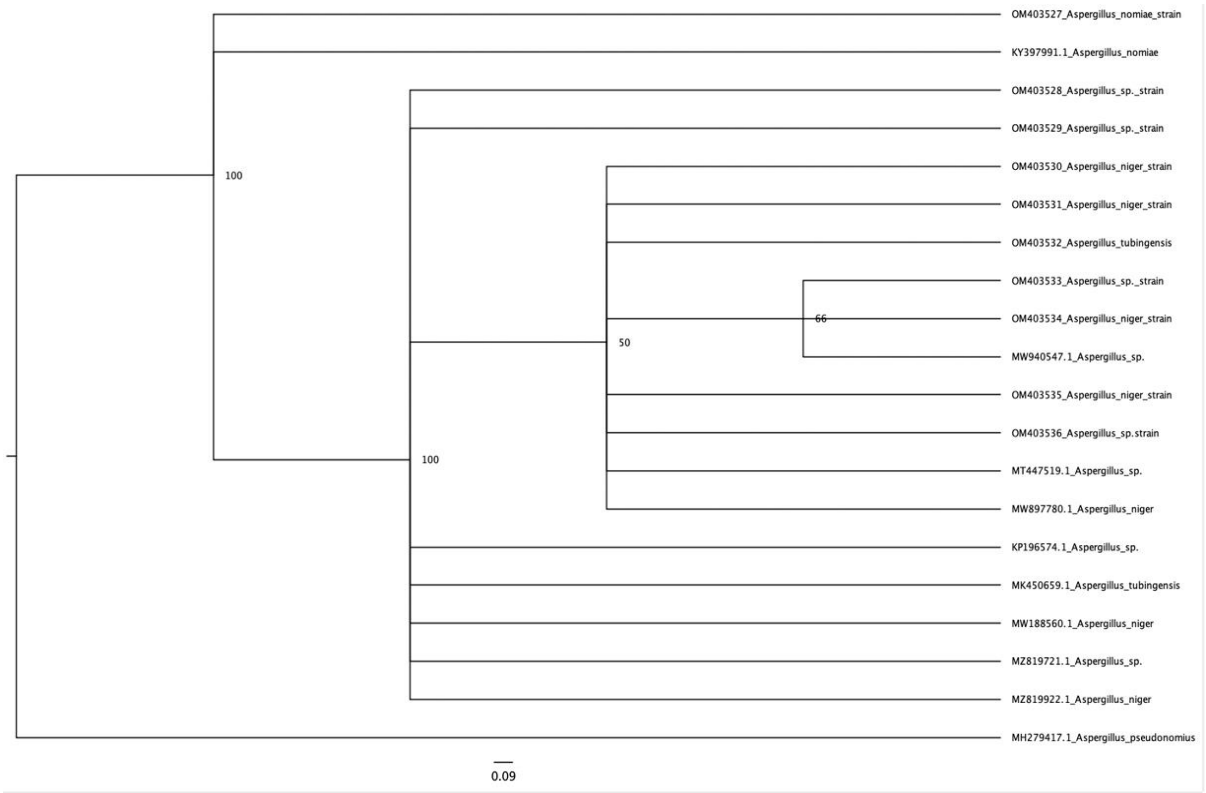

Figure 4. Phylogenetic consensus tree based on Bayesian inference analysis showing the relationship among fungi species isolated from mangroves; Bayesian posterior probabilities are expressed as percent probabilities in branches and nodes

In conclusion, *S. alba* harbored the most species of bacteria and fungi. Mangroves can serve as rich reservoir of Bacilli species that include medically important strains, *B. cereus*, *B. subtilis*, and *B. thuringiensis*. Diverse fungi were also recorded, including strains belonging to *Aspergillus* sp., *A. nomiae*, *A. Tubingensis*, and *A. niger*. Microbes and their secondary metabolites can be used for future medical and commercial applications. Identification based on DNA short sequence tags was effective.

ACKNOWLEDGEMENTS

Department of Science and Technology (DOST)-Philippine Council for Agriculture, Aquatic, Natural Resources Research and Development for the funding of this study.

Table 1. Plant hosts and isolated microbiome

Location	Host plant	Type of endophyte/ epiphyte	Species	Location
Lebak (Sultan Kudarat, Mindanao, Philippines)	*Rhizophora apiculata*	Bacteria	*Bacillus* sp.	Epiphyte
			Bacillus pumilus	
			Bacillus sp.	
			Bacillus subtilis	
			Bacillus cereus	Endophyte
			Bacillus velezensis	
		Fungi	*Aspergillus* sp.	Epiphyte
			Aspergillus niger	
	Avicennia marina	Bacteria	*Bacillus cereus*	Endophyte
			Bacillus sp.	Epiphyte
			Bacillus clausii	
		Fungi	*Aspergillus* sp.	Epiphyte
	Sonneratia alba	Bacteria	*Bacillus cereus*	Epiphyte
			Bacillus flexus	
			Bacillus sp.	
			Bacillus altitudinis	
			Bacillus subtilis	
			Bacillus sp.	
			Bacillus sp.	Endophyte
		Fungi	*Aspergillus nomiae*	Epiphyte
			Aspergillus tubingensis	
			Aspergillus niger	
			Aspergillus sp.	
	Avicennia alba	Bacteria	*Bacterium*	Endophyte
		Fungi	*Aspergillus niger*	Epiphyte
			Aspergillus sp.	Endophyte
Palimbang (Sultan Kudarat, Mindanao, Philippines)	*Rhizophora apiculata*	Bacteria	*Bacillus licheniformis*	Epiphyte
			Bacillus cereus	
			Bacillus sp.	
			Bacillus megaterium	
			Bacillus sp.	
			Bacillus sp.	
			Bacillus sp.	
	Sonneratia alba	Bacteria	*Bacillus* sp.	Epiphyte
			Bacillus sp.	
			Bacillus altitudinis	
			Bacillus megaterium	
			Bacillus cereus	
	Avicennia alba	Bacteria	*Bacillus thuringiensis*	Epiphyte
			Bacterium	
			Bacillus flexus	
			Uncultured bacterium	
			Bacillus thuringiensis	Endophyte
	Aegiceras corniculatum	Bacteria	*Bacillus subtilis*	Epiphyte
			Bacillus megaterium	
			Bacillus cereus	
	Avicennia marina	Bacteria	*Bacillus flexus*	Epiphyte
			Bacillus sp.	
			Bacillus subtilis	
			Brevibacterium sp.	Endophyte
	Rhizophora mucronata	Bacteria	*Bacillus subtilis*	Endophyte
			Bacillus sp.	
			Bacillus sp.	
			Bacillus sp.	Epiphyte
	Xylocarpus moluccensis	Bacteria	*Bacillus pumilus*	Epiphyte
			Bacillus sp.	
Kalamansig (Sultan Kudarat, Mindanao, Philippines)	*Avicennia marina*	Bacteria	*Bacillus firmus*	Epiphyte
			Bacillus cereus	
			Bacillus cereus	Endophyte
	Rhizophora apiculata	Bacteria	*Bacillus pumilus*	Epiphyte
	Avicennia alba	Bacteria	*Bacillus cereus*	Epiphyte
	Rhizophora mucronata	Bacteria	*Bacillus licheniformis*	Epiphyte
	Sonneratia alba	Bacteria	*Bacillus thuringiensis*	Epiphyte

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