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Antibacterial activities of mangrove leaf endophytic fungi from Luzon Island, Philippines

Ramirez CSP[1,2], Notarte KIR[2] and dela Cruz TEE[1,2,3*]

[1]The Graduate School, University of Santo Tomas, España Blvd. 1008 Manila, Philippines
[2]Fungal Biodiversity, Ecogenomics and Systematics Group, Research Center for the Natural and Applied Sciences, University of Santo Tomas, España Blvd. 1008 Manila, Philippines
[3]Department of Biological Sciences, College of Science, University of Santo Tomas, España Blvd. 1008 Manila, Philippines

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Abstract

Fungi associated with mangroves are untapped sources of bioactive secondary metabolites. In this study, 628 mangrove leaf endophytic fungi (MLEF) were recorded from 19 mangrove hosts collected from the provinces of Zambales, Batangas, Cavite, and Quezon in Luzon Island, Philippines. The MLEF morphospecies were identified as belonging to the genera *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Nigrospora*, *Penicillium*, *Pestalotiopsis*, *Phialophora*, and *Trichoderma*. Screening revealed that the MLEF crude culture extracts were promisingly potent against the gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus* (ZOI > 19 mm) with no or partial activity against the gram-negative bacteria *Klebsiella oxytoca* and *Pseudomonas aeruginosa*. Our findings showed that mangrove leaf endophytic fungi are potential sources of bioactive compounds.

Key words – bioactivities – fungal endophytes – mangrove forests – secondary metabolites

Introduction

Mangroves are hosts to many plant-associated microorganisms including fungi. The diversity of these mangrove-associated fungi, also known as manglicolous fungi, is influenced by numerous factors such as wide variety of substrates and level of salinity (Zhou et al. 2018). In the Philippines, studies have recently reported the isolation of fungal endophytes from mangrove leaves (Guerrero et al. 2018, Moron et al. 2018, Apurillo et al. 2019). Still, limited studies on fungal endophytes associated with Philippine mangroves have been done despite the numerous species of mangroves present in the country. Of the 65 species of mangroves known worldwide and of the 60 mangrove species found in the Indo-Pacific region, around 40 species inhabit the Philippines (Primavera 2000). However, overexploitation and conversion of mangrove forests to fishponds and human settlements have caused a steep decline in Philippine mangroves with about 70% habitat loss since 1951 (Primavera 2000). With the continued decline in the mangrove habitat, it is imperative to study the mangrove mycoflora in the Philippines before the loss of the available mangrove habitats becomes permanent.

Interestingly, the marine environment is tapped for the search for microorganisms capable of producing biologically active natural products. Marine-associated fungi have been shown to

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Corresponding Author: Thomas Edison E. dela Cruz – e-mail – tedelacruz@ust.edu.ph

produce secondary metabolites with anti-infective properties (Schulz et al. 2008, Lavadia et al. 2017, Notarte et al. 2017, 2018). Fungi from mangroves have also been tapped for their biological activities including anticancer compounds (Tan et al. 2015, Deshmukh et al. 2018, Moron et al. 2018, Apurillo et al. 2019). For example, the mangrove fungus *Diaporthe* produced the polyketide dicerandrol D that displayed a selective antimalarial activity (Calcul et al. 2013). In another study, the antiviral fumiquinazoline alkaloids neosartoryadins A and B were isolated from the mangrove fungus *Neosartorya udagawae* (Yu et al. 2016). The mangrove fungal endophyte *Penicillium chrysogenum* was found to produce an antibacterial compound containing an indole and a diketopiperazine moiety which was active against *Vibrio cholerae* (Devi et al. 2012). Tan et al. (2015) identified tyrosol C, cytosporone B, dothiorelone A, and dothiorelone C from culture extracts of *Phyllosticta* sp., an endophytic fungus identified from Philippine mangroves. With the emergence of new diseases due to factors such as agricultural changes, population growth, global migration, and the occurrence of antibiotic-resistant disease-causing microbes, it is vital to search for new sources of drugs. Interestingly, the mangroves in the Philippines are potential reservoir of highly diversified endophytic fungi that may have unique metabolic properties. Thus, in this study, the endophytic fungi were isolated from mangrove leaves collected from Batangas, Cavite, Quezon, and Zambales and screened for their antibacterial properties.

Materials & Methods

Identification of host mangroves

The vegetative and reproductive parts of healthy mangroves were collected from the coastal areas of Zambales, Batangas, Cavite and Quezon (Table 1). Herbaria of the mangrove samples were then prepared by mounting pressed, oven-dried specimens (branch, leaves, fruits, flowers) onto clean bristol board. Identification of the collected mangrove species was done following comparison of their morphology with published identification guides/keys, e.g. Primavera (2009) and Calumpong & Meñez (1997). Herbarium specimens were also sent to the Botany Division, National Museum in Manila for species identification and verification.

Isolation of mangrove leaf endophytic fungi

Healthy leaf samples from 19 mangrove hosts were transported to the laboratory in clean zip-locked plastic bags and processed within 24 h of collection. The mangrove leaves were initially washed with cold, running tap water to remove any soil debris and then dried with clean paper towels. The leaf samples were hole-punched (6 mm in diameter) using a flame-sterilized puncher and collected into sterile petri dishes. The leaf explants (30 explants per mangrove species per collection site) were then surface sterilized following the protocol of Toofanee & Dulymamode (2002). This was done by immersing the leaf explants in 75% ethyl alcohol (Univar) for 1 min, followed by 0.5% sodium hypochlorite solution for 3 min, and then, in 75% ethyl alcohol for an additional 30 sec. Hereafter, the explants were washed three times with sterile artificial seawater (ASW, 33 g commercially available marine salt dissolved in 1,000 mL distilled water). Following surface-sterilization, the leaf explants (6 leaf explants per culture plate) were placed on culture plates pre-filled with 2% Malt Extract Agar (Oxoid) supplemented with 33 g/L marine salt (Bioresearch) and 500 mg/L streptomycin sulphate (Sigma). A total of 5 plates were inoculated for each host plant species. All culture plates were then incubated at room temperature (25 ± 2°C) for 1-4 weeks. The fungi that grew out of the leaf explants were cut out and transferred to freshly prepared Potato Dextrose Agar (PDA, Pronadisa) plates supplemented with 33 g/L marine salt (PDAS) for purification.

Identification of mangrove leaf endophytic fungi

The isolated mangrove leaf endophytic fungi were initially cultured on freshly prepared PDAS plates and incubated at room temperature for 3-5 days. The fungal colonies were then described based on their colony appearance, e.g. texture, margin, color, reverse texture, and reverse

color. For the spore and hyphal morphology of the isolated MLEF, spores and hyphae were fixed with lactophenol in clean glass slides and viewed under a compound light microscope (Olympus model, 400-1000x) (Henrici 1930). Spore color, shape, and arrangement as well as hyphal morphology were noted. Identification of the isolated endophytic fungi was done based on published literature (Samson et al. 2010).

Table 1 The sampling localities for the collection of host mangroves.

Province	Collection sites	GPS coordinates	Host mangrove species
Zambales	San Agustin, Iba	N15°11′26.3″ E119°57′12.3″	*Avicennia marina* (Forssk.) Vierh. *Excoercaria agallocha* L. *Nypa fruticans* Wurmb. *Sonneratia alba* J. Smith *Xylocarpus granatum* Koen
	Felmida Diaz, Cabangan	N15°11′26.3″ E120°01′53.7″	*Rhizophora mucronata* Poir.
	Libaba, Palauig	N15°26′05.7″ E119°53′47.2″	*A. marina* *R. mucronata*
	Lipay, Palauig	N15°29′07.6″ E119°54′17.1″	*A. marina* *R. mucronata* *S. alba*
	Banban, Masinloc	N15°30′21.3″ E119°58′06.8″	*Osbornia octodonta* F. Muell. *R. mucronata*
	Taltal, Masinloc	N15°34′07.0″ E119°57′03.9″	*A. marina* *R. mucronata* *S. alba*
	Uacon, Candelaria	N15°40′17.4″ E119°56′11.7″	*Aegiceras floridum* Roem. & Schult. *A. marina* *R. mucronata* *S. alba*
	Subic Bay, Olongapo	N14°46′56.9″ E120°16′58.5″	*A. marina* *S. alba*
Batangas	Wawa, Nasugbu	N14°05′12.1″ E120°37′20.4″	*Acanthus ebracteatus* Vahl. *A. marina* *Ceriops tagal* (Perr.) C.B. Robinson *R. mucronata* *S. alba*
	Lian	N13°56′39.3″ E120°36′50.9″	*A. marina* *R. mucronata* *S. alba*
Cavite	Maragondon	N14°13′07.7″ E120°37′14.2″	*A. marina* *R. mucronata* *S. alba*
Quezon	Gumaca	N13°55′02.3″ E122°07′31.7″	*A. marina* *S. alba*
	Calauag	N13°57′37.1″ E122°17′23.0″	*A. marina* *R. mucronata*
	San Narciso	N13°33′49.6″ E122°34′49.8″	*A. marina* *R. stylosa* *S. alba*
	Unisan	N13°49′48.2″ E121°58′48.1″	*A. marina* *R. mucronata* *S. alba*

Table 1 Continued.

Province	Collection sites	GPS coordinates	Host mangrove species
Quezon	Pagbilao	N13°58′31.5″ E121°43′32.4″	*A. ebracteatus* *Aegiceras corniculatum* (L.) Blanco *A. floridum* *Avicennia officinalis* L. *A. marina* *Brugueira* sp. *B. sexangular* *Camptostemon philippinense* (Vidal) Becc. *Ceriops decandra* (Griff.) Ding Hou *C. tagal* *E. agallocha* *O. octodonta* *Rhizophora apiculata* Blume *R. mucronata* *R. stylosa* *Scyphiphora hydrophyllacea* C.F. Gaertn. *S. alba* *X. granatum*

To determine the ability of 19 representative fungi to grow in the presence and absence of marine salts, an agar block approximately 5 mm^2 were cut from the 7-day old MLEF and inoculated onto PDA and PDAS in triplicates. Culture plates were then incubated at room temperature. Colony radial growth was then measured for each of the isolates as the distance from the agar block to the margin of the growing fungal colony using a standard ruler. Two readings per plate were recorded on the 4^{th} and 6^{th} day to coincide with growths of both slow and fast-growing fungi. The colony extension rates (CER) were determined using the equation described by Notarte et al. (2017): CER = [length of hyphal growth after 6 incubation days (d_f)] – [length of hyphal growth after 4 incubation days (d_i)] / 2. To test for significant differences between the colony extension rates of the MLEF, one-way ANOVA was computed using SigmaStat ver.3.1 (Systat Software, USA).

Production and extraction of secondary metabolites from mangrove leaf endophytic fungi

Selected 17 mangrove leaf endophytic fungi were grown in PDAS slants for one week. Following incubation, spore and mycelial suspensions were prepared by adding 5 mL sterile distilled water on the culture slants. Then, the spore and mycelial suspensions were transferred into 100 mL Potato Dextrose Broth (PDB, Pronadisa) supplemented with 33 g/L marine salt (in duplicates) and incubated under static condition at room temperature for 3-4 weeks. After incubation, 100 mL ethyl acetate (RCI Labscan) was added to each culture bottle and soaked overnight. After 24 h, the fungal mycelia were filtered in filter paper and the ethyl acetate extracts were decanted. The culture filtrates per MLEF were combined and concentrated *in vacuo* at 35°C using a rotary evaporator. The crude culture extracts were transferred to pre-weighed amber bottles and allowed to dry. Then, the crude culture extracts were finally dissolved in methanol:acetone (1:1 v/v) to a final concentration of 100 mg/mL, and then stored inside the refrigerator until used in the assay.

Assay for the antibacterial activities

Standard test bacteria, *Klebsiella oxytoca* (ATCC 700524), *Micrococcus luteus* (environmental sample), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923), were acquired from the University of Santo Tomas Collection of Microbial Strains, Manila, Philippines. These bacteria were selected because of their pathogenicity. Initially, these bacteria were maintained and stored on Nutrient Agar (NA, HiMedia) plates. Prior to the assay, the test bacteria were grown on NA plates for 24 h. Following incubation, cell suspension was prepared

with sterile normal saline solution. The cell concentration was adjusted to 0.5 McFarland and then swabbed using sterile cotton swab onto culture plates pre-filled with 25 mL Mueller Hinton Agar (MHA, HiMedia). Due to insufficiency of the extracted metabolites in some species, 10 to 30 µL of the stock crude culture extracts (100 mg/mL) were added onto sterile paper disks (Whatman, 6 mm in diameter) giving a final concentration of 1-3 mg of extract per disk. The impregnated disks were then air-dried and placed onto the inoculated culture plates (in triplicates). As positive control for *K. oxyt*oca, *M. luteus*, *P. aeruginosa*, and *S. aureus*, commercially available standard antibiotic disks containing 10 µg/mL streptomycin (BBL) and 30 µg/mL tetracycline (BBL) were used in the assay. The solvent methanol:acetone was used as negative control. All culture plates were then incubated at 37°C for 24 h. After incubation, zones of inhibition (ZOI) were measured using a ruler and their antimicrobial activities were assessed using the following indices: (1) very active: >19 mm ZOI, (2) active: 13-19 mm ZOI, (3) partially active: 10-12 mm ZOI, and (4) inactive: <10 mm ZOI (Quinto & Santos 2005).

Results

The fungal endophytes associated with mangrove leaves

A total of 628 fungal isolates belonging to 20 different morphospecies were recorded from the 19 mangrove hosts that were collected from the provinces of Zambales, Batangas, Cavite and Quezon in Luzon Island, Philippines (Table 2). Morphocultural characterization identified the MLEF as belonging to the genera *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Pestalotiopsis*, *Phialophora*, *Trichoderma*, *Fusarium*, *Nigrospora,* and *Penicillium*. Two MLEF, however, were not identified by morphological methods and were labelled only as morphospecies 1 and morphospecies 2. One MLEF remained sterile even after prolonged incubation and was designated as *mycelia sterilia*.

Though the isolated mangrove leaf endophytic fungi were similar to known terrestrial fungal genera, 19 representative MLEF were tested for their growth in the absence or presence of marine salts (Fig. 1). Some of these selected MLEF were similar morphospecies but isolated from different localities. Results showed that some of the MLEF (*Nigrospora* sp. 2, *Penicillium* sp. 1, *Penicillium* sp. 3, *Penicillium* sp. 4, *Penicillium* sp. 5 and *mycelia sterilia*) grew significantly better in the presence of marine salts than in PDA without marine salts ($p < 0.01$). Interestingly, *Nigrospora* sp. 1 isolated from *R. mucronata* leaves collected from Batangas and Quezon showed varied growth patterns in PDA with and without marine salts. *Nigrospora* sp. 1 from Batangas demonstrated faster growth as compared to the same morphospecies from Quezon regardless of the presence of salt. The ability of MLEF to grow in the presence of marine salts indicated their possible adaptation in the marine habitat.

Production of antibacterial metabolites from mangrove leaf endophytic fungi

Seventeen selected MLEF isolates were extracted for their secondary metabolites and tested for their biological properties. These fungi were commonly isolated and exhibited unique colonial morphology and/or produced pigment in culture media. We have also chosen different fungal strains of the same morphospecies that were isolated either from different host mangroves and/or collection sites. Interestingly, the MLEF showed to be more biologically active against gram-positive than gram-negative bacteria (Table 3). Of the 17 MLEF, four isolates (*Phialophora*, *Cladosporium*, *Penicillium* sp.4 and *Penicillium* sp.5) were very active (>19mm ZOI) against *S. aureus*. Aside from targeting *S. aureus*, eight MLEF isolates (*Phialophora*, *Cladosporium*, *Penicillium* sp.3, *Penicillium* sp.4 and *Penicillium* sp.5) were also very potent against *M. luteus* (>19mm ZOI). Among these bioactive MLEF, *Phialophora* spp. isolated from different host mangroves showed varied biological activities. *Phialophora* sp. derived from *Sonneratia alba* showed very active antibacterial property against *S. aureus* (22 mm ZOI) and *M. luteus* (32 mm ZOI), while *Phialophora* sp. isolated from *Rhizophora mucronata* showed no antagonism to the same pathogens.

Table 2 Number of mangrove leaf endophytic fungi isolated from 19 mangrove species.

Mangrove Leaf Endophytic Fungi (MLEF)	Host Mangroves																		
	A. ebracteatus	***A. corniculatum***	***A. floridum***	***A. marina***	***A. officinalis***	***Bruguiera* sp.**	***B. sexangula.***	***C. philippinensis***	***C. decandra***	***C. tagal***	***E. agallocha***	***N. fruticans***	***O. octodonta***	***R. apiculata***	***R. mucronata***	***R. stylosa***	***S. hydrophyllacea***	***S. alba***	***X. granatum***
A. niger	-	-	-	19	1	-	-	-	6	6	-	-	-	-	-	-	-	3	-
A. ochraceus	3	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	14	6
Cladosporium sp.	-	1	11	6	-	-	-	-	1	1	-	1	-	-	13	2	-	11	-
Colletotricum sp.	12	-	-	4	-	11	-	-	-	-	-	-	-	-	2	-	-	-	-
Fusarium sp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-
Fusarium sp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
Morphospecies 1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Morphospecies 2	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
Mycelia sterilia	1	-	1	31	4	15	1	30	9	9	-	-	9	5	28	4	-	9	10
Nigrospora sp. 1	-	-	-	1	-	-	-	-	-	-	-	2	-	-	4	-	-	8	-
Nigrospora sp. 2	1	2	-	20	-	-	-	-	-	-	-	-	-	-	-	1	-	3	-
Penicillium sp. 1	-	-	-	-	-	-	-	-	-	-	27	-	2	-	-	-	-	-	-
Penicillium sp. 2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Penicillium sp. 3	-	-	-	-	-	-	-	-	13	13	-	-	-	-	-	-	-	5	-
Penicillium sp. 4	-	-	-	11	-	-	-	-	-	-	8	-	-	-	29	-	-	2	-
Penicillium sp. 5	-	-	-	-	-	-	-	-	-	-	19	-	-	-	2	-	-	1	-
Penicillium sp. 6	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pestalotiopsis sp.	-	-	-	3	-	-	-	-	-	-	-	1	-	-	5	-	-	-	-
Phialophora sp.	9	-	1	10	4	-	-	-	4	4	31	8	11	-	12	1	-	15	4
Trichoderma sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
Subtotal	26	3	14	122	9	26	1	30	33	33	85	12	22	7	104	8	0	73	20
Total										628									

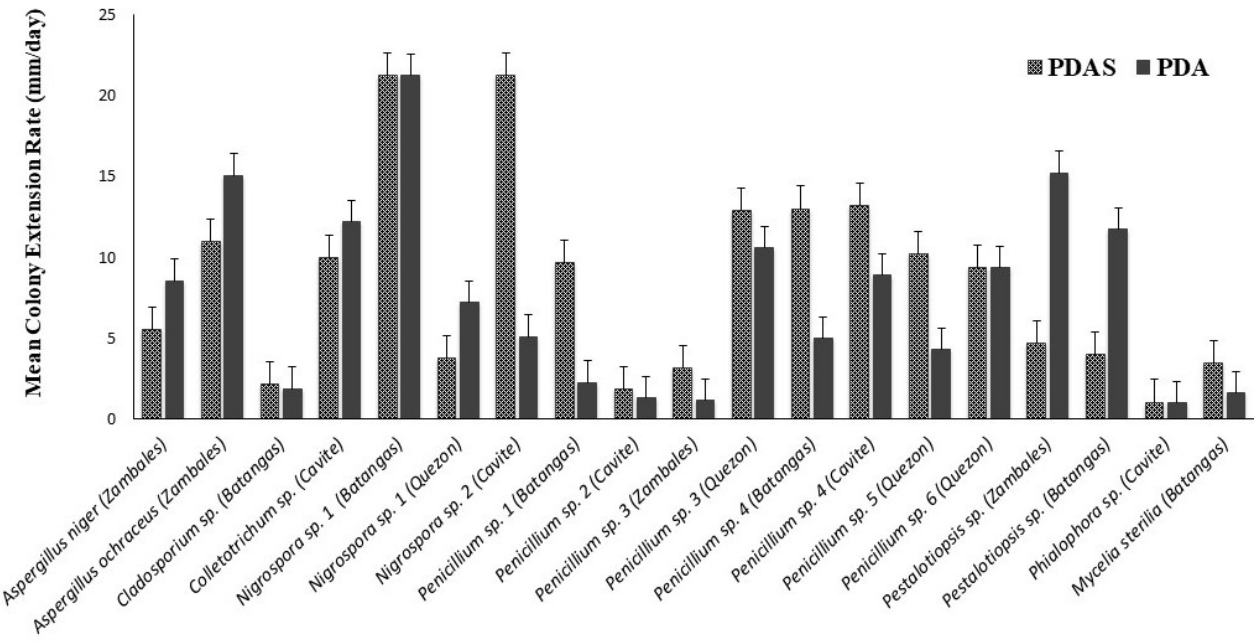

Fig. 1 – Mean colony extension rates (in mm/day) of MLEF isolates grown on PDA in the absence or presence of marine salts. Standard error of the mean was presented in error bars ($p < 0.01$).

Another interesting finding showed that the crude culture extracts from *Phialophora* sp. (22-32 mm ZOI), *Cladosporium* sp. (16-34 mm ZOI), *Penicillium* sp.4 (15-42 mm ZOI) and *Penicillium* sp.5 (20 mm ZOI) showed comparable or even more potent antibacterial property than the antibiotic controls streptomycin (11-25 ZOI) and tetracycline (22-40 ZOI) against *S. aureus* and *M. luteus*. The differences between the zones of inhibition exhibited by the MLEF crude culture extracts were found to be statistically significant ($p<0.001$). In contrast to the potency of the culture extracts against gram-positive bacteria, the MLEF showed partial activity or no antagonism to the gram-negative bacteria *P. aeruginosa* and *K. oxytoca*.

Table 3 Antibacterial properties of mangrove leaf endophytic fungi against gram-positive and gram-negative bacteria (n=3).

Province	**Host Mangrove**	**Fungal Endophytes**	**Test bacteria zone of inhibition, ZOI (mm ± SD)[a]**			
			S. aureus	***P. aeruginosa***	***K. oxytoca***	***M. luteus***
Zambales	*Sonneratia alba*	*Phialophora* sp.	22 ±0	-	-	32 ±0
	Rhizophora mucronata	*Phialophora* sp.	-	-	-	-
	Xylocarpus granatum	*A. ochraceus*	8 ±0	-	-	10 ±0
	Avicennia marina	*Cladosporium* sp.	21 ±0	-	-	30 ±0
	Sonneratia alba	*Cladosporium* sp.	16 ±0	-	-	34 ±2.3
	Sonneratia alba	*Penicillium* sp. 2	9 ±0	-	-	12 ±0
Batangas	*Avicennia marina*	*A. niger*	7 ±0	-	9 ±0	13 ±0.6
	Avicennia marina	*A. ochraceus*	-	-	-	8 ±0.6
	Acanthus ebracteatus	*A. ochraceus*	-	-	-	8 ±0
	Sonneratia alba	*Penicillium* sp. 4	20 ±0	-	10 ±0	35 ±0
	Avicennia marina	*Penicillium* sp. 4	15 ±0	-	-	42 ±0
	Sonneratia alba	*Penicillium* sp. 5	20 ±0	-	-	20 ±0
Cavite	*Rhizophora mucronata*	*Penicillium* sp. 4	18 ±0	-	7 ±0	35 ±0

Table 3 Continued.

Province	Host Mangrove	Fungal Endophytes	Test bacteria zone of inhibition, ZOI (mm ± SD)[a]			
			S. aureus	***P. aeruginosa***	***K. oxytoca***	***M. luteus***
Quezon	*Osbornia octodonta*	*Penicillium* sp. 1	18 ±0	-	-	16 ±0
	E. agallocha	*Penicillium* sp. 1	-	-		13 ±0.6
	Sonneratia alba	*Penicillium* sp. 4	15 ±1.2	-	-	33 ±0
	Sonneratia alba	*Mycelia sterilia*	-	-	-	14 ±1.7
Controls	Negative	MeOH: Acetone	-	-	-	-
		Streptomycin	11 ±0	13 ±0	24 ±0	25±0
	Positive	Tetracycline	22 ±0.6	11 ±0	22 ±0	40±0

[a] ZOI: inactive <10 mm, partially active 10-13 mm, active 14-19 mm, very active > 19 mm (Quinto & Santos 2005)

Discussion

Mangroves dominate one-fourth of the world's coastlines and comprised a total land area of about 181,000 km^2 (Thatoi et al. 2013). Considering their wide abundance, mangrove plants have been morphologically and physiologically adapted to extreme habitats of changing salinity, high temperature, tidal inundation, high wind velocity and faunal competition (Guerrero et al. 2018, Latha & Mitra 2004). Interestingly, mangrove-associated fungi, known as the second largest ecological group of marine-derived fungi, promote the survival of their plant hosts under extreme environmental conditions through long term plant-fungi interactions (Zhou et al. 2018). As a result of such interactions, mangrove fungi were able to produce unique and diverse natural products. For several years, mangrove fungi attracted researchers across the globe due to its bioactive secondary metabolites, which could be target compounds in drug discovery, particularly in producing antibiotics, antidiabetic, antiviral, anti-inflammatory, anticancer, antioxidant and immunosuppressive and therapeutic agents (Thatoi et al. 2013). Mangrove-associated fungi have also been reported for their applications in bioremediation (Torres & dela Cruz 2013).

Several studies have previously reported fungal endophytes associated with terrestrial plants collected in the Philippines, e.g. *Canarium ovatum* (Torres & dela Cruz 2015), *Ficus* species (Solis et al. 2015, 2016), *Pandanus amaryllifolius* (Bungihan et al. 2011, 2013), and even from medicinal plants (Eskandarighadikolaii et al. 2015). In this study, 628 mangrove leaf endophytic fungi (MLEF) belonging to 20 different morphospecies were recorded from mangrove hosts collected from the provinces of Zambales, Batangas, Cavite, and Quezon in Luzon Island. The MLEF belonged to the genera *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Nigrospora*, *Penicillium*, *Pestalotiopsis*, *Phialophora* and *Trichoderma*, with species capable of growth in culture media with marine salts (Table 2, Fig. 1). In addition, two morphospecies were not identified and one species was designated only as *mycelia sterilia* since the organism failed to produce any spores *in vitro*. Such high number of MLEF is not surprising, since, in general, leaves have a more diverse fungal endophytic community as compared to other parts of the plants (Hamzah et al. 2018). Cultivating the MLEF in PDA with and without marine salt revealed that most of the MLEF grew significantly better in the presence of marine salt, further supporting their adaptability to the marine environment (Fig. 1). Though the fungal strains were isolated from mangroves which are known halophytes, the isolated endophytic fungi belonged to known terrestrial fungal taxa. This was also observed by Kumaresan & Suryanarayanan (2001) and Tariq et al. (2006), in which common terrestrial fungal taxa, including those belonging to the genera *Alternaria*, *Aspergillus*, *Colletotrichum*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma*, were isolated from mangrove leaves, roots, and stems collected in India. However, obligate marine fungi, e.g. *Cirrenalia pygmea*, *Cumulospora marine*, *Lulworthia* sp., *L. grandispora*, *Trichocladium alopallonellum* and *Zalerion maritimum*, were also reported from the roots of mangroves (Ananda & Sridhar 2002, Maria & Sridhar 2003). In contrast, no obligate marine fungi were isolated from the collected mangrove leaves in this study. Spatafora et al. (1998) supported

via molecular evidence the linkages between terrestrial fungi and the marine ascomycetes. The study proved that marine organisms, more specifically marine fungi, can trace their ancestors back to terrestrial organisms. In addition, plants such as mangroves survive in the ecotone between terrestrial and marine ecosystems (Rajamani et al. 2018). It is therefore not surprising to isolate terrestrial fungal genera from substrates collected in marine waters. Solis et al. (2010) and Notarte et al. (2017) also isolated marine-derived fungi from seaweeds belonging to known terrestrial fungal genera which exhibited better growth on culture medium with marine salts.

Endophytic fungi are known to be good sources of numerous bioactive secondary metabolites with antimicrobial activities (Leylaie & Zafari 2018). Among the promising endophytes are the mangrove-associated fungi that produced novel compounds such as the trihydroxybenzene lactone cytosporone B that conferred antifungal activities against *Aspergillus niger*, *Trichoderma* sp. and *Fusarium* sp. (Xu et al. 2005) and the meroterpenes guignardones A and B that showed inhibitory activity against methicillin-resistant *S. aureus* (Mei et al. 2012). In the study of Silva et al. (2011), 34 ethyl acetate crude extracts of 70 strains of mangrove-associated fungi isolated from *Laguncularia racemosa* also showed to be active against a variety of pathogenic bacteria. Furthermore, the compounds chloropreussomerins A and B, isolated from the mangrove fungus *Lasiodiplodia theobromae*, were reported as effective antibacterial agent (Chen et al. 2016). In this study, 17 MLEF were selected and assayed for their antibacterial properties. Of the 17 MLEF, four isolates were found to be very active against *S. aureus*, while eight MLEF exhibited promising activity against *M. luteus* (Table 3). The bioactive MLEF were found to be belonging to the genera *Cladosporium*, *Phialophora* and *Penicillium*. Most of the MLEF were either partially active or inactive against the gram-negative bacteria *K. oxytoca* and *P. aeruginosa*. Similar results were also observed in the study of Maria et al. (2005) where little to no bioactivity was observed of their mangrove endophytic fungi against a gram-negative pathogen. However, their study also showed that the submerged state fermentation-derived extracts were active to partially active against various gram-positive bacteria. Comparing the results of this study and that of Maria et al. (2005), the metabolites from the mangrove fungal endophytes showed to favorably target the gram-positive bacteria as opposed to the gram-negative bacteria. Although in this study the bioactive MLEF, *Cladosporium*, *Phialophora* and *Penicillium*, were identified as fungal genera with known antibiotic activities, it is interesting to note that these were isolated from marine substrata, and thus, the metabolites may be different from their terrestrial counterparts. In fact, novel compounds have been isolated from these typical fungal genera of marine origin, including the antibacterial and antifungal polyketide malettinin E from *Cladosporium* (Silber et al. 2014) and the *Penicillium*-derived aromatic butenolides eutypoids B-E that inhibited glycogen synthase kinase-3β, a target for the treatment of diabetes 2 (Schulz et al. 2011). Despite the isolation of these novel molecules, marine-derived fungi include mangrove-associated fungi remained to be an underrepresented sources of new natural products and, therefore, screening further these microbes, especially the mangrove fungal endophytes, for biological activities is highly encouraging (Imhoff 2016).

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