Antimicrobial activities of crude culture extracts from mangrove fungal endophytes collected in Luzon Island, Philippines

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Mangrove fungal endophytes (MFE) are potential sources of bioactive metabolites that can be tapped by local pharmaceutical companies in the development of new drugs. In this study, 40 selected MFE, previously isolated from healthy stems and roots of 12 host mangrove species, were identified based on morphology and/or ITS sequence analysis and cultured for the production of bioactive secondary metabolites. The common MFE identified were *Arthrinium phaeospermum*, *Colletotrichum siamense, C. tropicale, Fusarium oxysporum, F. chlamydosporum, F. proliferatum, F. solani, Lasiodiplodia theobromae, Nodulisporium* sp., *Paecilomyces formosus, Penicillium citrinum,* and *Pestalotiopsis microspora*. The secondary metabolites were extracted from mycelial mass of MFE grown *in vitro* using liquid cultures and screened for antimicrobial activities against eight bacterial and two yeast species using the paper disk diffusion and microdilution assays. Twenty-two MFE exhibited inhibitory activities against at least one of these test microorganisms. Results also showed that *Pestalotiopsis microspora* (MFE 24) showed strong antimicrobial activities against gram-positive bacteria while species of *Lasiodiplodia* were very active against *Saccharomyces cerevisiae* with minimum fungicidal concentration of <7.8 µg/mL, indicating their potential as sources of bactericidal and/or fungicidal compounds.

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INTRODUCTION

Fungal endophytes are microorganisms that colonize internal tissues of plants without causing harm and disease (Astuti et al. 2014). These fungi play an important role in plant communities by providing protection against various herbivores and pathogens (Arnold and Engelbrecht 2003, Schulz and Boyle 2005, Saikkonen 2007, Vega et al. 2008, Rodriguez et al. 2009). Quite significantly, novel secondary metabolites from fungal endophytes have also been reported and even exploited for pharmaceutical products and drug development (Strobel 2006). Culture extracts from fungal endophytes have been found to exhibit antifungal, antibacterial, anticancer, antimalarial, and insecticidal activities (Johann et al. 2012, Calcul et al. 2013, Mousa and Raizada 2013, Ratnaweera et al. 2015, Chen et al. 2016, Vinale et al. 2017). These findings clearly indicate the potential economic importance of fungal endophytes as sources of lead compounds in drug development. The availability of these useful microorganisms in an ecologically-diverse country like the Philippines demands a greater effort to discover their usefulness.

Mangrove forests are referred to as rich biodiversity sites since they harbor a plethora of both macro- and micro-organisms among which are the mangrove fungi. These fungi have been reported to be widely distributed in leaves, twigs, trunks and roots of the following: *Aegiceras corniculatum*, *Avicennia marina*, *A. officinalis*, *Bruguiera gymnorrhiza, Excoecaria*

Table 1: Fungal endophytes isolated from roots (R) and stems (S) of mangroves.

Taxa	Nos. of Isolates	Collection Site	Substrate type	Host Mangroves
Alternaria sp.	1	Camarines Sur	S	*R. stylosa*
Arthrinium phaeospermum	1	Camarines Sur	S	*R. stylosa*
Aspergillus ochraceus	2	Zambales	R	*R. apiculata*
Colletotrichum siamense	1	Camarines Sur	S	*R. stylosa*
Colletotrichum sp.	1	Camarines Sur	S	*A. marina*
Colletotrichum tropicale	2	Camarines Sur	S	*R. stylosa, A. corniculatum*
Fusarium chlamydosporum	1	Pangasinan	R	*R. apiculata*
Fusarium oxysporum	2	Camarines Sur	S	*A. corniculatum, R. apiculata*
Fusarium proliferatum	2	Camarines Sur, Zambales	R, S	*L. littorea, R. stylosa*
Fusarium solani	1	Pangasinan	S	*R. apiculata*
Fusarium sp.	3	Camarines Sur, Zambales	S	*R. apiculata, R. stylosa*
Lasiodiplodia sp.	1	Camarines Sur	S	*R. stylosa*
Lasiodiplodia theobromae	1	Zambales	S	*A. lanata*
Nodulisporium sp.	1	Camarines Sur	S	*R. stylosa*
Paecilomyces formosus	1	Zambales	S	*L. littorea*
Penicillium citrinum	1	Pangasinan	S	*B. gymnorrhiza*
Pestalotiopsis microspora	1	Pangasinan	S	*R. apiculata*
Pestalotiopsis sp.	2	Camarines Sur	S	*E. agallocha, R. apiculata*
Phomopsis sp.	2	Zambales	R, S	*A. lanata, C. phillipinense*
Trichoderma sp.	2	Zambales	R, S	*A. lanata, A. officinalis*
Xylaria sp.	1	Camarines Sur	S	*Avicennia* sp.
Unidentified MFE	10	Camarines Sur, Zambales	S	*A. corniculatum, A. lanata, R. apiculata, R. stylosa, S. alba*

agallocha, and *Lumnitzera racemoa* (Kumaresan and Suryanarayanan 2001, Bhimba et al. 2012, Li et al. 2016). The Philippines is known for its numerous species of mangrove plants that can be hosts to a wide variety of fungal endophytes. Among the 70 known species of mangroves, about 35-40 have so far been reported in the country (Primavera et al. 2004). However, only *R. apiculata, A. officinalis, C. decandra,* and *E. agallocha* have been studied and reported to harbor the mangrove fungi *Halosarpheia marina, Rosellinia* sp., *Savoryella lignicola, Trichocladium achrasporum,* and *Zalerion varium* (Jones et al. 1988) indicating a dearth of studies despite the apparent diversity of Philippine mangroves. The present study reports on locally- isolated and identified fungal endophytes from stems and roots of different mangrove hosts from different sites in Northern Philippines with the end view of these fungal endophytes being future sources of new compounds in drug development by our local pharmaceutical companies.

MATERIALS AND METHODS

Sample collection

Thirty-five specimens of healthy roots, measuring approximately 15-20 cm, and stems from different species of mangroves were collected above water mark during low tide in five sampling sites in Luzon Island, Philippines, more specifically in Calabanga, Camarines Sur, Alaminos, Pangasinan, and in Iba, Masinloc, and Palauig, Zambales. The host mangroves were identified and recorded per sampling province as follows: (1) Zambales: *Avicennia officinalis, A. rumphiana, Aegiceras corniculatum, Bruguiera gymnorrhiza, Camptostemon philippinense, Excoecaria agallocha, Lumnitzera litorea, Rhizophora apiculata,* and *R. stylosa*, (2) Camarines Sur: *Avicennia marina, Avicennia* sp., *A. corniculatum*, *E. agallocha*, *R. apiculata*, *R. stylosa*, and *Sonneratia alba*, and (3) Pangasinan: *B. gymnorrhiza* and *R. apiculata*. All samples were placed in clean zip lock bags right after collection and stored at 4°C. The mangrove roots and stems

were initially washed with tap water to remove any adhering soil debris and then surface-sterilized within 24 hours of collection. Voucher specimens were also prepared from the host mangroves and later identified by comparing morphological characters with published identification keys, such as the Field Guide to Philippine Mangroves (Primavera 2009) and the Field Guide to the Common Mangroves, Seagrasses, and Algae of the Philippines (Calumpong and Meńez 1997). Herbarium specimens were also sent to the Botany Division of the National Museum for authentication and confirmation of the identity of the host species.

Isolation and purification of mangrove fungal endophytes (MFE)

Collected stem and roots samples were subjected to surface-sterilization using 10% sodium hypochlorite solution (5% active ingredient, commercially available bleach solution) for three minutes and washed with sterile distilled water thrice for three minutes. Surface-sterilized roots and stems were cut into small pieces approximately 5 mm in length (30 explants per substrate type per host mangrove, 6 explants per plate) and placed on Potato Dextrose Agar medium supplemented with 33 g/L marine salts (PDAS, Hi-media) and 500 mg/L streptomycin (Sigma). To confirm that the isolated fungus is an endophyte, surface-sterilized roots and stems were also tissue-printed on freshly prepared PDAS. Fungi growing on these tissue printing were excluded in the study. All plates were incubated for two weeks at room temperature. Fungal growth was examined every day for two weeks. Fungi growing out of the stem and root explants were cut out and transferred to freshly prepared PDAS using agar plug method or spore touch technique for further sub-culturing and later, for purification. The percent colonization rate as the number of explants with MFE over the total number of explants x 100 was computed for each sampling province and explant type. Subsequently, a total of 40 morpho-culturally distinct MFE isolates were selected for identification and bioassays, maintained as pure stock cultures on PDAS slants and stored at 4°C.

Characterization and identification of MFE

Initially, the 40 MFE isolates were cultured on PDAS plates and incubated at room temperature for 3-5 days. Identities of the isolates were determined following comparison of their morpho-cultural characters with published literature, specifically the Illustrated Genera of Imperfect Fungi, 4th Edition (Barnett and Hunter 1998). To confirm the identities of the MFE, molecular identification was done for selected strains, especially for those with promising bioactivities. The same method was also utilized for isolates that did not sporulate in culture. The fungal specific internal transcribed spacer (ITS) primers that amplify the entire 5.8S rDNA gene, both the ITS regions I and II, and a portion of the 18S nuclear small-subunit rDNA gene, were used. The ITS region is widely used in fungal identification and phylogenetic studies. It is also considered as the universal DNA barcode marker for fungi (Martin and Rygiewicz 2005, Schoch et al. 2012). The isolated mangrove fungi were grown on PDAS plates at room temperature for 3-5 days. Following culture, the DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Rehman et al. 2011). The internal transcribed spacer (ITS) was amplified through PCR with the fungal specific primer pair: forward ITS5 and reverse ITS4 (White et al. 1990). Purification of the PCR products was done using commercially-available kit GeneAll Biotechnology (South Korea). The purified amplicons were sequenced using corresponding PCR primers by Macrogen (Seoul, Korea) in both forward and reverse directions using an ABI Prism 3700 genetic analyzer (Life Technologies, Gaithersburg, Maryland, USA). Then, the homologous sequences of related fungal taxa were downloaded from the GenBank and aligned with the sequences of MFE using Clustal W in MEGA Ver. 5.1 software (Thompson et al. 1994, Tamura et al. 2011). The related sequences used from GenBAnk were either of type strains or from published sequences to ensure reliable fungal identification. The aligned sequences were used in the construction of phylogenetic tree using the Neighbor Joining analysis with a bootstrap of 1,000 replicates.

Production and extraction of secondary metabolites from MFE

Forty MFE (see Table 1 for the identities) were initially grown on Malt Extract Agar (MEA) slants supplemented with 33 g/L marine salts for 2 weeks at room temperature. Pre-filled sterile glass bottles containing 100 mL Malt Extract Broth (MEB) were each inoculated with the 14-day old culture. All bottles were further incubated at room temperature under stationary condition for four weeks. Following incubation, 100 ml analytical grade ethyl acetate (RCI Labscan) was added to each broth culture. The mycelial mass was macerated with a spatula to facilitate extraction of the secondary metabolites. After 24 hours, the ethyl acetate fraction was separated from the aqueous fraction and concentrated *in vacuo.* The concentrated crude culture extracts were air-dried overnight. Crude culture extracts of MFE were then dissolved in 1:1 methanol acetone to a final concentration of 10 mg/mL.

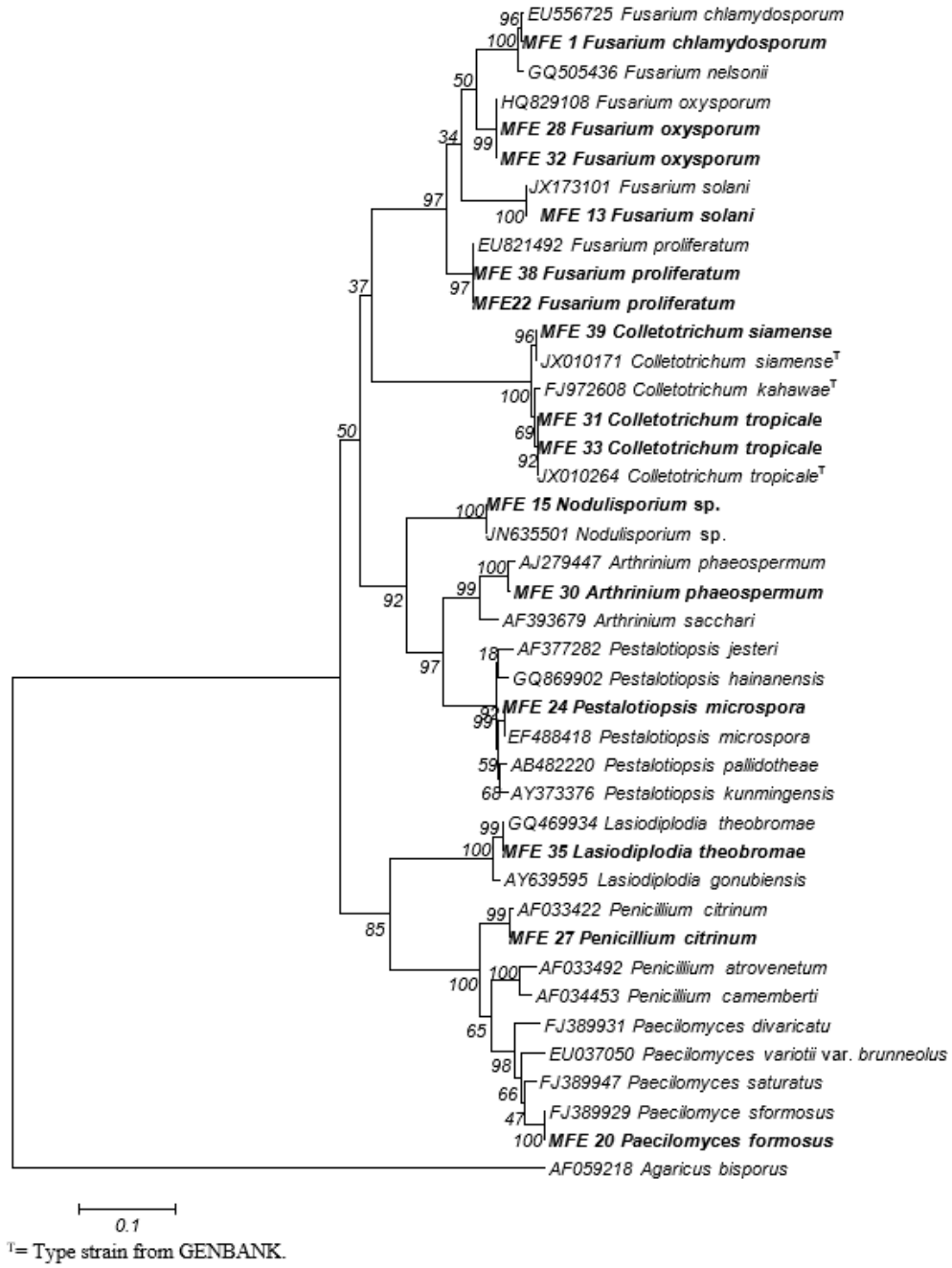
Assay for antimicrobial activities of MFE crude culture extracts

Test microorganisms. Gram-negative bacteria (*Escherichia coli* K12, *Klebsiella pneumoniae*, *Serratia marcescens*, *Vibrio harveyi*), gram-positive bacteria (*Bacillus megaterium*, *B. subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*), and yeasts (*Candida albicans, Saccharomyces cerevisiae*) were procured from the University of Santo Tomas-Collection of Microbial Strains and used as test microorganisms due to their clinical importance. Test bacteria were maintained on Nutrient Agar (NA) slants while yeast cultures were maintained on PDA slants. All test organisms were kept at 4°C.

Paper disk diffusion assay. Bacterial/yeast cell suspensions were prepared from 24-hr old cultures of test bacteria and yeasts. The inoculum concentration was adjusted to 0.5 McFarland standard, and swabbed on Mueller-Hinton Agar (MHA) plates for bacteria and Sabouraud Dextrose Agar (Merck) for yeasts. To each paper disk, 25 µL with a concentration of 250 µg of the MFE crude culture extracts was added and air-dried before placing onto the inoculated culture plates. Twenty five µL of methanol:acetone (1:1), streptomycin (250 µg), ampicillin (250 µg), and nystatin (25 µg) were used as negative and positive controls. All cultures, in triplicates, were incubated at 37°C for 24 hours with 48 hours for yeasts. Antimicrobial activities were assessed by measuring the average zones of inhibition (ZOI). Results were interpreted following the protocol of Quinto and Santos (2005) as follows: >19mm ZOI (very active), 14-19mm ZOI (active), 10-13mm ZOI (partially active, <10 mm ZOI (inactive).

Microdilution assay. Minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of the crude culture extracts exhibiting active to very active antimicrobial activities from the paper disk diffusion assay was determined using the microdilution method modified from Quinto & Santos (2005). Crude culture extracts of selected fungal endophytes were dissolved in 1:1 methanol:acetone to a final concentration of 4 mg/mL. A 2-fold dilution series in a 96-well microtiter plate was prepared with 100 µL Mueller Hinton broth (MHB) as diluent to give the following concentrations of the crude culture extracts: 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000, and 4000 µg/mL. To each well with different MFE crude culture extract concentrations (in triplicates), 100 µL of inoculum with cell density adjusted to 0.5 McFarland was finally introduced for a final volume of 200 µL. The microtiter wells

Table 2: Neighbor Joining tree based on the ITS sequences of selected species of mangrove associated fungal endophytes and other related fungal taxa. Phylogenetic tree was rooted with the basidiomycete *Agaricus bisporus*.



T= Type strain from GENBANK.

were then incubated at 37°C for 24 hours (48 hours for the yeasts). As positive controls, 100 µL with concentrations of 10 mg/mL ampicillin (Sigma), 10 mg/mL streptomycin (Sigma), and 1 mg/mL nystatin (Sigma) were used. Following incubation, all wells containing different concentrations of MFE crude culture extracts and test microorganisms were streaked on freshly prepared Nutrient Agar, for bacteria, or Potato Dextrose Agar, for yeasts, plates to determine cell viability. Inoculated culture plates were then incubated at 37°C for 24 hours (48 hours for yeasts). The least concentration of the crude culture extracts without any bacterial or yeast growth was considered as the MBC or MFC.

RESULTS AND DISCUSSION

Fungal endophytes associated with mangrove stems and roots

Out of the 1,380 stem and 720 root explants sampled, 72% and 30%, respectively, showed colonization by fungal endophytes. The colonization rate of 95% in the Camarines Sur collection sites was higher than those estimated in the Zambales and Pangasinan sites which yielded 48% and 34% rates, respectively. The high number of fungal endophytes estimated suggests that mangroves are easily colonized by fungi, particularly those in Camarines Sur where the mangroves are of a younger age, a situation that seemed to be the factor for its high colonization rate. The frequency of endophytes is also dependent on the host tissue type (Kumar and Hyde 2004) with different parts of mangroves harboring different fungal communities (Vittal and Sarma 2006). For instance, in the studies of Tariq et al. (2006), roots from Indian mangroves had

Table 2: Antimicrobial activities exhibited by the mangrove fungal endophytes against at least one of the test microorganisms.

Taxa	Gram-negative Bacteria			Gram-positive Bacteria				Yeasts		
	E. coli	*K. pneumoniae*	*S. marcescens*	*V. harveyi*	*B. subtilis*	*B. megaterium*	*M. luteus*	*S. aureus*	*C. albicans*	*S. cerevisiae*
A. ochraceus MFE 23	I	I	I	I	I	PA	I	VA	I	I
A. phaeospermum MFE 30	I	I	I	I	I	I	PA	VA	I	I
C. siamense MFE 39	I	I	I	PA	PA	I	PA	PA	I	I
C. tropicale MFE 33	PA	PA	I	A	A	A	PA	I	I	I
F. chlamydosporum MFE 1	I	I	I	I	PA	PA	I	I	I	I
F. oxysporum MFE 28	I	I	I	I	PA	PA	I	I	I	I
F. oxysporum MFE 32	PA	I	I	I	PA	A	PA	I	VA	I
F. proliferatum MFE 38	A	I	I	I	PA	VA	I	I	PA	I
Fusarium solani MFE 13	I	I	I	I	I	PA	PA	I	I	I
Fusarium sp. MFE 37	I	I	I	I	PA	I	I	I	I	I
L. theobromae MFE 35	I	I	I	I	I	I	I	I	I	VA
Lasiodiplodia sp. MFE 11	I	I	I	I	I	I	I	I	I	VA
Pestalotiopsis sp. MFE 18	I	I	I	I	I	I	PA	I	I	I
P. microspora MFE 24	I	I	I	I	I	VA	VA	VA	I	I
Phomopsis sp. MFE 21	I	I	I	I	I	I	PA	I	I	I
Trichoderma sp. MFE 2	I	I	I	I	I	PA	I	I	I	I
Trichoderma sp. MFE 14	I	I	I	I	I	I	PA	I	I	I
Xylaria sp. MFE 29	A	A	PA	A	A	VA	I	I	I	I
unidentified MFE 4	I	I	I	PA	I	PA	I	I	I	I
unidentified MFE 8	I	I	I	I	I	PA	I	I	I	I
unidentified MFE 25	I	I	I	I	PA	I	I	I	I	I
unidentified MFE 34	I	I	I	I	PA	I	I	I	I	I
Ampicillin	VA	I	VA	VA	ND	ND	ND	ND	ND	ND
Streptomycin	ND	ND	ND	ND	VA	VA	VA	VA	ND	ND
Nystatin	ND	ND	ND	ND	ND	ND	ND	ND	A	A
Methanol:Acetone	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] inactive (I) = <10mm ZOI, partially active (PA) = 10-13mm ZOI, active (A) = 14-19mm ZOI, very active (VA) = >19mm ZOI, not determined (ND)
[b] Eighteen (18) MFE did not exhibited any activity in any of the test microorganisms, and thus, were not listed in this table.

Table 3: Number of MFE exhibiting inhibitory activities against test microorganisms.

Test microorganisms	Inactive	Partially Active	Active	Very Active
Gram-negative bacteria				
Escherichia coli K12	36	2	2	0
Klebsiella pneumoniae	38	1	1	0
Serratia marcescens	39	1	0	0
Vibrio harveyi	36	2	2	0
Gram-positive bacteria				
Bacillus subtilis	30	8	2	0
Bacillus megaterium	28	7	3	2
Micrococcus luteus	31	8	0	1
Staphylococcus luteus	37	0	0	3
Yeasts				
Candida albicans	38	1	0	1
Saccharomyces cerevisiae	38	0	0	2

[a] Inactive = <10mm ZOI, Partially active = 10-13mm ZOI, Active = 14-19mm ZOI, Very Active = >19mm ZOI
[b] Activities exhibited by the control antibiotics: Ampicillin = *E. coli* (VA), *K. pneumoniae* (IA), *S. marcescens* (VA), *V. harveyi* (VA)
Streptomycin = *B. subtilis* (VA), *B. megaterium* (VA), *M. luteus* (VA), *S. aureus* (VA)

higher number of fungi compared to stems. Likewise, fungal diversity and abundance in xylem parts are lower than in the bark, shoot, and foliar parts (Stone et al. 2000). The difference can be attributed to the fact that fungal endophytes which colonize different tissue types have different substrate utilization abilities which may enable partitioning of the substrate resources by the fungal endophytes (Carroll and Petrini 1983).

Of the 40 fungal isolates from 12 mangrove host species chosen and used in the bioassays (Table 1) utilizing morphocultural characterization and ITS sequence analysis, majority of the MFE isolated belonged to the following genera: *Fusarium*, *Xylaria*, *Colletotrichum*, *Aspergillus*, *Lasiodiplodia*, *Trichoderma, Penicillium*, *Paecilomyces*, *Pestalotiopsis*, *Alternaria*, and *Phomopsis*. BLAST searching and phylogenetic analysis of their gene sequences resulted in the confirmation of the identities of 12 species of mangrove fungal endophytes (Figure 1). Bootstrapping of 1,000 replicates, used to assess clade support, resulted in high bootstrap values in the constructed phylogenetic tree indicating that the topology of MFE clustering in a particular taxon is highly probable and that the identification of the fungi was accurate. As a general rule, if 95% or higher bootstrap value is observed for a given interior branch, the topology of that branch is considered appropriate for species identification (Nei and Kumar 2000).

Species of fungal endophytes are reported to be ubiquitous (Vega et al. 2010) and can actually change their life strategies and become saprophytes after host senescence. This life strategy change might explain their cosmopolitan distribution and possible role in the environment (Rodriguez and Redman 2005). Their ubiquitous distribution and highly adaptive life strategies could explain why similar fungal genera in the present study were observed in different sites and mangrove parts (Table 1). The fungal genera *Colletotrichum, Fusarium, Guinardia, Phomopsis*, and *Nodulisporium* reported in this study have also been isolated from leaves, roots, and stems of mangroves (Xing et al. 2010, Gilna and Khaleel 2011, Wanderley Costa et al. 2012). The host mangrove species *A. marina, B. gymnorrhiza, E. agallocha, S. alba,* and *L. racemosa* reported in these previous studies were the same species found in the sites of the present study. Notably, some genera were also present in leaves and stems of the wild rubber tree, *Hevea brasiliensis* (Gazis and Chaverri 2010) and of tropical halophytes *Acanthus ilicifolius*, *Arthrocnemum indicum*, *Suaeda maritima*, and *Sesuvium portulacastrum* (Kumaresan and Suryanaranayan 2001). Similar fungal endophytes from *Musa* spp. and *Canarium ovatum* were also reported by Dagamac et al. (2008) and Torres and dela Cruz (2015). The similarity of the collected fungal endophytes with those from other plants indicates that these endophytic fungi are not host-specific. A more detailed study on the physiological products of different host plants and how these stimulate growth and/or colonization by fungal endophytes may also explain this observation and should be a topic for future research.

Antimicrobial activities of mangrove-associated fungal endophytes

Fungal endophytes from terrestrial plants, including mangroves, have previously been recognized as potential sources of novel, natural bioactive compounds that can be exploited in the field of medicine and agriculture (Strobel and Daisy 2003, Kumar and Sagar 2007, Bungihan et al. 2011, 2013a, 2013b), and of enzymes with industrial and environmental applications (Torres and dela Cruz, 2012). In the present study, four MFEs: *F. oxysporum* (MFE 32), *F. proliferatum* (MFE 38), *Lasiodiplodia* sp. (MFE 11), and *L. theobromae* (MFE 35), showed activities against yeasts while six MFEs: *Xylaria* sp. (MFE 29), *F. oxysporum* (MFE 32), *C. tropicale* (MFE 33), *F. proliferatum* (MFE 38), *C. siamense* (MFE 39), and an unidentified fungal endophyte (MFE 4), inhibited gram-negative bacteria (Table 2). Antimicrobial activities were more directed against gram-positive bacteria than to the other two groups of microorganisms indicating higher degree of susceptibility to the bioactive metabolites produced by the MFEs (Table 3). Differences in cell wall composition may also explain this group-directed activity but this requires further testing of their mechanism/s of action.

The crude extracts from *Pestalotiopsis microspora* (MFE 24) showed very active results against *Bacillus megaterium, Micrococcus luteus,* and *Staphylococcus aureus* with minimum bactericidal concentrations (MBC) of 250 µg/mL, 62.5 µg/mL, and 1,000 µg/mL, respectively. *Xylaria* sp. (MFE 29) was also very active against *Bacillus megaterium* while *Aspergillus ochraceus* (MFE 23) and *Arthrinium phaeospermum* (MFE 30, 500 µg/mL MBC) were highly inhibitory against *Staphyloccus aureus* (Table 2). The highly effective inhibitory effect of the crude culture extract of *P. microspora* shown in this study confirms the results of the studies of Strobel et al. (2002) and Xu et al. (2010). Crude and purified extracts of *Pestalotiopsis* spp. have been previously shown to exhibit a wide spectrum antimicrobial activity (Subban et al. 2013, Sharma et al. 2016). Against yeasts, only three crude extracts showed very active results. *Fusarium oxysporum* (MFE 38) showed activities against *Candida albicans* with MFC value of 125 µg/mL while two culture extracts belonging to the same genus, *Lasiodiplodia* sp. (MFE 11, <7.8 µg/mL MFC) and *Lasiodiplodia theobromae* (MFE 35, <7.8 µg/mL MFC), yielded very active results against *Saccharomyces cerevisiae.* Only few proved highly inhibitory to the eight test microorganisms but a number of crude culture extracts showed partial to active results. It is suggested that these fungal crude culture extracts be further tested against other test microorganisms not included in this study as the fungal endophytes they were extracted from are known to synthesize bioactive compounds, e.g. *Colletotrichum* (colletotric acid), *Phomopsis* (dicerandrols), and *Fusarium* (fusaristatins) as reported by Zou et al. (2000), Wagenaar and Clardy (2001), and Shiono et al. (2007). These bioactive compounds are part of the defense or resistance mechanism against the invasion of pathogens (Tan and Zou 2001; Joseph and Priya 2011). These secondary metabolites are synthesized via various metabolic pathways (Tkacz 2000). It is noteworthy that majority of the reported fungal endophytes in this study have earlier been reported to produce antimicrobial secondary metabolites (Schulz et al. 2002, Bungihan et al. 2013a). For instance, *Aspergillus ochraceus* was reported to synthesize aspinonene and dihydroaspyrone metabolites (Rateb and Ebel 2011) while *Pestalotiopsis microspora,* a common species present in tropical and subtropical plants, had been widely isolated as an endophyte with several bioactivities (Metz et al. 2000, Strobel et al. 2002). It is of significance that this species was reported as a producer of taxol, an anticancer compound (Strobel et al. 1996). Clearly, the screening of fungal endophytes for antimicrobial compounds particularly those associated with many unique host plants such as mangroves, is a very promising approach in the search for ways to counter the increasing threat of emerging and re-emerging infectious diseases and drug-resistant bacterial or fungal pathogens.

In conclusion, the results showed that Philippine mangroves, including rare mangrove species such as *C. philipinense,* harbored endophytic fungi. Many of these fungal endophytes have also been isolated reported from other tree hosts indicating that they are not host-specific. The crude culture extracts of these MFE exhibited antimicrobial activities against bacteria and yeasts. Among the MFE, the crude culture extracts of *Pestalotiopsis microspora* showed very active inhibitory activities against gram-positive bacteria. *Fusarium oxysporum* and *Lasiodiplodia theobromae* have also been reported as highly inhibitory against yeasts. The present study showed the potential of locally- isolated fungal endophytes as new sources of secondary metabolites that can be further exploited in the search for bactericidal or fungicidal compounds. An investigation on endophytic fungi associated with other mangrove hosts or other plants endemic to the Philippines is hereby recommended.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Llewelyn S. Moron and Thomas Edison E. dela Cruz did the sampling, planning, experimentation, data analysis, and manuscript writing. Young Woon Lim assisted in the molecular identification of the fungal isolates and in writing the manuscript.

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