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Diversity and biofilm inhibition activities of algicolous fungi collected from two remote islands of the Philippine archipelago

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Abstract

Algicolous fungi are valued for their pharmaceutically and agrochemically useful secondary metabolites. However, very few studies on algicolous fungi have been carried out in the Asia Pacific region, particularly in the Philippines, in spite of the country's rich macroalgal flora. In this study, a total of 212 algicolous fungi belonging to 29 morphospecies were recorded from seaweeds (macroalgae) collected from Potipot Island and Lubang Island, northern Philippines. These fungi were identified as species of *Aspergillus*, *Alternaria*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Nigrospora*, *Pestalotia*, *Penicillium*, and *Trichoderma*. Diversity assessment among collection sites and among algal groups was determined. Lubang Island registered higher species richness than Potipot Island, while brown algae had the highest compared to red and green algae. Species diversity measured by Shannon Index and Simpson Index showed no significant difference between the two study sites, while brown algae had the highest species diversity among the algal groups. Comparison of communities shows that the morphospecies clustered more based on the site they were collected, and not based on their algal host. Communities have more similarities between those isolated from the smaller island of Potipot than in Lubang, which may show that proximity and anthropogenic activity might affect the distribution of fungal communities. Extracts of the AF isolates were used against biofilm-forming *S. aureus* to determine whether AF can inhibit biofilm formation. The results of the assay showed that algicolous fungi extracts can successfully reduce biofilm formation as much as ≥99%.

Key words – algicolous fungi – biofilm – biodiversity – macroalgae – seaweeds

Introduction

Fungi from marine habitats are valuable sources of biologically active secondary metabolites with potential pharmaceutical importance (Schulz et al. 2008). Reports from 2013 to 2014 showed a total of 541 new compounds isolated from fungi in marine habitats (Blunt et al. 2016). Biological activities of these compounds include antihelminthic, antibacterial, anticoagulant, antifungal, anti–inflammatory, antimalarial, antiplatelet, antituberculosis and antiviral activities (Mayer et al. 2007, Blunt et al. 2016). One particular group of marine fungi that can be tapped for production of secondary metabolites is the macroalgae–inhabiting fungi, also known as algicolous fungi (AF). According to the study made by Bugni and Ireland in 2004, 27% of the novel marine–derived

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compounds were isolated from these fungi. Some produce pharmaceutically significant metabolites such as fungistatic macrodiolides and sesquiterpenes, cytotoxic dimeric diketopiperazines and lactones and radical-scavenging epoxycylohexenone. In the Asia Pacific, especially the Philippines, current studies about marine fungi are mostly focused on substrates other than macroalgae, i.e. mangroves, marine sediments, seagrass and seawater (Yao et al. 2009, Besitulo et al. 2010, Ramirez et al. 2010, Torres et al. 2011, Jones et al. 2013, Torres & dela Cruz 2013, Su et al. 2014, Notarte et al. 2017), although an earlier study reported the potential role of marine fungi in ice-ice disease formation among cultivated seaweeds (Solis et al. 2010). Additional research focusing solely on algicolous fungi in the Philippines is highly advantageous for the discovery of novel metabolites since both the area and the substrate has not yet been fully explored.

The Philippine archipelago is part of the biodiversity hotspot, the Indo–Australian Archipelago (IAA). The IAA is the largest and the epicenter of marine biodiversity, having a richness of ~250 to 300 genera of benthic macroalgae (Kerswell 2006, Lohman et al. 2011, Cowman & Bellwood 2013). The Philippines itself is blessed with more than 820 species of macroalgae (Trono Jr. 1999). Although there are a lot of probable macroalgal hosts in the country, only a small number of local studies have been done. The diversity of algicolous fungi in the Philippines is yet to be determined, and since underexplored, the country is a good candidate for the isolation and study of less exploited but biologically active microorganisms with an array of novel metabolites.

The discovery of novel metabolites is of high importance due to the continuous emergence of antimicrobial resistance among clinically relevant pathogens. Due to several mechanisms of the bacterial cells, they are able to withstand antibacterial agents rendering them hard to eradicate and treatments become unsuccessful. One of the known mechanisms is the formation of biofilms. Biofilm-embedded microorganisms are more capable of surviving harsh conditions than their planktonic counterparts, making them more difficult to eradicate. The extracellular polymeric substances (EPS) in biofilms aid in collecting nutrients from the environment, and protect the cells against desiccation, ultraviolet radiation, and even several protozoan gazers (Flemming & Wingender 2010). The structure of biofilms can also affect the transport of antimicrobial agents via several mechanisms, such as decreasing the amount of antibiotics that penetrates the biofilms, and increasing the activity of multi–drug efflux pumps (Fux et al. 2005, Leid 2009). The host's immune response is also impaired by affecting its normal function such as a decrease in the ability of leukocytes to engulf biofilm–embedded bacteria, and suppression of leukocyte effector function (Leid 2009). One of the leading causes of biofilm–associated infections is the bacterium *Staphylococcus aureus*. Its ability to colonize the human skin and mucous surfaces provides easy access of the bacteria to infect medical devices (Otto 2004). Due to the increase in prevalence of biofilm-related infections related to Staphylococci, different strategies and methods have been proposed to control biofilms, which include directly using topical antimicrobial ointments, using antibiotics for their control, coating the catheter lumen with bactericidal or bacteriostatic substances, and utilizing matrix–targeting enzymes (Smith 2005, Chen et al. 2013). Sometimes the only way to treat the infection is to remove the implant, resulting to more trauma to the patient and a higher cost of treatment (Mah & O'toole 2001). Kwon et al. (2008) showed a worse scenario wherein Methicillin-resistant *S. aureus* (MRSA) and other multi-drug resistant *S. aureus* form biofilms on medical devices. Since antibiotics appear to have lesser effect against these biofilms, new compounds that may exhibit inhibitory activities are of high value. Thus, this research study aims to answer the following questions: (1) How diverse are the algicolous fungal communities collected between different remote islands and among different macroalgal types? and (2) Is there any potential antibiofilm activities these algicolous fungi can exhibit?

Materials & Methods

Collection Sites: Potipot and Lubang Islands

Seaweed samples were collected during summer season from Potipot Island and Lubang Island. Potipot Island (15°40'39"N, 119°55'19"E) is located in the town of Candelaria, Zambales Province in Northwestern Luzon, Philippines. The entire island has an area of approximately 500 x 200 m^2. The majority of the island's flora consists of *Cocos nucifera*. The coastal area is populated mainly by seaweeds specifically *Sargassum* spp. Potipot Island is a popular tourist spot famous for its white shores and blue waters. Lubang Island (13°47'N, 120°12'E) is located northwest of the main island of Mindoro in Southwestern Luzon, Philippines. The island has a land area of about 25,000 × 10,000 m^2. The fishing industry is the main economic activity in the island.

A total of ten species of seaweeds were collected from the two study sites, six from Potipot Island and four from Lubang Island. All freshly collected seaweeds were initially placed in clean Ziplock bags with approximately 1 liter (L) of seawater. These were kept inside a cooler and were brought to the Microbiology Laboratory of the Research Centre for the Applied and Natural Sciences (RCNAS) of the University of Santo Tomas within 24 h of collection and were processed immediately. The seaweed thalli were washed with running water to remove any adhering soil particles and epiphytic algae. Collected algal samples were identified based on their gross thallus morphology.

Isolation and Identification of Algicolous Fungi

Initially, the algal thalli were surface sterilized by dipping into 70% ethanol for 3 s, followed by rinsing with sterile artificial seawater. Tissue imprints on Potato Carrot Agar (PCA) plates were done to ensure effectiveness of surface sterilization. Control plates were exposed under the hood to confirm that there was no fungal contamination. The thalli were aseptically cut into pieces (10 mm), and plated on Potato Carrot Agar with 33 g/L marine salt (PCAS). The plates were then incubated at room temperature for 14 days. Fungal hyphae growing out of the algal explants were further isolated until pure colonies were obtained. A total of 212 algicolous fungal (AF) strains were then inoculated on Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) for morphocultural characterization. Identification at least up to genus level was done based on their spore morphology, hyphal characteristics and colonial description. Codenames were used for those isolates that did not sporulate, and designated as *mycelia sterilia*.

Diversity Assessment of the Algicolous Fungi

Fungal growth from each algal explant was recorded and observed daily for 14 days. Each growth from an explant was counted as one fungal strain, and strains with similar characteristics (spore, hyphae, and colony morphology) were considered to be one morphospecies. Here, a morphospecies is defined as a morphoculturally distinct fungal isolate that failed to sporulate in culture. The use of this taxonomic concept is well supported by the study of Lacap et al. (2003) which showed that similar morphotypes clustered together and are distinctly separated from each other based on comparison of gene sequences. Thus, these records were taken into account to assess the diversity of the AF. Species accumulation curves were initially constructed according to the rarefaction formula using the program EstimateS (Version 9.1) (Colwell 2016, 100 randomizations), which computes also a number of estimators of species richness. The computation of several α-diversity measurements, its corresponding statistical tests and the comparisons of abundance data using Kruskal Wallis test between the two collecting localities and among the three algal types were all employed using the software PAST Version 3.1 (Hammer et al. 2001). Moreover, to visualize the patterns of species composition, a clustering analysis and a non-metric multidimensional scaling ordination was employed in the same software using the paired group algorithm of the Bray Curtis similarity measurement.

Assay for Inhibition of Biofilm Formation of Staphylococcus aureus

Production and extraction of secondary metabolites was done by initially inoculating the 29 AF morphospecies to culture bottles containing 150 mL of Potato Dextrose Broth with 0.8% Plant Tissue Culture Agar. These bottles were incubated at room temperature under ambient light

conditions. After 4 weeks of incubation, the mycelial growth and the culture media were macerated, to which 150 ml ethyl acetate was added and soaked overnight. The culture filtrates were then concentrated *in vacuo*, air-dried and stored in the refrigerator until used in the assay. Prior to the bioassays, the crude culture extracts were first diluted with 1:1 methanol–acetone to a final concentration of 500 mg/ml.

Inhibition of biofilm formation was then conducted with the crude culture extracts following the methods of Polonio et al. (2001) and Peeters et al. (2008). Biofilm–forming strain of *S. aureus* ATCC 25923 was initially inoculated onto Tryptic Soy Broth (TSB) and incubated at 37°C for 24 h. The suspension was adjusted to 0.5 McFarland standard prior to inoculation and 100 µl was transferred to each well of the 96–well microtiter plates. The inoculated microtiter plates were then incubated at 37°C for 4 h to allow bacterial cells to adhere to the wells. After incubation, the TSB in each well was removed by pipetting, and the wells were rinsed with 100 µl phosphate buffered saline (PBS). Freshly prepared TSB was supplied to each well (100 µL) after rinsing. Crude culture extracts (50 µl) of the algicolous fungi were added to three wells to perform triplicates, and the set up was further incubated at 37°C for 48 h. The culture media were then removed by decantation and the wells were rinsed with 100 µl PBS. One hundred microliters of 0.1% aqueous crystal violet solution was added to each well for fifteen minutes, and then the stain was decanted. The microtiter plates were then air-dried and 200 µl of 30% glacial acetic acid were added to each well, solubilizing the crystal violet in the wells. After 10 min, the glacial acetic acid-crystal violet solution was transferred to new microtiter plates and the optical density of each well were read at a wavelength of 630 nm (OD_{630}) using a microplate reader. The percent biofilm inhibition of the crude extracts was computed as $100 - [(OD_{630}$ of treated well/OD_{630} of reference well) × 100]. The reference wells were performed in the same manner with the exception of the addition of crude extracts. Growth controls are wells with TSB + solvents inoculated with *S. aureus*, and wells with TSB only.

Results

Isolated Algicolous Fungi

Based on morphology, seaweed samples collected in Potipot Island were identified as *Codium tenue*, *Halicoryne wrightii*, *Hydroclathrus clathratus*, *Hypnea vernicornis*, *Padina japonica*, and *Sargassum ilicifolium*, while seaweed samples in Lubang Island were identified as *Gracillaria arcuata*, *Padina australis*, *Sargassum polycystum*, and *Ulva reticulata*. From these, a total of 212 algicolous fungi (AF) belonging to 29 morphospecies were recorded. One hundred thirty two (62%) AF isolates belonging to 17 morphospecies were isolated from seaweeds of Potipot Island, while 80 AF (38%) isolates belonging to 18 morphospecies were isolated from seaweeds of Lubang Island (Table 1, Fig. 1A). Twelve AF morphospecies were found exclusively in Lubang Island while 11 AF were found only in Potipot Island. It should be noted that tissue imprint plates and air control plates did not present any growth of bacteria nor fungi.

Among the seaweeds collected, 47 (22%) of the total AF isolates were obtained from three species of green algae, 30 AF isolates (14%) from two species of red algae, and 135 AF isolates (64%) from five species of brown algae (Table 2). Higher number of morphospecies were also noted in brown algae (n=23) followed by green algae (n=13) and then, in red algae (n=12).

Within the green algal hosts, C. tenue harbored 16 AF isolates (8%) belonging to six morphospecies. *H. wrightii* harbored 27 AF isolates (13%) belonging to six morphospecies, while *U. reticulata* harbored four AF isolates only (2%) belonging to two morphospecies. Of the 13 morphospecies isolated from these three green algae, one morphospecies grew exclusively on *C. tenue*, similarly with *H. wrightii*. Within the red algal hosts, 14 AF isolates (7%) belonging to five morphospecies were obtained from *H. cervicornis*, while 16 (8%) AF belonging to eight morphospecies was isolated from *G. arcuata*. Among these eight morphospecies, three were exclusively isolated from this red alga. The brown alga *S. polycystum* had the most number of AF isolates (38 or 18%). Three morphospecies was found exclusively on this seaweed host. *S.*

ilicifolium had 25 AF isolates (12%). Three of the six morphospecies were only isolated from the said algal host. *H. clathratus*, on the other hand, harbored 26 AF isolates (12%) belonging to eight morphospecies. Twenty two AF isolates (10%) belonging to eight morphospecies and 24 AF (11%) isolates belonging to six morphospecies were isolated from *P. australis* and *P. japonica*, respectively. Four species was found exclusively in *P. australis*, while no species was found exclusively in *P. japonica* (Table 2, Fig. 1B).

Table 1 Fungal morphospecies isolated from algal samples collected in Potipot Island, Zambales and Lubang Island, Occidental Mindoro.

Taxon	Isolate Codes	No. of AF Isolates		
		Potipot Island	Lubang Island	Total no of isolates
Mycelia sterilia	AF01	1	0	1
Mycelia sterilia	AF02	1	0	1
Mycelia sterilia	AF04	2	0	2
Mycelia sterilia	AF10	8	0	8
Mycelia sterilia	AF11	8	0	8
Mycelia sterilia	AF12	1	0	1
Mycelia sterilia	AF17	1	0	1
Mycelia sterilia	AF21	0	3	3
Mycelia sterilia	AF28	0	4	4
Alternaria sp.	AF25	0	10	10
Aspergillus flavus	AF08	15	5	20
Aspergillus niger	AF06	6	0	6
Aspergillus sp. 1	AF05	7	3	10
Aspergillus sp. 2	AF13	12	6	18
Aspergillus sp. *3*	AF18	0	3	3
Aspergillus sp. 4	AF23	0	3	3
Aspergillus sp. 5	AF26	0	4	4
Aspergillus sp. 6	AF27	0	1	1
Aspergillus terreus	AF15	1	17	18
Chaetomium sp.	AF16	2	1	3
Cladosporium sp. 1	AF09	23	0	23
Cladosporium sp. 2	AF14	17	0	17
Colletotrichum sp.	AF29	0	2	2
Nigrospora sp.	AF03	7	0	7
Penicillium sp. 1	AF07	20	8	28
Penicillium sp. 2	AF19	0	1	1
Pestalotia sp.	AF20	0	2	2
Trichoderma sp. 1	AF22	0	4	4
Trichoderma sp. 2	AF24	0	3	3
	Total	**132**	**80**	**212**

Morphocultural characterization identified the 29 AF morphospecies as belonging to nine genera: *Aspergillus*, *Alternari*a, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Nigrospora*,

Pestalotia, *Penicillium*, and *Trichoderma*. AF codes were used for the nine AF isolates that did not sporulate (*mycelia sterilia*).

Table 2 Number of the algicolous fungi isolated from different algal hosts.

AF Code	Taxon	No. of Isolated AF Isolates										
		Brown Algae					Red Algae		Green Algae			
		H. clathratus	*S. japonica*	*A. australis*	*S. ilicifolium*	*S. polycystum*	*H. cervicornis*	*G. arcuata*	*U. reticulata*	*C. tenue*	*A. wrightii*	Total no. of isolates
AF01	*Mycelia sterilia*	0	0	0	1	0	0	0	0	0	0	1
AF02	*Mycelia sterilia*	0	0	0	1	0	0	0	0	0	0	1
AF04	*Mycelia sterilia*	0	0	0	2	0	0	0	0	0	0	2
AF10	*Mycelia sterilia*	1	0	0	4	0	1	0	0	2	0	8
AF11	*Mycelia sterilia*	3	0	0	0	0	0	0	0	5	0	8
AF12	*Mycelia sterilia*	0	0	0	0	0	0	0	0	1	0	1
AF17	*Mycelia sterilia*	0	0	0	0	0	0	0	0	0	1	1
AF21	*Mycelia sterilia*	0	0	0	0	3	0	0	0	0	0	3
AF28	*Mycelia sterilia*	0	0	4	0	0	0	0	0	0	0	4
AF25	*Alternaria* sp.	0	0	10	0	0	0	0	0	0	0	10
AF08	*Aspergillus flavus*	1	6	0	0	5	2	0	0	0	6	20
AF06	*Aspergillus niger*	5	0	0	0	0	1	0	0	0	0	6
AF05	*Aspergillus* sp.1	3	0	1	0	0	0	2	0	1	3	10
AF13	*Aspergillus* sp.2	0	11	0	0	3	0	3	0	0	1	18
AF18	*Aspergillus* sp.3	0	0	0	0	0	0	3	0	0	0	3
AF23	*Aspergillus* sp.4	0	0	0	0	3	0	0	0	0	0	3
AF26	*Aspergillus* sp.5	0	0	1	0	0	0	0	3	0	0	4
AF27	*Aspergillus* sp.6	0	0	1	0	0	0	0	0	0	0	1
AF15	*Aspergillus terreus*	0	1	1	0	14	0	2	0	0	0	18
AF16	*Chaetomium* sp.	0	1	0	0	0	0	1	0	0	1	3
AF09	*Cladosporium* sp.1	5	2	0	13	0	0	0	0	3	0	23
AF14	*Cladosporium* sp.2	0	0	0	0	0	2	0	0	0	15	17
AF29	*Colletotrichum* sp.	0	0	2	0	0	0	0	0	0	0	2
AF03	*Nigrospora* sp.	0	3	0	4	0	0	0	)	0	0	7
AF07	*Penicillium* sp.1	8	0	0	0	6	8	2	0	4	0	28
AF19	*Penicillium* sp.2	0	0	0	0	0	0	1	0	0	0	1
AF20	*Pestalotia* sp.	0	0	0	0	0	0	2	0	0	0	2
AF22	*Trichoderma* sp.1	0	0	0	0	4	0	0	0	0	0	4
AF24	*Trichoderma* sp.2	0	0	2	0	0	0	0	1	0	0	3
		26	24	22	25	38	14	16	4	16	27	212

Diversity Assessment of Algicolous Fungi

For the two study sites, 132 records from 17 morphospecies were observed in the smaller island of Potipot, while 80 records from 18 morphospecies were found in the bigger island of

Lubang (Fig. 2A). The accumulation curves for the two islands did not show an expected asymptotic accumulation curve. Nevertheless, the richness of species showed higher in Lubang than Potipot (rarefied species numbers: 18.0 vs. 14.7). In terms of the three algal types, the brown algae had the highest number of morphospecies (23 morphospecies, 135 records), and the accumulation curves ascertain that it is also the most species-rich (Fig. 2B).

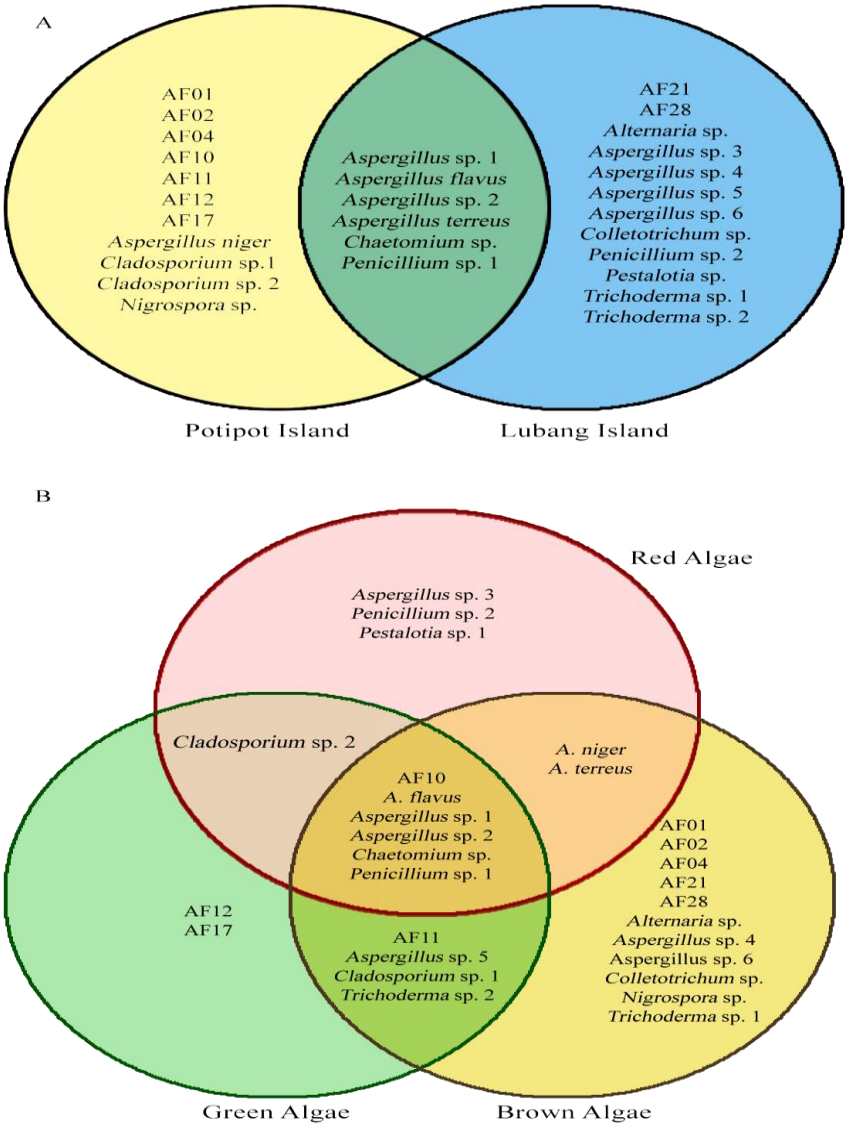

Fig. 1 – Venn diagram illustrating the common and unique morphospecies of AF in (A) Potipot Island and Lubang Island and (B) per taxonomic groups of the algae.

α diversity were measured using two heterogeneity indices that considered both richness and evenness, namely, (1) the Shannon (SHA) index and (2) the Simpson (SIM) index. Higher SHA and SIM values were observed from Lubang (SHA = 2.138; SIM = 0.736) than in Potipot (SHA = 2.110; SIM = 0.735), but the diversity t–test showed no significant difference for both Shannon ($p = 0.937$, $\alpha = 0.05$) and Simpson ($p = 0.994$, $\alpha = 0.05$) index. Moreover, the non–parametric Kruskal Wallis test for the abundance records between the two sites also did not register a significant difference (H (chi2) = 0.049, $p>\alpha = 0.05$). In terms of the diversity among algal types, the indices show that the highest values were observed from the brown algae (SHA = 2.261; SIM = 0.739). The non-parametric Kruskal Wallis test for the abundance records showed significant difference (H (chi2) = 6.886, $p<\alpha = 0.05$). A pairwise Mann-Whitney test affirms a significant difference in abundance between brown and red ($p = 0.006$, $\alpha = 0.05$) and brown and green ($p = 0.012$, $\alpha = 0.05$).

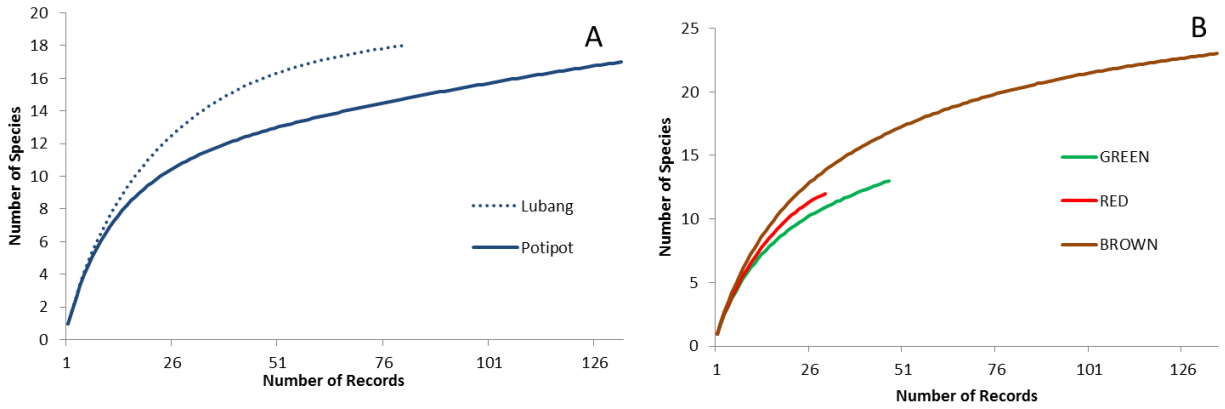

Fig. 2 – Species accumulation curves (e.g. species richness) generated from Estimates between the two remote islands (A) and the three different algal types (B).

In terms of β diversity, communities of algicolous fungal morphospecies clustered more together based on the collection sites and not based on algal types as shown in Fig 3. In comparison, the communities from the three different algal types in Lubang are more distant than the three algal types in Potipot.

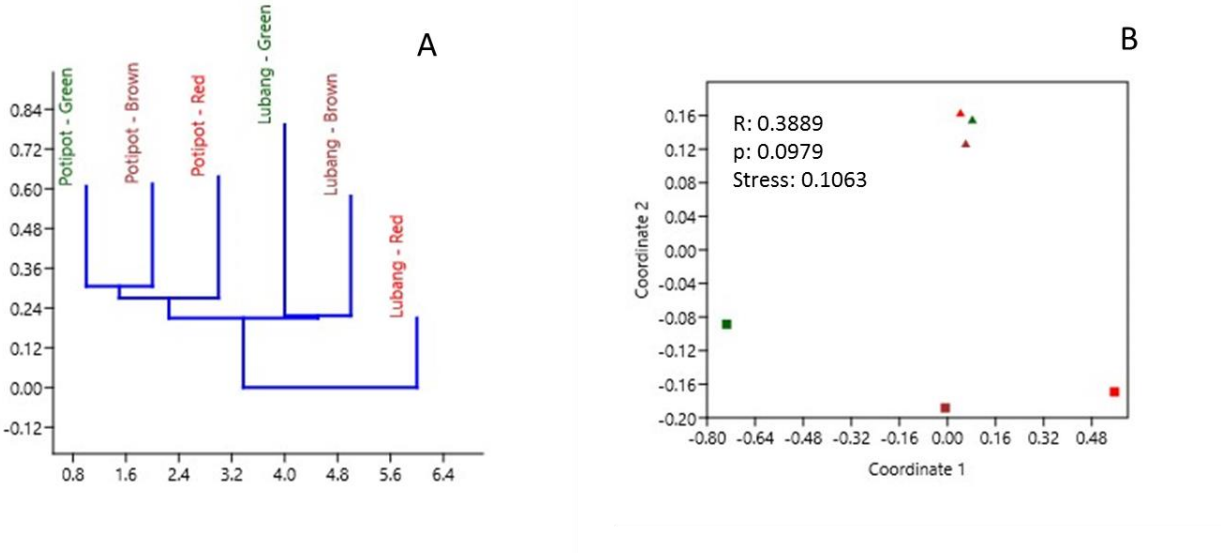

Fig 3 – β diversity using the Bray Curtis similarity measurement between collecting sites and algal types that show the clustering method (A) and the NMDS or nonmetric multidimensional scaling ordination (B). Triangles in the NMDS ordination represent Potipot while the squares represent Lubang. The color of each shape represents the specific algal type. *Biofilm Assay.*

The results of the assay showed that most of the crude culture extracts exhibited biofilm inhibitory activities (Table 3). Five of the AF crude culture extracts exhibited >90% biofilm inhibition against *S. aureus*. AF 17 (*mycelia sterilia*) inhibited 99.76% biofilm formation, followed by *Aspergillus* sp. 5 with 97.67% inhibition, *Aspergillus* sp. 3 with 96.97% inhibition, *Trichoderma* sp. 1 with inhibition 96.63%, and *Aspergillus* sp. 6 with 94.18% inhibition. Thirteen other AF isolates showed positive for biofilm inhibitory properties, and 11 isolates did not show antibiofilm properties against *S. aureus*.

Discussion

Species diversity show clearer trend when we compare the fungal morphospecies based on their algal types. As observed, brown macroalgae harbored the highest record and species count,

Table 3 Percent inhibition of biofilm formation in *S. aureus* by the crude culture extracts of algicolous fungi.

Codes	Taxon	Inhibition of biofilm production (Mean %)
AF17	*Mycelia sterilia*	99.76
AF26	*Aspergillus* sp.5	97.67
AF18	*Aspergillus* sp.3	96.97
AF22	*Trichoderma* sp.1	96.63
AF27	*Aspergillus* sp. 6	94.18
AF19	*Penicillium* sp.2	88.15
AF23	*Aspergillus* sp.4	87.47
AF21	*Mycelia sterilia*	82.79
AF10	*Mycelia sterilia*	78.08
AF04	*Mycelia sterilia*	71.00
AF28	*Mycelia sterilia*	67.33
AF07	*Penicillium* sp.1	63.33
AF20	*Pestalotia* sp.	62.26
AF01	*Mycelia sterilia*	61.67
AF15	*Aspergillus terreus*	41.33
AF06	*Aspergillus niger*	38.33
AF09	*Cladosporium* sp.1	36.79
AF05	*Aspergillus* sp.1	11.00

while green macroalgae had the lowest. A screening study of Suryanarayanan et al. (2010) suggests a similar trend. In their study from Tamilnadu coast in India, 281 AF isolates belonging to 72 species were studied, and the species diversity showed that brown macroalgae supported the highest diversity and green had the lowest diversity of marine fungi. On the other hand, the study of fungal isolates from the macroalgae of Shetland Islands, UK (Flewelling et al. 2013) showed a dissimilar trend, wherein the green algae had the highest diversity. Unfortunately, there is no consensus yet on the diversity of AF in terms of algal groups since the number of studies on this particular topic is still very limited. Therefore, more research should focus to establish a trend and to further understand the underlying factors that affect the diversity of algicolous fungi among algal groups.

The highest number of isolates among the seaweed species was obtained from *S. polycystum*. This showed that more than one fungal strain can be isolated from explants of this brown alga. On the other hand, the lowest number of isolates was observed from *U. reticulata*, which indicated that lesser number of fungi can be isolated to this alga as compared to the other algal hosts. Thus, the isolation for the ten host algae were as follows: *S. polycystum* > *H. wrightii* > *H. clathratus* > *S. illicifolium* > *P. japonica* > *P. australis* > *G. arcuata* = *C. tenue* > *H. cervicornis* > *U. reticulata*. It is interesting to note here that species of green algae showed the lower isolation rates as the other species. Other studies indicated that *U. reticulata* has antifungal activities, which may explain why this macroalgae has the lowest number of isolates (Kolanjinathan 2011, Omar et al. 2012). Dobretsov & Qian (2002) deliberated why *U. reticulata* from the Hong Kong waters often remained free from biofouling. The results of their study showed that the green alga indeed has

antifouling properties made possible by compounds from *U. reticulata*, as well as the epibiotic bacteria, *Vibrio* sp., living on its algal surface. These inhibited larval attachment and metamorphosis on the surface of *U. reticulata*, thus, preventing biofouling. Further investigation is encouraged to determine whether the same phenomenon affects the attachment of fungal spores or the thriving of endophytes and epiphytes on the thalli of *U. reticulata*.

Size of land mass may also contribute in shaping the distribution difference in the community structure of the algicolous fungi. It was observed (Fig. 3) that the smaller island showed more similarity in terms of community composition than the bigger island. The smaller island of Potipot is highly exposed to tourist attraction and is prone to anthropogenic disturbance. The island is a popular tourist spot where boats often dock on one side of the island. Two-thirds of the island, including the dock, also served as a swimming area, and one thirds served as a conserved area where most marine organisms, e.g. seaweeds, were allowed to grow. The conserved area is small, and the space between seaweed species is minimal. Thus, fungal spores disperse and are expected to land on seaweeds of closer proximity. In addition to this, the anthropogenic activity, such as the boats coming to and from the island, and the tourists strolling on the coastal area, may play as spore vectors that help distribute different fungal communities. This supposition is affirmed by several studies that relates human impact with various spore producing organisms, i.e. fungi (Tsui et al. 1998, Zhang et al. 2016), myxomycetes (Dagamac et al. 2017, Macabago et al. 2017) and bryophytes (Jägerbrand & Alatalo 2015). Nonetheless, investigations of other environmental factors that could influence the distribution and diversity patterns of algicolous fungi in the Asia Pacific region merits further research focus.

In this study, several AF extracts showed higher percentage of biolfilm inhibition than those of the antibiotics. Moxifloxacin, a broad-spectrum fourth generation quinolone, inhibited only 70% of biofilm formation of *S. aureus*, and Daptomycin, a broad spectrum lipopetide showed 86% biofilm inhibition (Roveta et al. 2007, Roveta et al. 2008), while seven of the AF crude extracts showed 87%. This shows that antibiotic treatment of biofilms does not guarantee a hundred percent successful approach to biofilm inhibition. This is due to the fact that bacteria in a biofilm state greatly affect the mechanisms which antibiotics work. It is therefore more effective if the treatment includes compounds that directly target the formation of biofilm. One approach to do this is to study the organism's communication network. Quorum sensing (QS) is the process of bacterial cell to cell communication associated with the ability of certain bacterial populations to execute synchronized activities such as biofilm–formation. QS in *S. aureus* allows bacterial cells to signal when they should enter a biofilm state, and when they should trade it off for production of other virulence factors. This renders QS a good target for the inhibition of biofilm formation (Otto 2004, Rutherford & Bassler 2012). A study proves that marine fungi produce metabolites that have the capacity to interfere in bacterial quorum sensing (Martín–Rodríguez et al. 2014). Several compounds present in the extracts mentioned in the study are beauvericin, emericellamide A, and linoleic acid. These compounds were previously isolated from marine-derived *Aspergillus* spp. (Chiang et al. 2008, Deng et al. 2013, Martín–Rodríguez et al. 2014).

Most of the isolates with >90% antibiofilm activity are *Aspergillus* spp. These fungal species are known to produce a number of bioactive compounds such as nucleases and proteolytic enzymes. These types of compounds have proven effects against biofilms (Blackledge et al. 2013). A number of diketopiperazine compounds were also derived from *Aspergillus* spp., and these compounds were reported to have a wide array of biological activities including antiviral, antibacterial, antifungal and antibiofilm activities. Diketopiperazines influence the quorum-sensing systems of bacteria, and therefore has an effect in the biofilm formation (P de Carvalho & Abraham 2012). Although there was no mention of an *Aspergillus*–derived diketopiparizene against *S. aureus* biofilms from published studies, it is possible that a similar compound is present in our crude extracts. It is therefore recommended that further studies be continued on algicolous fungal–derived compounds.

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References

Besitulo A, Moslem MA, Hyde KD. 2010 – Occurrence and distribution of fungi in a mangrove forest on Siargao Island, Philippines. Botanica Marina 53, 535–543.

Blackledge MS, Worthington RJ, Melander C. 2013 – Biologically-inspired strategies for combating bacterial biofilms. Current Opinion in Pharmacology 13, 699–706.

Blunt JW, Copp BR, Keyzers RA, Munro MH et al. 2016 – Marine natural products. Natural Product Reports 33, 382–431.

Bugni TS, Ireland CM. 2004 – Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Natural Product Reports 21, 143–163.

Chen M, Yu Q, Sun H. 2013 – Novel strategies for the prevention and treatment of biofilm related infections. International Journal of Molecular Sciences 14, 18488–18501.

Chiang YM, Szewczyk E, Nayak T, Davidson AD et al. 2008 – Molecular genetic mining of the *Aspergillus* secondary metabolome: discovery of the emericellamide biosynthetic pathway. Chemistry and Biology 15, 527–532.

Colwell RK. 2016 – EstimateS: Statistical estimation of species richness and shared species from samples. http://purl.oclc.org/estimates (accessed 25 October 2016).

Cowman PF, Bellwood DR. 2013 – The historical biogeography of coral reef fishes: global patterns of origination and dispersal. Journal of Biogeography 40, 209–224.

Dagamac NHA, dela Cruz TEE, Rea–Maninta MAD, Aril–dela Cruz JV et al. 2017 – Rapid assessment of myxomycete diversity in Bicol Peninsula. Nova Hedwigia 104, 31–46.

Deng CM, Liu SX, Huang CH, Pang JY et al. 2013 – Secondary metabolites of a mangrove endophytic fungus *Aspergillus terreus* (No. GX7–3B) from the South China Sea. Marine Drugs 11, 2616–2624.

Dobretsov SV, Qian PY. 2002 – Effect of bacteria associated with the green alga *Ulva reticulata* on marine micro-and macrofouling. Biofouling 18, 217–228.

Flemming HC, Wingender J. 2010 – The biofilm matrix. Nature Reviews Microbiology 8, 623–633.

Flewelling AJ, Johnson JA, Gray CA. 2013 – Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Botanica Marina 56, 287–297.

Fux CA, Costerton JW, Stewart PS, Stoodley P. 2005 – Survival strategies of infectious biofilms. Trends in Microbiology 13, 34–40.

Hammer O, Harper DAT, Ryan PD. 2001 – PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4, 1–9.

Jägerbrand AK, Alatalo JM. 2015 – Effects of human trampling on abundance and diversity of vascular plants, bryophytes and lichens in alpine heath vegetation, Northern Sweden. Springerplus 4, 95. doi: 10.1186/s40064–015–0876–z.

Jones EG, Alias S, Pang KL. 2013 – Distribution of marine fungi and fungus like organisms in the South China Sea and their potential use in industry and pharmaceutical application. Malaysian Journal of Science 32, 119–130.

Kerswell AP. 2006 – Global biodiversity patterns of benthic marine algae. Ecology 87, 2479–2488.

Kolanjinathan K. 2011 – Comparative studies on antimicrobial activity of *Ulva reticulata* and *Ulva lactuca* against human pathogens. International Journal of Pharmaceutical and Biological Archive 2, 1738–1744.

Kwon AS, Park GC, Ryu SY, Lim DH et al. 2008 – Higher biofilm formation in multidrug–resistant clinical isolates of *Staphylococcus aureus*. International Journal of Antimicrobial Agents 32, 68–72.

Lacap DC, Hyde KD, Liew ECY. 2003 – An evaluation of the fungal 'morphotype'concept based on ribosomal DNA sequences. Fungal Diversity 12, 53–66.

Leid J. 2009 – Bacterial biofilms resist key host defenses. Microbe 4, 66–70.

Lohman DJ, de Bruy M, Page T, von Rintelen K et al. 2011 – Biogeography of the Indo–Australian archipelago. Annual Review of Ecology, Evolution and Systematics 42, 205–226.

Macabago SAB, Dagamac NHA, dela Cruz TEE, Stephenson SL. 2017 – Implications of the role of dispersal on the occurrence of litter-inhabiting Myxomycetes in different vegetation types after a disturbance: a case study in Bohol Islands, Philippines. Nova Hedwigia 104, 221–236.

Mah TC, O'Toole GA. 2001 – Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology 9, 34–39.

Martín–Rodríguez AJ, Reyes F, Martín J, Pérez-Yépez J et al. 2014 – Inhibition of bacterial quorum sensing by extracts from aquatic fungi: first report from marine endophytes. Marine drugs 12, 5503–5526.

Mayer AMS, Rodriguez AD, Berlinck RGS, Hamman MT. 2007 – Marine pharmacology in 2003 – 2004: Marine Compounds with anthelminthic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, anti–tuberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 145, 553–581.

Notarte KI, Nakao Y, Yaguchi T, Bungihan M et al. 2017 – Trypanocidal activity, cytotoxicity and histone modifications induced by malformin A1 isolated from the marine-derived fungus *Aspergillus tubingensis* IFM 63452. Mycosphere 8, 111–120.

Omar HH, Gumgumji NM, Shiek HM, El–Kazan MM et al. 2012 – Inhibition of the development of pathogenic fungi by extracts of some marine algae from the red sea of Jeddah, Saudi Arabia. African Journal of Biotechnology 11, 13697–13704.

Otto M. 2004 – Quorum-sensing control in Staphylococci – a target for antimicrobial drug therapy. FEMS Microbiology Letters 241, 135–141.

P de Carvalho M, Abraham WR. 2012 – Antimicrobial and biofilm inhibiting diketopiperazines. Current Medicinal Chemistry 19, 3564–3577.

Peeters E, Nelis HJ, Coenye T. 2008 – Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of Microbiological Methods 72, 157–165.

Polonio R, Mermel L, Paquette G, Sperry J. 2001 – Eradication of biofilm–forming *Staphylococcus epidermidis* (RP62A) by a combination of sodium salicylate and vancomycin. Antimicrobial Agents and Chemotherapy 45, 3262–3266.

Ramirez CS, Go C, Hernandez SM, Ruiz H et al. 2010 – Characterization of marine yeasts isolated from different substrates collected in Calatagan, Batangas. Philippine Journal of Systematic Biology 4, 1–11.

Roveta S, Schito AM, Marchese A, Schito GC. 2007 – Activity of moxifloxacin on biofilms produced in vitro by bacterial pathogens involved in acute exacerbations of chronic bronchitis. International Journal of Antimicrobial Agents 30, 415–421.

Roveta S, Marchese A, Schito GC. 2008 – Activity of daptomycin on biofilms produced on a plastic support by *Staphylococcus* spp. International Journal of Antimicrobial Agents 31, 321–328.

Rutherford ST, Bassler BL. 2012 – Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harbor Perspectives in Medicine 2, a012427. doi: 10.1101/cshperspect.a012427.

Schulz B, Draeger S, dela Cruz TE, Rheinheimer J et al. 2008 – Screening strategies for obtaining novel, biologically active, fungal secondary metabolites from marine habitats. Botanica Marina 51, 219–234.

Solis MJ, Draeger S, dela Cruz TEE. 2010 – Marine-derived fungi from *Kappaphycus alvarezii* and *K. striatum* as potential causative agents of ice-ice disease in farmed seaweeds. Botanica Marina 53, 587–594.

Smith A. 2005 – Biofilms and antibiotic therapy: Is there a role for combating bacterial resistance by the use of novel drug delivery systems? Advanced Drug Delivery Reviews 57, 1539–1550.

Su GS, Dueñas K, Roderno K, Sison MA et al. 2014 – Distribution and diversity of marine fungi in Manila Bay, Philippines. Annual Research & Review in Biology 4, 4166–4173.

Suryanarayanan TS, Venkatachalam A, Thirunavukkarasu N, Ravishankar JP et al. 2010 – Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and biotechnological potential. Botanica Marina 53, 457–468.

Torres JMO, Cardenas CV, Moron LS, Guzman APA et al. 2011 – Dye decolorization activities of marine–derived fungi isolated from Manila Bay and Calatagan Bay, Philippines. Philippine Journal of Science 140 (2), 133–143.

Torres JMO, dela Cruz TEE. 2013 – Production of xylanases by mangrove fungi from the Philippines and their application in enzymatic pretreatment of recycled paper pulps. World Journal of Microbiology and Biotechnology 29 (4), 645–655.

Trono Jr GC. 1999 – Diversity of the seaweed flora of the Philippines and its utilization. Hydrobiologia 398, 1–6.

Tsui KM, Fryar SC, Hodgkiss U, Hyde KD et al. 1998 – The effect of human disturbance on fungal diversity in the tropics. Fungal Diversity 1, 19–26.

Yao MLC, Villanueva JDH, Tumana MLS, Calimag JG et al. 2009 – Antimicrobial activities of marine fungi isolated from seawater and marine sediments. Acta Manilana 57, 19–27.

Zhang Y, Ni J, Tang F, Pei K et al. 2016 – Root-associated fungi of *Vaccinium carlesii* in subtropical forest of China: intra- and inter-annual variability of the impacts of human disturbances. Science Reports 6, 22399. doi:10.1038/srep22399.