Species of *Aspergillus* Section *Fumigati* from the Coral Reefs in the Gulf of Thailand and Andaman Sea and their Antagonistic Effects Against Plant Pathogenic Fungi

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Abstract

Twenty seven isolates of *Aspergillus* section *Fumigati* were obtained from five marine organisms and sediments from the Gulf of Thailand and the Andaman Sea. The samples were collected underwater by SCUBA diving. The tissue transplanting and soil plate methods using malt extract agar supplemented with 70% sea water and streptomycin were used for isolation. All Petri dishes were incubated at 28°C for 5 days. The fungi were identified based on their macro- and microscopic characteristics including SEM photomicrographs and phylogenetic analylsis and most are reported for the first time from a marine environment. Twenty-seven isolates of *Aspergillus* section *Fumigati* were found including seven known species and one unidentified species. These were *Aspergillus fischeri*, *A. hiratsukae*, *A. laciniosus*, *A. pualistensis*, *A. siamensis*, *A. spinosus*, *A. takakii* and *Aspergillus* sp. (KUFC 7922). The fungal species were found mainly from various species of sponges. The dual culture tests of the eight *Aspergillus* species with nine plant pathogenic fungi showed strong inhibitory effects against *Phytophthora palmivora* and *Pythium aphanidermatum,* and moderate inhibitory effects to various other plant pathogens. However all species failed to inhibit *Sclerotium rolfsii*. Ethyl acetate crude extracts of the eight *Aspergillus* species grown on rice based medium at 10,000 ppm completely suppressed mycelial growth of nine plant pathogenic fungi. The crude extract at 1,000 ppm completely suppressed mycelial growth of *P. palmivora*, and reduced the growth of *Alternaria brassicicola* and *P. aphanidermatum* 60% and 70%, respectively. These eight species of *Aspergillus* may be the source of novel fungicidal compounds and are potential biocontrol agent against various plant pathogens including the two most important plant pathogens, *P. palmivora* and *P. aphanidermatum.*

Keywords: marine-derived fungi, *Aspergillus* section *Fumigati,* coral reefs, biological control, plant pathogenic fungi

Introduction

Fungi in the coral reefs exist as endoliths, endobionts, saprotrophs and as pathogens. These fungi play an important role in the coral reef ecosystem. Fungi associated with sponges and their role in either the production or induction of secondary host metabolites are of primary interest as

potential pharmaceutical compounds. Fungal enzymes capable of degrading coral mucus and plant detritus hold great promise in biotechnological applications. Examining the fungal diversity in corals and associated reef organisms using a unique culturing approach is a subject gaining attention from researchers worldwide (Raghukumar and Ravindran, 2012).

Aspergillus section *Fumigati* and its teleomorphic state *Neosartorya* are a very important group of filamentous fungi in the environment causing allergies, food spoilage, and human disease such as aspergillosis. Most of the species produce thermoresistant ascospores and are ubiquitously distributed in soils, house dust, marine sediments, sponges, plant tissues, air, foods and organic materials. Several species have been reported to be either pathogenic or mycotoxigenic in animals and humans. In addition, many species are used for the production of various metabolites such as antibiotics, organic acids, medicines, enzymes, and as agents in many food fermentations (Samson et al., 2007). The fungi in the group are characterized by uniseriate aspergilli, columnar conidial heads in shades of green and flask shaped vesicles (Raper and Fennell, 1965). Its teleomorphic state is *Neosartorya* which has been widely used in the past and is now invalid according to The International Code of Nomenclature for algae, fungi, and plants (The Melbourne Code). However, the International Commission of *Penicillium* and *Aspergillus* decided to keep the *Aspergillus* name and provide an updated accepted species list for the genus which now contains 339 species (Samson et al., 2014).

Frisvad et al. (2008) stated that *Aspergillus fumigatus* is the most important species in *Aspergillus* causing infective lung diseases. This species has been reported to produce a large number of extrolites, including secondary metabolites, acids, and proteins such as hydrophobins and extracellular enzymes including fumigatins, fumigaclavines, and fumiquinazolines as well at others. Velmurugan and Lee (2011) reviewed the literature and reported that the enzymes β-1-3-glucanase, β-glucosidase, N-acetyl-β–glucosaminidase and amylase originated from marine-derived fungi *A. fumigatus* in Japan. Hong et al. (2006) reported several novel extrolites from *Neosartorya* spp. from Korea.

Recently, several new species within the *Aspergillus* section *Fumigati* and its *Neosartorya* teleomorph have been described from soil and marine sponges. Hong et al. (2006, 2008) described five new species, including *N. assulata, N. coreana*, *N. laciniosa* from soil, from Buyeo, North Korea, *N. denticulate* from soil under *Elaeis guineensis* in Suriname and *N. galapagensis* from soil in Equador. Yaguchi et al. (2010) described two new *Neosartorya* species from soil: *N. shendaweii* from Xinjiang, China and *N. tsunodae* from Pernambuco, Brazil. Eamvijarn et al. (2013b) recorded *A. siamensis* sp. nov. from coastal forest soil in Samaesarn Island, Thailand. Hubka et al. (2013) described *A. waksmanii* from New Jersey soil and *A. marvanovae* from contaminated water in the Czech Republic. Nováková et al. (2014) described *A. brevistipitatus*, *A. conversis*, and *A. wyomingensis* from reclamation site soils in Wyoming, USA. Matsuzawa et al. (2014a, 2014b, 2015) described *A. caatingaensis* and *A. pernambucoensis* from semi-desert soil in Brazil and *A. huiyaniae* from desert soil in Xinjiang, China. Dethoup et al. (2016) reported *A. similanensis* sp. nov. from a marine sponge *Rhabdermia* from a coral reef of the Similan Island, Phang Nga Province, Thailand.

Prompanya et al. (2015) reviewed the literature and stated that among the marine fungal stains investigated, the genus *Aspergillus* are the most prolific source of bioactive secondary metabolites including sterols, cerebrosides, sesquiterpenoids, sesterterpenoids, diterpenoids, meroterpenoids, anthraquinone derivatives, nucleoside derivatives, indole alkaloids, prenylated indole alkaloids, quinazolinone alkaloids, pyrrolidine alkaloids, and cyclic peptides. Some these of compounds possess the antibacterial activity as well as the cytotoxicity against human cancer cell lines. Tan et al. (2012) reported bioactive metabolites from a marine-derived fungus *Neosartorya fischeri* strain 1008F1 that displayed potent inhibitory effect on the replication of tobacco mosaic virus (TMV) and inhibition of the cell proliferation of human gastric and hepatic cancer cell lines. Prompanya, et al. (2014) found two new isocoumarin derivatives from the marine sponge-associated fungus *Aspergillus similanensis* sp. nov. KUFA 0013, including a new 5-hydroxy-8-methyl-2*H*, 6*H*-pyrano[3,4-*g*] chromen-2,6-dione and 6,8-dihydroxy-3,7 dimethylisocouma-

rin. Sample records for marine-derived fungus *Aspergillus*, bioactive secondary metabolites and their role as antitumor and antimicrobial agents were summarized (http://www.science.gov/topicpages/m/marine+fungus +aspergillus.html#).

New species of fungi are considered to have a high probability to produce new compounds (Strobel and Daisy, 2003; Buttachon et al., 2012). This search would necessarily involve seeking for species of *Aspergillus* section *Fumigati* in highly unexpected places such as the coral reefs in the Gulf of Thailand and the Andaman Sea. Thus this report describes the results of a concerted search for fungi having novel and unique taxonomies as well as useful biological activities.

Materials and Methods

Samples from Coral Reefs

Marine organisms and marine sediments were collected by SCUBA diving on coral reefs in the Gulf of Thailand and in the Andaman Sea (Table 1, Figures 1 and 2). The samples were labeled and placed in the clean polyethylene bags containing seawater. They were put in an ice box for one night before transporting to the laboratory for isolation.

Isolation *Aspergillus* Species of Marine Fungi

Aspergillus species were isolated from marine organisms such as sponges, coral, sea fans, zoanthids, and algae using the tissue transplanting method. The samples were washed in 0.06% sodium hypochlorite for 1 min, rinsed three times in sterilized seawater, then cut into 0.5x0.5 cm sections and placed on malt extract agar (MEA) with 70% sea water and incubated at 28°C for 5 days (Höller et al., 2000). Resulting hyphal tips was transferred from the marine of the colony to slant MEA with 70% sea water and kept as pure cultures for further investigations. The soil plate method and MEA supplemented with 70% sea water were used for isolation of fungi from marine sediment (Taboonpong et al., 2014). The pure cultures were maintained at Kasetsart University Fungal Collection (KUFC), Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Table 1 Eight species of *Aspergillus* section *Fumigati* isolated from marine organisms and marine sediments at various locations.

Aspergillus spp.	Marine organism and marine sediment	KUFC	Location	
			Island	Province
A. fischeri	Sponge (*Aka coralliphaga*)	7916	Similan	Pang-Nga **
	Sea fan (*Subergorgia* sp.)	7917	Surin	Pang-Nga **
	Sponge (*Hyrtios erecta*)	7918	Rok Nai	Krabi **
	Sponge (*Petrosia* sp.)	7919	Khram Yai	Chonburi *
A. hiratsukae	Sponge (*Acanthella cavernosa*)	7920	Rok Nai	Krabi **
A. laciniosus	Coral *Porites lutea* showing white spot disease	7896	Lan	Chonburi *
	Sponge (*Rhabdermia* sp.)	7909	Similan	Pang-Nga **
	Sea fan (*Rumphella* sp.)	7910	Similan	Pang-Nga **
	Sponge (*Halichondia* sp.)	7911	Haa Yai	Krabi **
A. pualistensis	Sponge (*Chondrilla australiensis*)	7897	Sak	Chonburi *
	Marine sediment from the coral reef	7921	Sichang	Chonburi *
	Sea fan (*Rumphella* sp.)	7899, 7900	Similan	Pang-Nga **
	Soft coral (*Dendronephthya* sp.)	7901, 7902, 7903, 7904, 7905, 7906, 7907, 7908	Similan	Pang-Nga **
A. siamensis	Zoanthid (*Palythoa caesia*)	7912, 7913	Sichang	Chonburi*
	Sea fan (*Rumphella* sp.)	7914	Similan	Pang-Nga**
A. spinosus	Sponge (*Rhabdermia* sp.)	7915	Similan	Pang-Nga**
A. takakii	Alga (*Amphiroa* sp.)	7898	Samaesarn	Chonburi*
Aspergillus sp. (KUFC 7922)	Marine sediment from the coral reef	7922	Yak-Lek	Trat*

*Golf of Thailand, **Andaman Sea

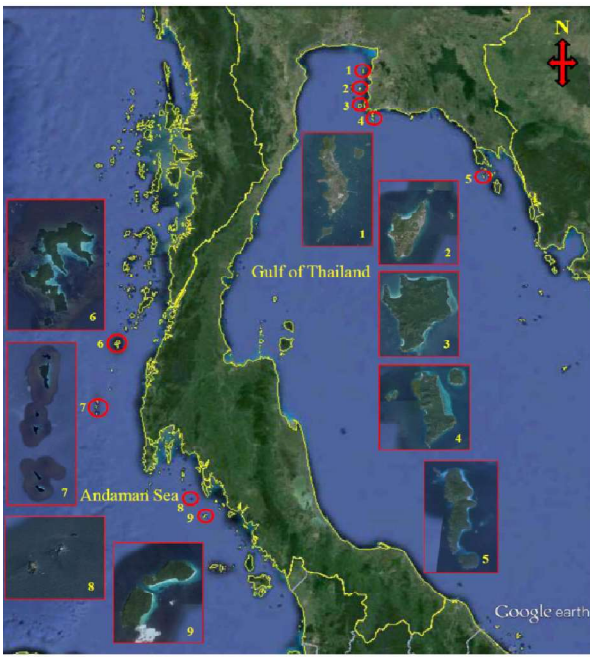

Figure 1 Map of samples collected from difference locations site of coral reefs from in the Gulf of Thailand and in the Andaman Sea.

Morphological Studies

After incubation for 7 to 14 days at 28°C on standard media such as Czapek yeast autolysate agar (CYA) Czapek agar (CZA) and malt extract agar (MEA), the morphological characteristic of colonies were determined including growth pattern and texture. Colony diameters were measured in millimeters, most effectively by transmitted light and from the reverse side. Studies on ascospore ornamentation were conducted using scanning electron microscopy (SEM: JEOL JSM 6400) (Manoch et al., 2009). Colony colors were recorded according to the mycological color chart (Rayner, 1970).

DNA Extraction and Sequencing Analysis

DNAs of *A. fischeri*, *A. laciniosus*, *A. pualistensis*, *A. siamensis*, *A. spinosus* and *Aspergillus* sp. (KUFC 7922) were prepared using the Gentorukun® (Takara Bio Inc., Ltd., Otsu, Japan) from approximately 100 µL volume of fungal mass cultured at 25°C for 5 days on PDA slants. Their β-tubulin genes were sequenced directly from PCR products using primer pair Bt2a and Bt2b (Glass and Donaldson, 1995). The PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM® 3130ABI Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions.

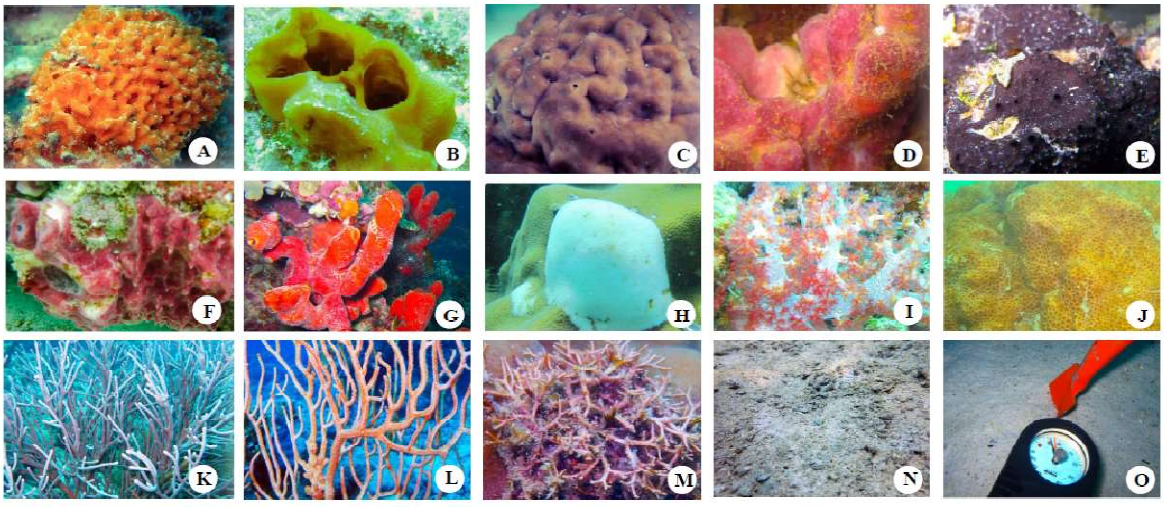

Figure 2 Marine organisms and marine sediments associated *Aspergillus* species: sponges *Acanthella cavernosa* (A), *Aka coralliphaga* (B), *Chondrilla australiensis* (C), *Halichondia* sp. (D), *Hyrtios erecta* (E), *Petrosia* sp (F), *Rhabdermia* sp. (G); coral *Porites lutea* showing white spot disease (H); soft coral *Dendronephthya* sp. (I); zoanthid *Palythoa caesia* (J); sea fans *Rumphella* sp.(K), *Subergorgia* sp. (L); algae *Amphiroa* sp. (M); marine sediments from coral reefs (N-O).

DNAs of *A. hiratsukae* and *A. takakii* were extracted using the DNeasyTM Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. Their nuclear ribosomal internal transcribed spacer (ITS) regions were amplified with primer pair ITS1 (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). DNA sequencing analyses were carried out by Macrogen Inc., Korea.

Molecular Phylogenetic Analyses

DNA sequences were edited using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan). The GenBank database at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were then searched using the sequences of β-tubulin genes and ITS regions, and the BLAST algorithm.

Antagonistic Tests of Marine-Derived *Aspergillus* Section *Fumigati*

Dual culture testing

Aspergillus species were cultivated in dual cultures on PDA for 14 days at 28°C. The young mycelium from the colony margin of *Aspergillus* spp. and from the colony margin of the selected plant pathogenic fungi was cut with sterile cork borer (0.5 cm diam) and placed on PDA 7 cm apart. All Petri-dishes were incubated at 28°C for 14 days. The inhibition levels were calculated by using the formula: G1-G2/G1 x100 where G1 = colony radius of plant pathogenic fungi in the control and G2 = colony radius of plant pathogenic fungi in the dual culture test (Intana et al., 2003; Kokaew et al., 2011). Each treatment was performed with three replicates.

Crude extract preparation and testing

The *Aspergillus* species were cultured at 28˚C on autoclaved rice in 1,000 mL flasks (200 g of rice in 200 ml of water). After 30 days incubation, 800 mL of ethyl acetate was added to the culture and all flasks were kept for 10 days. The organic phase was evaporated to dryness under reduced pressure with a rotary evaporater. A dark brown viscous mass of crude ethyl acetate extract was collected and kept at 28ºC until used (Kokaew et al., 2011).

One gram of dark brown crude extract of *Aspergillus* section *Fumigat*i was dissolved in ten mL of ethyl acetate (100,000 ppm). This stock solution was serially diluted to four concentrations (10 ppm, 100 ppm, 1,000 ppm, 10,000 ppm). One mL of each concentration of crude extract was added to nine ml of warm PDA, mixed, and poured into Petri dishes. The young mycelia of the nine plant pathogenic fungi (Table 2) were transferred to the PDA plates containing various concentrations of the crude extract solution. All Petri dishes were incubated at 28°C. The colony diameters were recorded at 7 days. The inhibition levels were calculated by comparing with a control (Kokaew et al., 2011). Each treatment was performed with two replicates.

Results and Discussion

Diversity and Distribution of *Aspergillus* section *Fumigati* from the Gulf of Thailand and the Andaman Sea

Twenty-seven isolates of *Aspergillus* section *Fumigati* were found associated with thirteen marine organisms including seven sponges: *Acanthella cavernosa*, *Aka coralliphaga*, *Chondrilla australiensis*, *Halichondia* sp., *Hyrtios erecta*, *Petrosia* sp., *Rhabdermia* sp.; two sea fans, *Rumphella* sp., *Subergorgia* sp.; one sample each of coral, *Porites lutea* showing white spot disease and a soft coral (*Dendronephthya* sp.), a zoanthid (*Palythoa caesia*), an alga (*Amphiroa* sp.) and two sediment samples from coral reefs in Srichang and Yak-Lek islands in Chonburi and Trat Provinces in the Gulf of Thailand (Table 1, Figures 1 and 2). Eight species of *Aspergillus* section *Fumigati* were identified comprising seven known, and one unidentified species namely *A. fischeri*, *A. hiratsukae*, *A. laciniosus*, *A. pualistensis*, *A. siamensis*, *A. spinosus*, *A. takakii* and *Aspergillus* sp. (KUFC 7922). The seven species of *Aspergillus* obtained in this study are similar to all previously described species in section *Fumigati* based on morphology, growth temperature, and sequence data (Malloch and Cain 1972; Udagawa et al., 1991; Raper and Fennell, 1965; Hong et al., 2006; Eamvijarn et al., 2013b; Horie et al., 2001). However *Aspergillus* sp. (KUFC 7922) is different from all described species and further studies on it are needed. *A. pualistensis* occurred at a high frequency, and twelve isolates were found, mostly from the sponges and a sea fan at Similan Island, Pang-gNa Province followed by *A. fischeri* (4 isolates), *A. laciniosa* (4), *A. siamensis* (3), and one isolate each of *A. hiratsukae*, *A. spinosus*, *A. takakii*, *Aspergillus* sp. (KUFC 7922) (Table 1, Figure 3).

Table 2 Results of blast research eight species of *Aspergillus* section *Fumigati*.

KUFC No.	Similarities to sequence in GenBank database
7917	100% to EF669796 (*Aspergillus fischeri* NRRL 181^T)
7920	100% to AB185257 (*Aspergillus hiratsukae* IFM 47035^T)
7896	100% to AY870754 (*Aspergillus laciniosus* CBS 315.89)
7897	99.8% to AB488758 (*Aspergillus paulistensis* IFM 46585^T)
7912	100% to AB646989 (*Aspergillus siamensis* KUFC 6349^T)
7915	99.6% to EF669816 (*Aspergillus spinosus* NRRL 32569)
7898	No high similarity to sequence of *Aspergillus* spp.
7922	95.5% to AY870739 (*Aspergillus* sp. CBS 112.55)

The comparison between the occurrence of the fungal strains between the two sites showed that *A. fischeri, A. laciniosa, A. paulistensis* and *A. siamensis* were found at both sites, whereas *A. takakii* and *Aspergillus* sp. were only found on alga and sediment at Samaesarn Island, Chonburi Province and Yak-lek Island, Trat Province, respectively. *A. hiratsukae* and *A. spinosus* were only found on sponges at Rok Nai Island, Krabi Province and Similan Island, Pang-Nga Province in the Andaman Sea, respectively (Table 1, Figures 4 and 5).

Aspergillus fischeri Malloch & Cain [teleomorph: *Neosartorya fischeri*], Can. J. Bot. 50: 2621. 1972.

In the present study, four strains of *A. fischeri* (KUFC 7916, 7917, 7918, 7919) were obtained from the sponge (*Aka coralliphaga*) Similan Island and the sea fan (*Subergorgia* sp.), Surin Island, Pang-Nga Province; from the sponge (*Hyrtios erecta*), Rok Nai Island, Krabi Provinve, in Andaman sea, whereas one strain was obtained from Khram Yai Island, Chonburi Provinve in the gulf of Thailand (Table 1, Figures 6A-E).

Samson et al. (2007) stated that *A. fischer*i produced reticulate ascospores, has a worldwide in distribution, and can be found in soil, (milled) rice, cotton, potatoes, groundnuts, leather, paper products, canned products and human. A similar species is *N. tatenoi*. Having such extrolites as terrein, fumitremorgins A & C, tryptoquivaline A, trypacidin, TR-2, verruculogen, sarcin, aszonalenins, fischerin, neosartorin, fiscalins, and helvolic acid and this fungus has been reported as pathogenic to animals and humans.

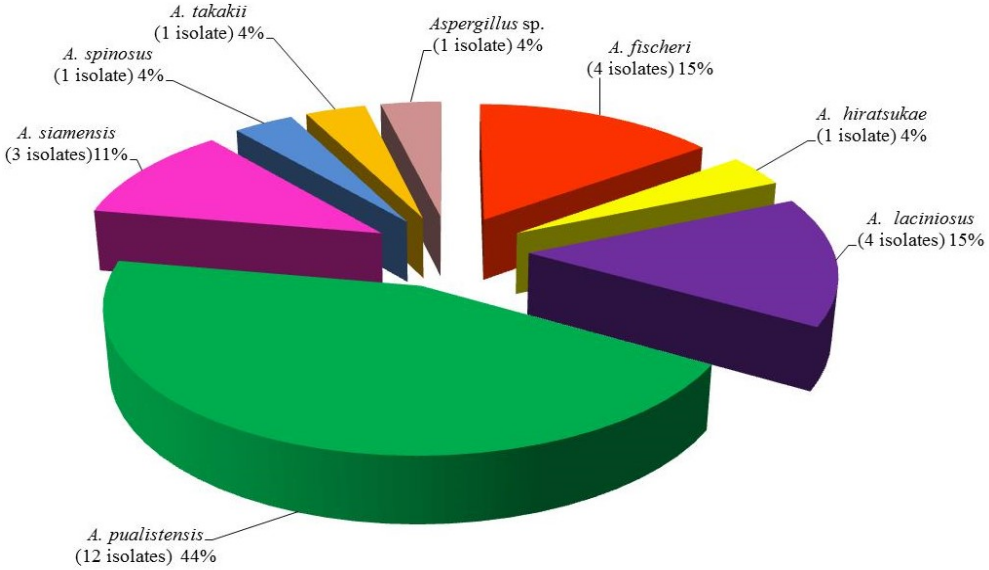

Figure 3 Percentage of the eight species of marine derived *Aspergillus* section *Fumigati* found from thirteen kinds of marine organisms and two sediment samples in the coral reef at nine islands in the Gulf of Thailand and Andaman Sea.

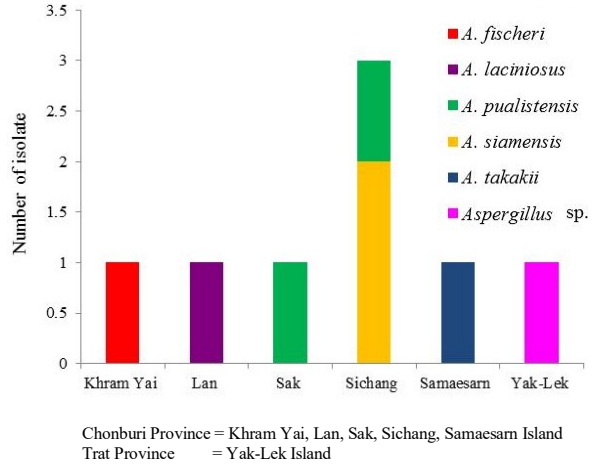

Figure 4 Occurrence of six species of *Aspergillus* section *Fumigati* from marine organisms and sediment at coral reefs in the Gulf of Thailand.

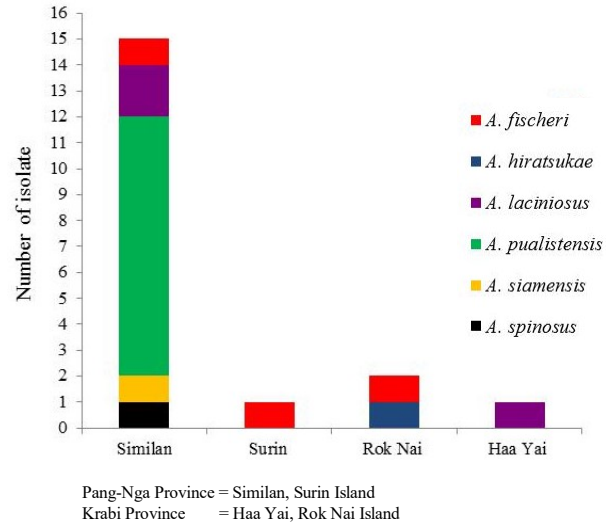

Figure 5 Occurrence of six species of *Aspergillus* section *Fumigati* from marine organisms at coral reefs in the Andaman Sea.

In Thailand, *Neosartorya fischeri* was widely distributed in soil and termite mounds in Chiang Rai, Chon Buri, Mae Hong Son, Ratchaburi, Sakon Nakhon and Si Sa Ket Provinces (Manoch et al., 2004, 2007; Manoch and Chana, 1995). Bussarakam (2002) reported *N. fischeri* from rhizosphere soil of the terrestrial orchid, *Goodyera procera*, collected from Queen Sirikit Botanic Garden, Chiang Mai Province. Moreover, twenty-seven isolates of this fungus were found from dung of buffalo, cow, deer, eld's deer, elephant, gaur, goat, rat and toad in Bangkok, Suphanburi, Surat Thani and Surin Provinces (Jeamjitt, 2007; Sudsanguan, 2012). Eamvijarn (2013) found 41 isolates of *N. fischeri* mainly from forest soils.

Fujimoto et al. (1993) reported a new toxic metabolite named fischerin (1, 4–dihydroxy-3, 5-disubstitute-2(1H)-pyridone) from *Neosartorya fischeri* var. *fischeri* which caused lethal peritonitis in mice. Wong et al. (1993) recorded three new compounds, named fiscalin A, B and C, produced by *Neosartorya fischeri* in culture broth. These compounds inhibit the binding of radiolabeled substance P ligand to the human neurokinin (NK-1) receptor, with Ki values of 57, 174, and 68 μM, respectively. Proksa et al. (1998) found a new metabolite called neosartorin in the mycelium of *Neosartorya fischeri* isolated from Vah river sediments in Slovakia. Ugwuanyi and Obeta (1999) stated that *Neosatorya fischeri, N. fischeri* var. *spinosa*, and *N. quadricincta* isolated from Nigerian soil produced pectinolytic and cellulolytic enzymes able to macerate mango (*Mangifera indica*) and African mango (*Irvingia gabonensis*). Shen et al. (2009) found that three crude extracts of the marine fungus *Neosartorya fischeri* inhibited TMV and two tumor cell lines. Tan et al. (2012) studied a marine-derived fungus *Neosartorya fischeri* strain 1008F producing two new compounds named fischeacid and fischexanthone, together with eight known compounds from the culture. Bioassays indicated that AGI-B4 and 3,4-dihydroxybenzoic acid showed a potent inhibitory effect on the replication and TMV, and AGI-B4 also inhibited cell proliferation of human gastric cancer cell line SGC-7901 and hepatic cancer cells BEL-7404. Eamvijarn et al. (2013a) found two new metabolites including a new aszonalenin analogue (1c) and a new meroditerpene (3), together with aszonalenin (1a), acetylaszonalenin (1b), 13-oxofumitremorgin B (2), aszonapyrone A (4b) and helvolic acid, from the culture of the soil fungus *Neosartorya fischeri* (KUFC 6344).

Wyatt et al. (2014) reported the mannitol has a role in sexual development of *N. fischeri* and in stress resistance of conidia. Wyatt et al. (2015) also reported oligosaccharides of *Neosartorya fischeri* consist of an α, α-trehalose backbone, extended with one [α-D-Glc*p*-(1/6)-a-D-Glc*p*-(141)-a-D-Glc*p*; isobemisiose], two [α-D-Glc*p*-(1/6)-α-D-Glc*p*-(1/6)-α-D-Glc*p*-(141)-α-D-Glc*p*] or three [α-DGlc*p*-(1/6)-α-D-Glc*p*-(1/6)-α-D-Glc*p*-(1/6)-α-D- Glc*p*-(141)-α-D-Glc*p*] glucose units. The novel saccharides, tetra- and pentasaccharide, dubbed neosartose and

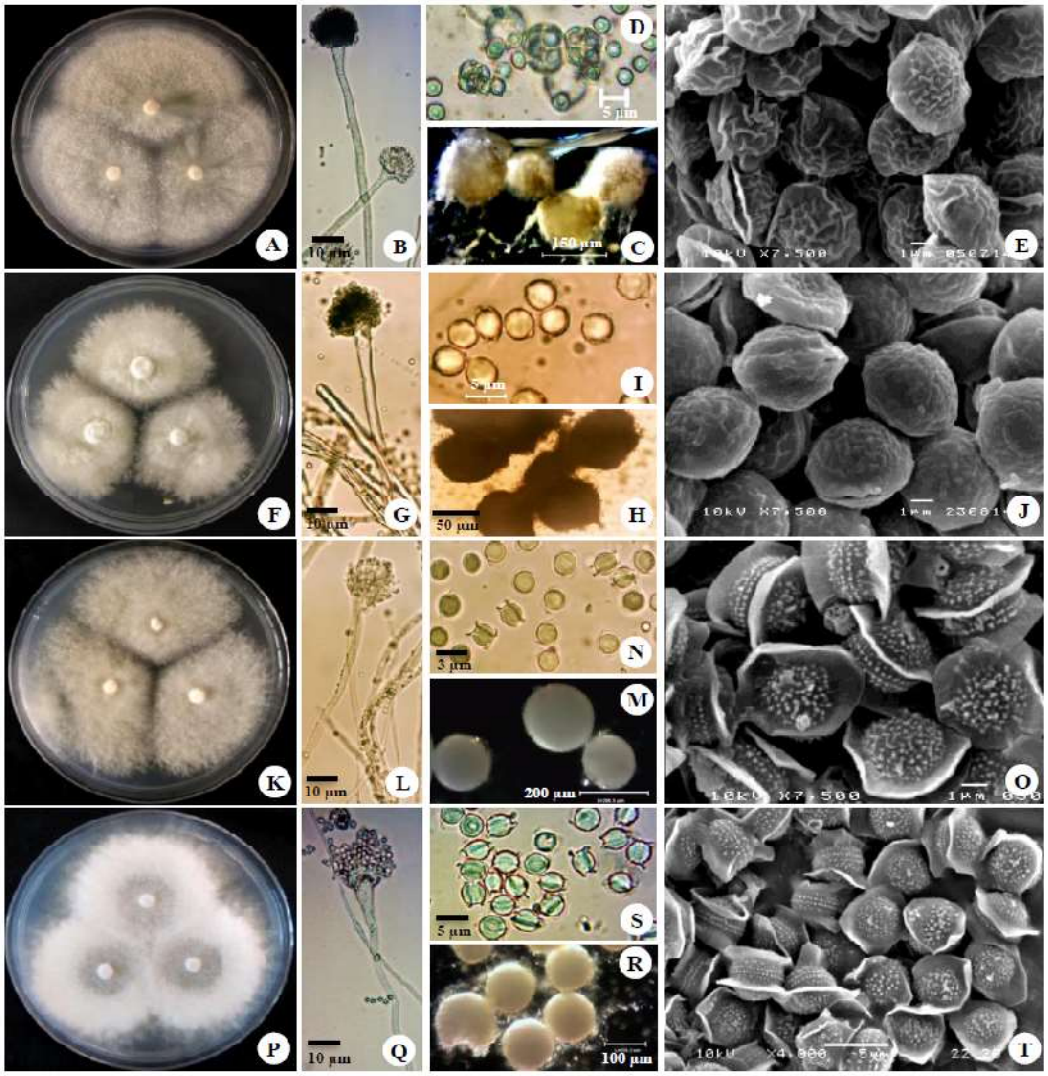

Figure 6 *A. fischeri* (KUFC 7917): Colonies on CZA, 28 °C, 7 d (A) conidial heads (B); cleisthothecia (C); asci and ascospores (D); ascospores (SEM) (E). *A. hiratsukae* (KUFC 7920): Colony on CZA, 28 °C, 7 d (F); conidial head (G); cleisthothecia (H); ascospores (I); ascospores (SEM) (J). *A. laciniosus* (KUFC 7896): Colony on CZA, 28 °C 7 d (K) conidial head (L); cleisthothecia (M); ascospores (N); ascospores (SEM) (O). *A. pualistensis* (KUFC 7897): Colony on CZA, 28 °C, 7 d (P); conidial head (Q); cleisthothecia (R); ascospores (S); ascospores (SEM) (T).

fischerose, respectively, have not found before to occur in nature.

Ramos et al. (2016) reported soil fungus *N. fischeri* KUFC 6344 possess anticancer activities in human colon carcinoma, breast adenocarcinoma, and melanoma cells, indicating a potential for identifying molecular targets involved in the anticancer activity. The above preview reports suggest the possibility for further investigations on anticancer activities of our marine derived *Aspergillus* spp. beside the mycelial growth inhibition of plant pathogenic fungi.

Aspergillus hiratsukae Udagawa, Tsub. & Y. Horie [teleomorph: *Neosartorya hiratsukae*], Udagawa et al., Trans. Mycol. Soc. Japan 32: 23, 1991

In the present study, only one strain of *A. hiratsukae* (KUFC 7920) was obtained from sponge (*Acanthella cavernosa*), Rok Nai Island, Krabi Province in the Andaman Sea (Table 1, Figures 6F-J).

Udagawa et al. (1991) described *Neosartorya hiratsukae* sp. nov. (anamorphic state: *Aspergillus hiratsukae*) from aloe beverage while studying a

spoilage outbreak in commercial foods of heat resistant molds. *A.hiratsukae* is characterized by its very restricted growth on CZA and ascospores with low, closely apprised equatorial crests and with convex surfaces bearing numerous close-anastomosing ridges arranged in a fine reticulate ornamentation. The marine-derived fungus *A. hiratsukae* (KUFC 7920) from the present study is similar to the species described by Udagawa et al. (1991).

Samson et al. (2007) stated that *A. hiratsukae* reported from Japan, Brazil, and South Korea, produced finely reticulate ascospores and, can be found in soil, fruit juice, indoor air, and humans. The similar species are *N. fischeri* and *N. tatenoi*. One extrolite is avenaciolide and it is toxic to humans.

Aspergillus laciniosus Hong, Frisvad & Samson [teleomorph: *Neosartorya laciniosa* Hong, Frisvad & Samson], Int. J. Syst. Evol. Microbiol. 56: 477. 2006.

In our study, four strains of *A. laciniosus* (KUFC 7896, 7909, 7910, 7911) were obtained from the sponge (*Rhabdermia* sp.) and the sea fan (*Rumphella* sp.), Similan Island, Pang-Nga Province; from the sponge (*Halichondia* sp.) Haa Yai Island, Krabi Province in the Andaman Sea, whereas one strain was obtained from coral *Porites lutea* showing white spot disease at Lan Island, Chonburi Province in the Gulf of Thailand (Table 1, Figures 6K-O).

Hong et al. (2006) described *Neosartorya laciniosa* as a new species from soil in Korea planted with perilla, tomato, pepper, grapes, and from strawberry pulp. Phylogenetic analyses of *N. laciniosa* was based on β-tubulin and calmodulin gene sequences.

Samson et al. (2007) stated that *A. laciniosus* produced microtuberculate ascospores with two bent crests and two distinct equatorial rings of small projections, found in soil in South Korea, U.S.A., Pakistan, Netherlands, Suriname, Dominican Republic and Kenya. Species similar to this are *N. spinosa* and *N. coreana.* Its extrolites are aszonalenins, tryptoquivaline and tryptoquivalone.

Eamvijarn et al. (2013a) studied the ethyl acetate extract of the culture of our strain, the diseased coral-derived fungus *N. laciniosa* (KUFC 7896) furnished aszonapyrone B (4a), aszonapyrone A (4b), tryptoquivaline L and 30-(4-oxoquinazolin-3-yl) spiro [1H-indole-3,50-oxolane]-2,20-dione. Gomes et al. (2014) reported the diseased coral-derived fungus *N. laciniosa* (KUFC 7896) led to isolation of a new tryptoquivaline derivative tryptoquivaline T (1d). Compounds 1a–d, 2, 3, and 5, together with aszonapyrones A (4a) and B (4b), chevalones B (6) and C (7a), sartorypyrones B (7b) and A (8), were tested for their antibacterial activity against four reference strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*), as well as the environmental multidrug-resistant isolates.

Ramos et al. (2016) reported that our strain of marine-derived fungi *N. laciniosa* (KUFC 7896) possess anticancer activities in human colon carcinoma, breast adenocarcinoma, and melanoma cells, validating the interest for an identification of molecular targets involved in the anticancer activity.

Aspergillus pualistensis Horie, Miyaji & Nishimura [teleomorph: *Neosartorya pualistensis* Horie, Miyaji & Nishimura], Mycoscience. 36: 161. 1995.

In the present study, twelve strains of *A. pualistensis* (KUFC 7897, 7899, 7900, 7901, 7902, 7903, 7904, 7905, 7906, 7907, 7908, 7921) were recovered from the sea fan (*Rumphella* sp.) and soft coral (*Dendronephthya* sp.) at Similan Island, Pang-Nga Province in Andaman Sea; from the sponge (*Chondrilla australiensis*), Sak Island and marine sediments from coral reefs at Lan Island, Chonburi Province in the Gulf of Thailand. *A. pualistensis* occurred at a high frequency, and twelve isolates were found from both sites, but mainly found in the Andaman Sea (Table 1, Figures 6P-T).

Kokaew (2011) recorded *N. pualistensis* as an endophytic fungus in the healthy plant tissue of *Dracaena conferta* (Dracaenaceae) from Mu Ko Similan National Park, Phang-Nga Province and this is the same location that we found *A. pualistensis* at high frequency.

Gomes et al. (2014) reported that the culture of our strain of marine sponge-associated fungus *N. paulistensis* (KUFC 7897) produced a new meroditerpene, sartorypyrone C (5), was isolated, together with the known tryptoquivalines L (1a), H (1b), F (1c), 3′-(4-oxoquinazolin-3-yl) spiro [1*H*-indole-3,5′]-2,2′-dione (2) and 4(3*H*)-quinazolinone (3), were tested for their antibacterial activity against

four reference strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*), as well as the environmental multidrug-resistant isolates.

Ramos et al. (2015) reported that the crude ethyl acetate extracts of our strain of marine fungus *N. paulistensis* (KUFC 7897) has selective anti-proliferative and cell death activities in HepG2, HCT16 and A375 cells. The bioactivity of these extracts suggests a potential for biotechnological applications and substantiates that both should be further considered for the elucidation of the molecular targets and signal transduction pathways involved.

Aspergillus siamensis sp. nov Manoch & Eamvijarn. In Eamvijarn et al. 2013 Mycoscience 54: 403, 2013.

In our study, three strains of *A. siamensis (*KUFC 7912, 7913, 7914) were obtained from the sea fan (*Rumphella* sp.) from Similan Island, Pang-Nga Province in the Andaman Sea and from a zoanthid (*Palythoa caesia*), Sichang Island, Chonburi Province in the Gulf of Thailand (Table 1, Figures 7A-E).

Eamvijarn et al. (2013b) described *Neosartorya siamensis* Manoch & Eamvijarn sp. nov. (*A. siamensis* Manoch & Eamvijarn) as a new species from soil at Samaesarn Island, Chonburi Province in the Gulf of Thailand. It can be distinguished from other known species of *Neosartorya* by producing pinkish mycelia with pale pink exudates on CZA medium. Eamvijarn, (2013) recorded forty-five isolates of *N. siamensis* widely distributed in the forest and agricultural soil in various parts of Thailand.

Buttachon et al. (2012) reported seven new indole alkaloids including indoloazepinone: sartorymensin, two quinazolinone: tryptoquivaline O and *3′-(4-Oxaquinazolin-3-yl) spiro[1H-indole-3,5′-oxalane] -2,2′-dione*, and four new pyrazinoquinazolinone derivatives: *epi*-fiscalin C, *epi*-fiscalin A, neofiscalin A and *epi*-neofiscalin A. Seven known compounds comprising 4-dihydroxy-3-methylacetophenone tryptoquivaline, tryptoquivalines L, H, F, fiscalins A and C were isolated from cultures of the fungus *N. siamensis* (KUFC 6349) collected from forest soil at Samaesarn Island, Chonburi Province, Thailand. Eight compounds were evaluated for their *in vitro* growth inhibitory activity on the human U373 and Hs683 glioblastoma, the A549 non-small cell lung cancer, the MCF-7 breast cancer, and the SKMEL-28 melanoma cell lines. Sartorymensin displayed a moderate *in vitro* growth inhibitory activity on the five cell lines.

Ramos et al. (2015) reported the crude ethyl acetate extracts of *N. siamensis* KUFA 0017 provided selective anti-proliferative and cell death activities in HepG2, HCT16 and A375 cells. The bioactivity of these extracts suggested a potential for biotechnological applications and substantiated that both should be further considered for the elucidation of the molecular targets and signal transduction pathways involved.

Bessa et al. (2016) reported ten indole alkaloids were obtained from the marine sponge-associated fungus *Neosartorya siamensis* KUFA 0017. They studied the antimicrobial properties of these and of three other compounds previously isolated from the soil fungus *N. siamensis* KUFC 6349. Only neofiscalin A showed antimicrobial activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE); with a minimum inhibitory concentration (MIC) of 8 μg mL^{-1} against both strains. Another compound, fiscalin C, presented synergistic activity against MRSA when combined with oxacillin, although alone it showed no antibacterial effect. Moreover, neofiscalin A, when present at sub-MICs, hampered the ability of both MRSA and VRE strains to form a biofilm. Additionally, the biofilm inhibitory concentration values of neofiscalin A against the MRSA and VRE isolates were 96 and 80 μg mL^{-1}, respectively. At a concentration of 200 μg mL^{-1}, neofiscalin A was able to reduce the metabolic activity of the biofilms by approximately 50%. One important fact is that results also showed that neofiscalin A had no cytotoxicity against a human brain capillary endothelial cell line.

Aspergillus spinosus (Raper & Fennell) Kozakiewicz [teleomorph: *Neosartorya spinosa*], Raper & Fennell, 1965.

In our study, a single strain of *A. spinosus* (KUFC 7915) was obtained from marine sponge (*Rhabdermia* sp.), Similan Island, Pang-Nga Province in the Andaman Sea (Table 1, Figures 7F-J).

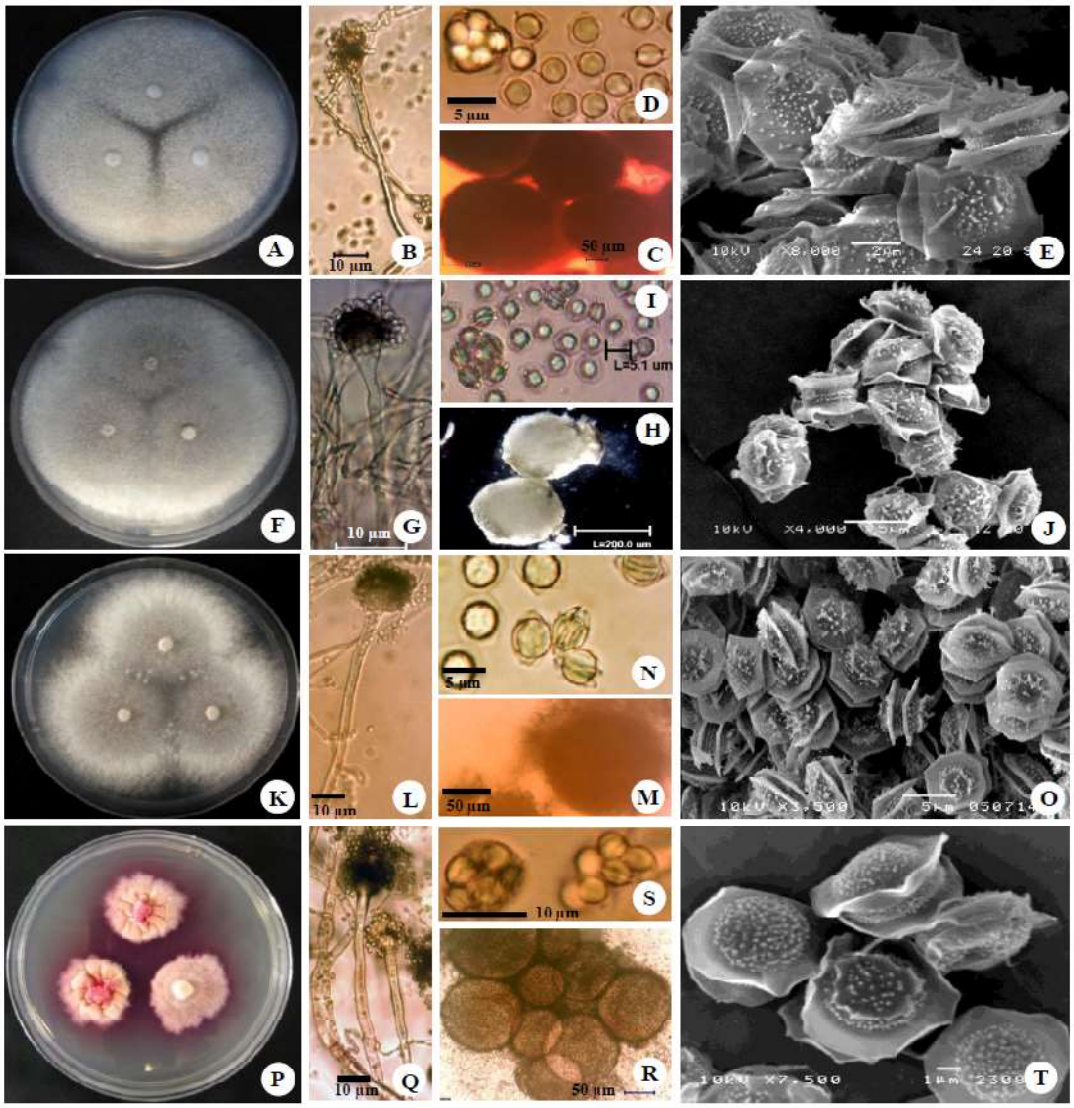

Figure 7 *A. siamensis* (KUFC 7912): Colonies on CZA, 28 °C, 7 d (A) conidial heads (B); cleisthothecia (C); asci and ascospores (D); ascospores (SEM) (E). *A. spinosus* (KUFC 7915): Colony on CZA, 28 °C, 7 d (F); conidial head (G); cleisthothecia (H); ascospores (I); ascospores (SEM) (J). *A. takakii* (KUFC 7898): Colony on CZA, 28 °C, 7 d (K) conidial head (L); cleisthothecia (M); ascospores (N); ascospores (SEM) (O). *Aspergillus* sp. (KUFC 7922): Colony on CZA, 28 °C, 7 d (P); conidial head (Q); cleisthothecia (R); ascospores (S); ascospores (SEM) (T).

In Thailand, Manoch and Chana (1995) reported *N. spinosa* on cow dung from Kanchanaburi Province. Manoch et al. (2007) found *N. spinosa* from soil and termite mounds from Ratchaburi Province. Kokaew (2011) recorded *N. spinosa* as an endophytic fungus in the healthy plant tissue of *Dracaena conferta* (Dracaenaceae) from Mu Ko Similan National Park, Phang-Nga Province in the Andaman Sea. Eamvijarn (2013) reported 79 isolates *of N. spinosa*, frequently found from forest soil in Thailand.

Samson et al. (2007) stated that *A. spinosus* produced echinulate ascospores with spines ranging or with verruculose and small triangular, sometimes circularly arranged, projections, reported from Nicaragua, Kenya, Denmark, Dominican Republic, U.S.A., Belgium, Sudan, Japan, India, Pakistan and South Korea, can be found in soil, fruit juice and humans. The similar species are *N. coreana, N. laciniosa.* Extrolites production: aszonalenins, 2-pyrovoylaminobenzamide, pseurotin.

Ferulic acid producing fungus *N. spinosa* NRRL 185 releases a full complement of enzymes from corn bran and corn fibers (Shin et al. 2006). On the other hand, *N. spinosa* was reported as pathogenic to humans. Summerbell et al. (1992) described the first case of endocarditis caused by *N. fischeri* var. *spinosa* in a child who received a calf pericardium graft.

Aspergillus takakii Y. Horie, Abliz & K. Fukush. [teleomorph: *Neosartorya takakii*], Horie et al Mycoscience 42: 91, 2001.

In our study, only one strain of *A. takakii* (KUFC 7898) was isolated from the alga (*Amphiroa* sp.), Samaesarn Island, Chonburi Province in the Gulf of Thailand (Table 1, Figures 7K-O).

Horie et al. (2001) described *N. takakii* sp.nov. (anamorphic state: *Aspergillus takakii*) from grassland soil in Roraima State, Brazil. *N. takakii* differs from the other known species of the genus in having lenticular ascospores with two distinct equatorial crests and with roughly circularly arranged projections on the convex walls.

Zin et al. (2015) reported a new meroditerpene sartorenol (1), a new natural product takakiamide (2) and a new tryptoquivaline analog (3) from our strain of *N. takakii* (KUFC 7898). Compounds 1, 2 and 3 were evaluated for their antimicrobial activity against Gram-positive and Gram-negative bacteria, and multidrug-resistant isolates from the environment; however, none exhibited antibacterial activity (MIC > 256 mg mL^{-1}). The three new compounds did not show any quorum sensing inhibition in the screening protocol based on the pigment production by *Chromobacterium violaceum* (ATCC 31532).

***Aspergillus* sp. (KUFC 7922)**

We found a single strain of *Aspergillus* sp. (KUFC 7922) from the sediment in a coral reef at Yak-Lek Island, Trat Province in the Gulf of Thailand (Table 1, Figures 7P-T).

This fungal strain produced a unique reddish colony on agar media. Furthermore, the morphology and phelogenic data do not fit with any described species.

Colonies on Czapek agar (CZA) white pink, attaining a diameter of 25-30 and 40-45 mm after 7 and 14 days at 28°C, white pink , consisting of thin mycelial layer; pinkish cleistothecia abundantly produced; conidiogenesis few in number; zonate, irregular margin, colorless exudates; reverse red pink (R 2).

Homothallic, ascomata superficial, yellowish brown to yellowish pink, globose to subglobose, 100-200 µm in diameter, surrounded by a loose covering of pale yellowish brown aerial hyphae, peridium hyaline to pinkish yellow, thin; asci 8-spored, globose to subglobose, 10.5-12 x 13-14 µm in diameter, evanescent at maturity; ascospores hyaline, lenticular, spore body 4.5-5 x 5-5.5 µm, provided with two wide equatorial crests measuring 0.5-1 µm wide, with convex surfaces microtuberculate ornamentation; mycelium composed of hyaline, branched, septate, smooth-walled hyphae.

Conidiophores arising from surface and aerial mycelium, hyaline to pale green, smooth-walled, 80-110 µm long and 4.0-5.0 µm wide, conidial heads columnar, pale blue-grey, anamorph uniseriate; vesicles flask-shaped, 16-18 µm in diameter, phialides hyaline to pale greyish-green, 4.0-5.0 x 3.5-4.5 µm, conidia, globose to subglobose, smooth-walled, 2.0-3.0 µm in diameter, pale greyish-green.

The morphological characteristics and the sequence data of the *Aspergillus* sp. (KUFC 7922) were not identical to those of known *Aspergillus* spp. In addition, *Aspergillus* sp. (KUFC 7922) produced unique reddish colonies on the obverse and reverse sides of the agar media.

Molecular Identification

Their sequences of the β-tubulin genes and ITS regions were determined and their results of the database search were shown at Table 2.

Mycelial Growth Inhibition of Plant Pathogenic Fungi (*Dual Culture Test*)

Eight strains of *Aspergillus* including *A. fischeri*, *A. hiratsukae*, *A. laciniosus*, *A. pualistensis*, *A. siamensis*, *A. spinosus*, *A. takakii* and *Aspergillus* sp. (KUFC 7922) were tested for antagonistic activity against nine isolates of plant pathogenic fungi (Table 3).

Results from the Table 4 and Figures 8 and 9 indicated that *A. fischeri* inhibited mycelial growth of *P. palmivora* and *P. aphanidermatum* by 98.5% and 97.8% respectively, whereas *A. hiratsukae*, *A.*

Table 3 Nine species of plant pathogenic fungi from various diseased plants used for antagonistic activity test.

Plant pathogenic fungi	Host plant	Disease
Alternaria brassicicola	*Brassica albograbra* (chinese kale)	Leaf spot
Colletotrichum gloeosporioides	*Capsicum frutescens* (chili)	Anthracnose
Curvularia lunata	*Oryza sativa* (rice)	Leaf spot
Fusarium oxysporum	*Musa sapientum* (banana)	Fusarium wilt
Lasiodiplodia theobromae	*Citrus maxima* (pomelo)	Fruit rot
Phytophthora palmivora	*Durio zibethinus* (durian)	Root rot
Pythium aphanidermatum	*Brassica albograbra* (chinese kale)	Damping-off
Rhizoctonia solani	*Oryza sativa* (rice)	Sheath blight
Sclerotium rolfsii	*Solanum tuberosum* (potato)	Stem rot

Table 4 Percent inhibition on mycelial growth of eight species of *Aspergillus* against nine species of pathogenic fungi in dual culture tests on PDA incubated at 28°C for 14 days.

Fungal isolate	Mycelial growth inhibition of plant pathogenic fungi *in vitro* (%)								
	Alternaria brassicicola	*Colletotrichum gloeosporioides*	*Curvularia lunata*	*Fusarium oxysporum*	*Lasiodiplodia theobromae*	*Phytophthora palmivora*	*Pythium aphanidermatum*	*Rhizoctonia solani*	*Sclerotium rolfsii*
A. fischeri (KUFC 7917)	53.1 (±3.26)	56.1 (±0.78)	45.0 (±2.35)	44.7 (±0.94)	36.7 (±4.71)	98.5 (±0.00)	97.8 (±0.00)	35.0 (±3.92)	0*
A. hiratsukae (KUFC 7920)	41.7 (±0.00)	44.4 (±0.00)	44.4 (±0.00)	41.2 (±0.00)	36.1 (±3.92)	50.0 (±0.00)	55.6 (±0.00)	33.3 (±0.00)	0*
A. laciniosus (KUFC 7896)	50.0 (±2.02)	38.9 (±0.00)	44.4 (±0.00)	43.3 (±1.88)	33.3 (±0.00)	71.4 (±0.00)	88.9 (±0.00)	38.9 (±0.00)	36.1 (±3.92)
A. pualistensis (KUFC 7897)	53.6 (±0.00)	47.2 (±3.92)	50.6 (±0.78)	40.0 (±0.00)	38.3 (±0.78)	57.9 (±1.01)	80.6 (±3.92)	46.1 (±2.35)	33.3 (±0.00)
A. siamensis (KUFC 7912)	44.0 (±3.16)	46.6 (±2.43)	46.7 (±3.14)	42.3 (±2.52)	30.6 (±2.35)	85.1 (±0.00)	79.4 (±2.35)	37.8 (±1.57)	0*
A. spinosus (KUFC 7915)	48.4 (±0.00)	38.9 (±1.57)	46.7 (±2.28)	39.5 (±2.44)	30.6 (±3.92)	75.8 (±0.00)	97.8 (±0.00)	56.1 (±0.78)	0*
A. takakii (KUFC 7898)	32.2 (±2.76)	46.7 (±3.14)	48.9 (±3.14)	40.0 (±3.53)	36.1 (±2.07)	81.1 (±2.64)	78.3(±2.07)	54.4 (±1.57)	0*
Aspergillus sp. (KUFC 7922)	34.6 (±2.43)	35.3 (±0.83)	36.1 (±3.92)	38.2 (±2.15)	16.7 (±0.00)	43.3 (±2.71)	0*	5.6 (±1.57)	0*

0* = plant pathogenic fungus overgrew colony of *Aspergillus* spp.; standard deviations of three replicates (N=3)

takakii, A. siamensis, *A. pualistensis*, *A. laciniosus*, and *A. spinosus*, inhibited mycelial growth of *P. aphanidermatum* by 55.6%, 78.3%, 79.4%, 80.6%, 88.9% and 97.8% respectively. In addition, *A. fischeri* could inhibit mycelial growth of *A. brassicicola*, *C. gloeosporioides*, *C. lunata, F. oxysporum* and *L. theobromae* by 36-56%. However, *A. fischeri*, *A. hiratsukae*, *A. siamensis*, *A. spinosus*, *A. takakii* and *Aspergillus* sp. (KUFC 7922) did not inhibit mycelial growth of *S. rolfsii*.

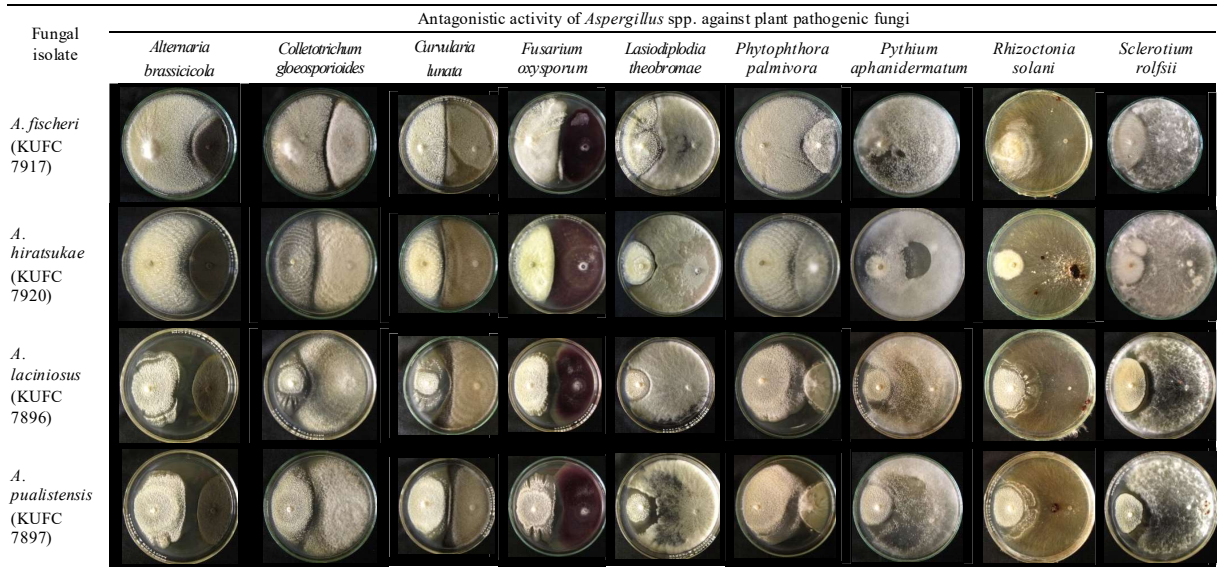

Figure 8 Dual culture tests of four *Aspergillus* species (left) against nine species of plant pathogenic fungi (right) on PDA, incubated at 28°C for 14 days.

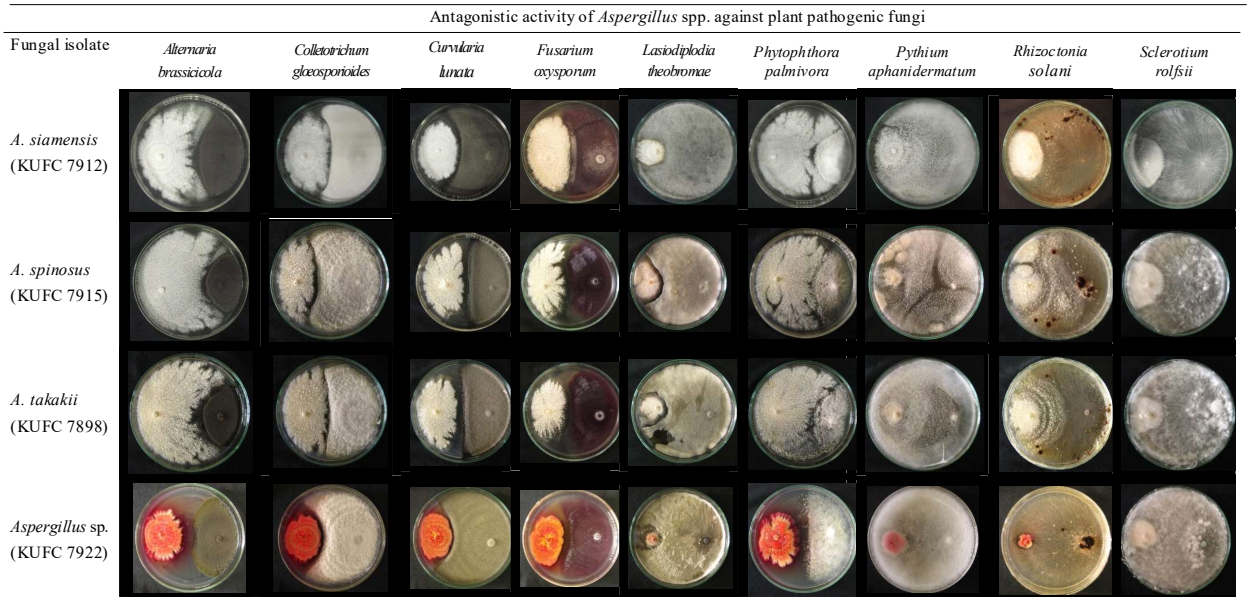

Figure 9 Dual culture tests of four *Aspergillus* species (left) against nine species of plant pathogenic fungi (right) on PDA, incubated at 28°C for 14 days.

Eamvijan (2013) reported *N. fischeri* isolated from soil inhibited 53.9%-58.3% of radial growth of *Fusarium oxysporum*, *Alternaria brassicicola*, *Colletotrichum gloeosporioides* and *Curvularia oryzae*. Moreover, *N. tatenoi* provided of 62.2 and 57.1% growth inhibition of *P. palmivora* and *P. aphanidermatum*. Taboonpong et al. (2014) reported *Neosartorya* sp. isolated from marine sediment inhibited 83.9% of mycelial growth of *Pyricularia oryzae* and did not inhibit mycelial growth of two basidiomycota anamorphs, *Rhizoctonia oryzae* and *Sclerotium rolfsii*.

Antagonistic Activity Test of Crude Extracts Against Plant Pathogenic Fungi

The crude extracts of eight *Aspergillus* species at 10,000 ppm completely inhibited (100%) nine plant pathogenic fungi on PDA. At 1,000 ppm of crude extracts derived from most species of *Aspergillus* spp. effectively inhibited mycelial growth of *P. palmivora* and *P. aphanidermatum* by 100% and over 70%, respectively. All *Aspergillus* crude extracts at 10 and 100 ppm did not inhibit mycelial growth of *C. lunata*, *R. solani* and *S. rolfsii* (Tables 5-7; Figures 10-12).

Eamvijarn (2013) reported that at 1,000 ppm crude extracts from six *Neosartorya* spp. completely inhibited mycelial growth of *Pythium aphanidermatum, Phytophthora palmivora* and *S. rolfsii*, whereas at 10,000 ppm concentration of these extracts on strongly suppressed (100%) mycelial growth of eight species of plant pathogenic fungi, but failed to control *Lasiodiplodia theobromae*. Shen et al. (2009) reported a marine derived fungus *N. fischeri* and the inhibitory effects of their crude extract on TMV and two tumor cell lines. Dethoup et al. (2015) reported that the ethyl acetate crude extracts of marine-derived fungi, *N. fischeri* (KUFA 0107), displayed a moderate antifungal activity against *S. rolfsii* and *P. aphanidermatum*, by 62.96% and 51.11% of mycelial growth inhibition respectively, while *N. pseudofischeri* (KUFA 0108) crude extract provided a moderate antifungal activity over 50% of mycelial growth inhibition. The crude ethyl acetate extracts of our strain *N. paulistensis* KUFC 7897 and *N. siamensis* KUFA 0017 provided selective anti-proliferative and cell death activities in HepG2, HCT16 and A375 cells. The bioactivity of these extracts suggested a potential for biotechnological applications and substantiated that both should be further considered for the elucidation of the molecular targets and signal transduction pathways involved (Ramos et al., (2015). Ramos et al. (2016) also reported our marine-derived fungus *N. laciniosa* KUFC 7896 and soil fungus *N. fischeri* KUFC 6344 possess anticancer activities in human colon carcinoma, breast adenocarcinoma, and melanoma cells, validating the interest for an identification of molecular targets involved in the anticancer activity. The above preview reports suggest the possibility for further investigations of the anticancer activities of our marine derived *Aspergillus* spp. are warranted.

Table 5 Percent inhibition on mycelial growth of *Alternaria brassicicola*, *Colletotrichum gloeosporioides* and *Curvularia lunata* using crude extractions (ppm) of eight *Aspergillus* spp. on PDA at 28˚C for 7 days.

Fungal isolate	Mycelial growth inhibition (%) at concentrations (ppm)											
	Alternaria brassicicola				*Colletotrichum gloeosporioides*				*Curvularia lunata*			
	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
A. fischeri (KUFC 7917)	11.7 (±0.66)	22.8 (±2.22)	59.9 (±3.66)	100	0	0	13.9 (±3.92)	100	0	0	7.2 (±0.78)	100
A. hiratsukae (KUFC 7920)	50.0 (±2.12)	56.7 (±2.51)	57.5 (±2.94)	100	22.8 (±0.78)	27.2 (±0.78)	31.7 (±2.35)	100	0	0	17.8 (±0.00)	100
A. laciniosus (KUFC 7896)	32.3 (±5.21)	44.6 (±3.87)	46.9 (±6.90)	100	0	0	14.7 (±0.83)	100	0	0	14.4 (±3.14)	100
A. pualistensis (KUFC 7897)	40.0 (±4.08)	48.0 (±6.09)	49.6 (±4.42)	100	0	1.7 (±2.41)	6.3 (±2.84)	100	0	0	6.1 (±0.78)	100
A. siamensis (KUFC 7912)	47.8 (±5.15)	55.7 (±5.00)	64.3 (±3.80)	100	13.3 (±0.00)	16.7 (±0.00)	34.4 (±1.15)	100	0	0	27.8 (±0.00)	100
A. spinosus (KUFC 7915)	45.8 (±1.17)	50.8 (±1.17)	65.0 (±0.00)	100	14.4 (±1.57)	17.2 (±0.78)	38.9 (±0.00)	100	0	0	63.3 (±0.00)	100
A. takakii (KUFC 7898)	41.5 (±2.02)	48.1 (±5.49)	74.1 (±3.88)	100	11.1 (±0.00)	22.2 (±0.00)	50.0 (±0.00)	100	0	0	68.3 (±2.35)	100
Aspergillus sp. (KUFC 7922)	47.8 (±3.21)	47.0 (±2.03)	82.6 (±1.07)	100	2.2 (±0.83)	8.8 (±0.83)	75.3 (±4.70)	100	0	0	69.4 (±0.79)	100

Remark: standard deviations of two replicates (N=2)

Table 6 Percent inhibition on mycelial growth of *Fusarium oxysporum*, *Lasiodiplodia theobromae* and *Phytophthora palmivora* using eight crude extractions (ppm) of *Aspergillus* spp. on PDA at 28˚C for 7 days.

Fungal isolate	Mycelial growth inhibition (%) at concentrations (ppm)											
	Fusarium oxysporum				*Lasiodiplodia theobromae*				*Phytophthora palmivora*			
	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
A. fischeri (KUFC 7917)	0	0	25.0 (±3.92)	100	0	0	0	100	30.0 (±3.33)	56.0 (±1.88)	100	100
A. hiratsukae (KUFC 7920)	33.5 (±3.94)	34.8 (±3.88)	35.5 (±2.94)	100	0	2.2 (±3.14)	100	100	85.6 (±1.57)	100	100	100
A. laciniosus (KUFC 7896)	0	0	31.7 (±2.35)	100	0	0	100	100	87.2 (±2.35)	91.7 (±0.78)	100	100
A. pualistensis (KUFC 7897)	0	13.3 (±3.14)	30.6 (±0.78)	74.4 (±1.57)	0	0	100	100	86.1 (±2.77)	88.9 (±0.00)	100	100
A. siamensis (KUFC 7912)	22.4 (±1.61)	22.4 (±1.61)	30.9 (±2.96)	100	0	0	5.6 (±0.00)	100	90.6 (±0.78)	100	100	100
A. spinosus (KUFC 7915)	24.0 (±1.88)	26.7 (±0.00)	35.3 (±0.94)	100	0	100	100	100	90.0 (±0.00)	100	100	100
A. takakii (KUFC 7898)	21.2 (±3.38)	23.6 (±3.27)	42.4 (±1.81)	100	0	100	100	100	94.4 (±0.00)	100	100	100
Aspergillus sp. (KUFC 7922)	6.3 (±0.00)	12.5 (±6.25)	37.5 (±0.00)	100	0	0	100	100	93.3 (±0.00)	100	100	100

Remark: standard deviations of two replicates (N=2)

Table 7 Percent inhibition on mycelial growth of *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rolfsii* using eight crude extractions (ppm) of *Aspergillus* spp. on PDA at 28˚C for 7 days.

Fungal isolate	Mycelial growth inhibition (%) at concentrations (ppm)											
	Pythium aphanidermatum				*Rhizoctonia solani*				*Sclerotium rolfsii*			
	10	100	1000	10000	10	100	1000	10000	10	100	1000	10000
A. fischeri (KUFC 7917)	0	0	100	100	0	0	0	100	0	0	83.3 (±2.71)	100
A. hiratsukae (KUFC 7920)	0	0	100	100	0	0	0	100	0	0	0	100
A. laciniosus (KUFC 7896)	0	0	100	100	0	0	0	100	0	0	0	100
A. pualistensis (KUFC 7897)	0	0	71.7 (±0.78)	100	0	0	0	100	0	0	0	100
A. siamensis (KUFC 7912)	0	0	100	100	0	0	0	100	0	0	40.6 (±3.88)	100
A. spinosus (KUFC 7915)	0	0	100	100	0	0	0	100	0	0	73.9 (±0.78)	100
A. takakii (KUFC 7898)	0	0	100	100	0	0	75.0 (±3.92)	100	0	0	88.9 (±0.00)	100
Aspergillus sp. (KUFC 7922)	0	0	100	100	0	0	80.0 (±5.55)	100	0	0	100	100

Remark: standard deviations of two replicates (N=2)

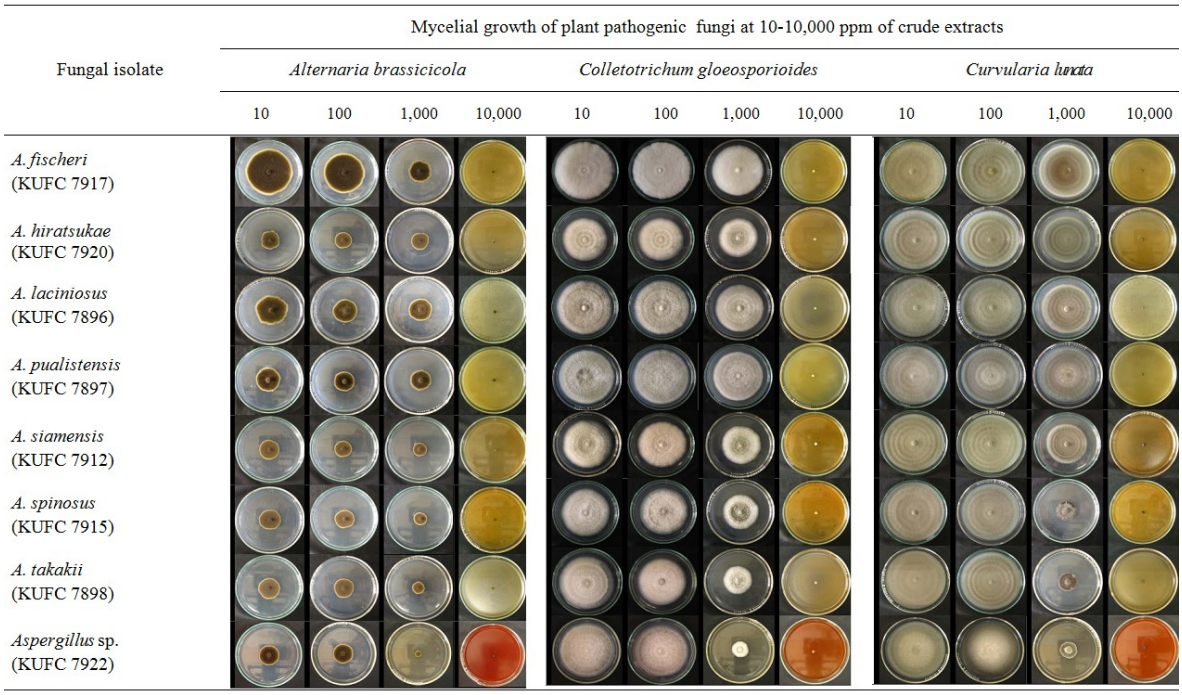

Figure 10 Antagonistic activity tests of four concentrations of crude extracts from eight species of *Aspergillus* against *Alternaria brassicicola*, *Colletotrichum gloeosporioides* and *Curvularia lunata* incubated on PDA at 28˚C for 7 days.

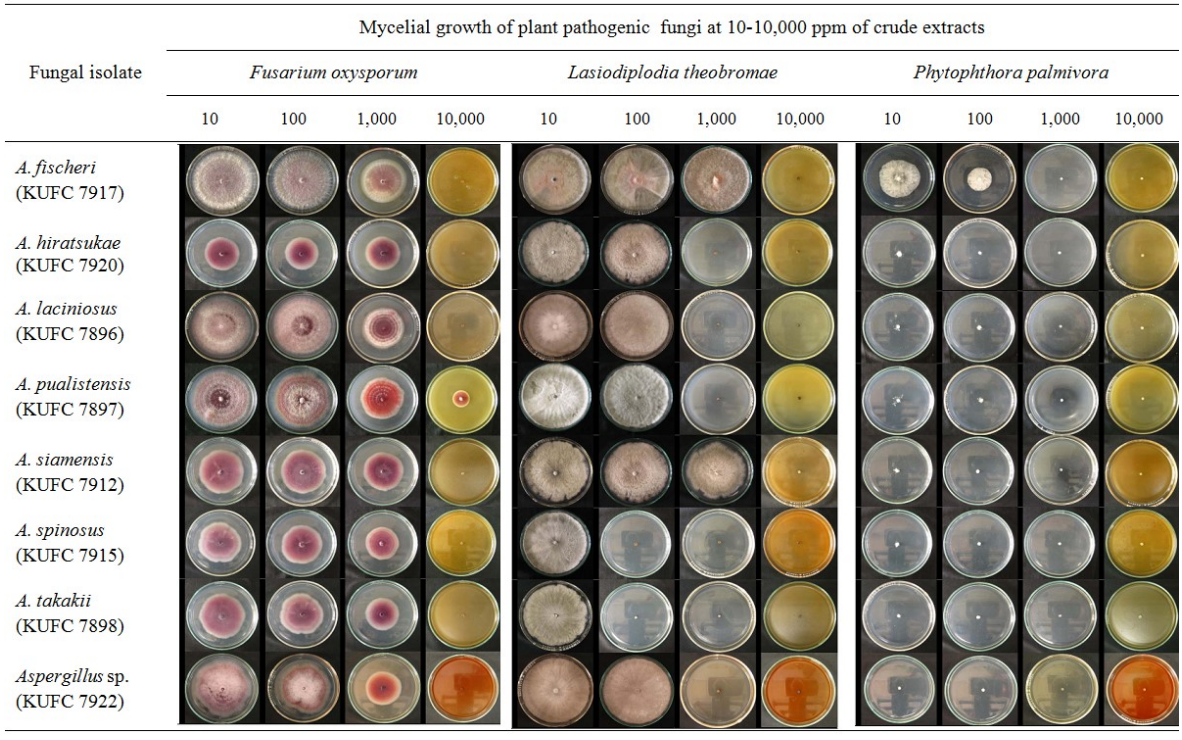

Figure 11 Antagonistic activity tests of four concentrations of crude extracts from eight species of *Aspergillus* against *Fusarium oxysporum*, *Lasiodiplodia theobromae* and *Phytophthora palmivora* incubated on PDA at 28˚C for 7 days.

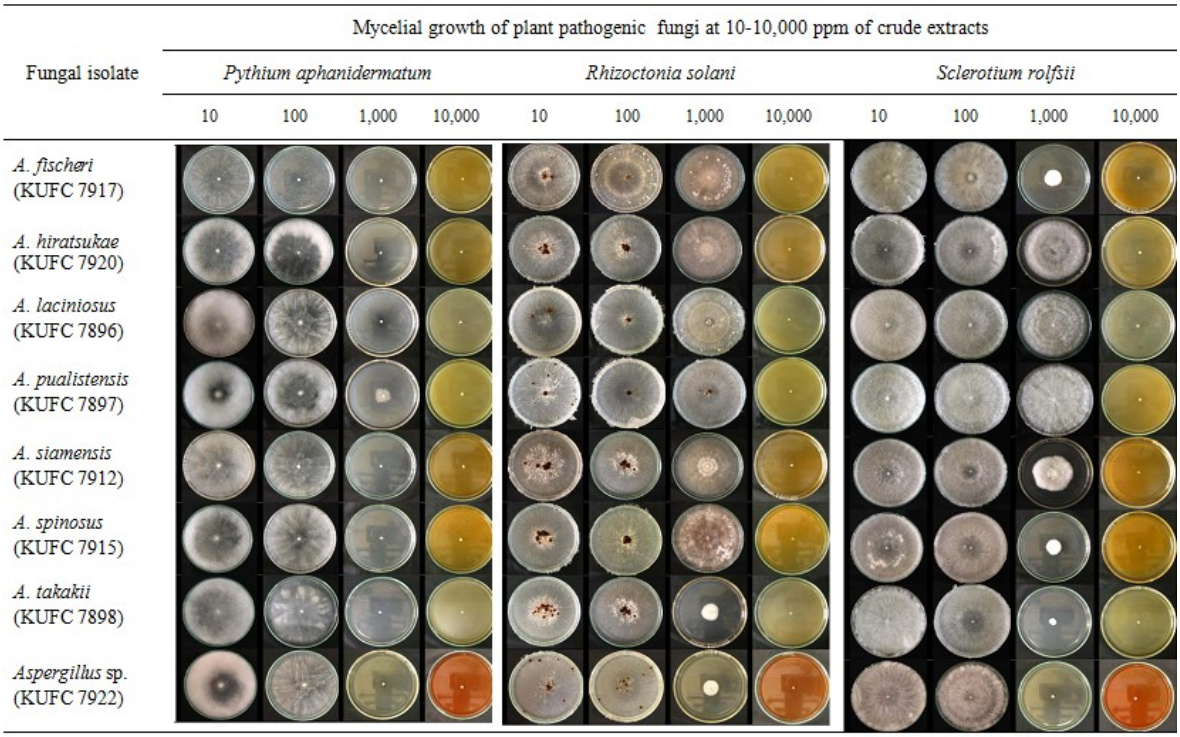

Figure 12 Antagonistic activity tests of four concentrations of crude extracts from eight species of *Aspergillus* against *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rolfsii* incubated on PDA at 28˚C for 7 days.

Conclusions

Two hundred and twenty-four samples of marine organisms mainly sponges and sediment were collected from nine islands of four provinces in the Gulf of Thailand and the Andaman Sea. Twenty seven isolates of *Aspergillus* section *Fumigati* were obtained including seven known species and one unidentified species, including *Aspergillus fischeri, A. hiratsukae, A. laciniosus, A. pualistensis, A. siamensis, A. spinosus, A. takakii* and *Aspergillus* sp. (KUFC 7922). *A. pualistensis* was the dominant species, whereas *A. fischeri* widely distributed at both sites. These fungal species have never been reported in marine habitats except *A. fischeri.* Thus it is the first report of 7 species of *Aspergillus* section *Fumigati* from sponges and other marine organisms from Thailand.

The antagonistic activity tests of eight species of *Aspergillus* section *Fumigati* against nine species of plant pathogenic fungi in dual culture showed that *A. fischeri* inhibited 53.1-98.5% of radial growth of *Alternaria brassicicola, Colletotrichum gloeosporioides, Phytophthora palmivora* and *Pythium aphanidermatum. Aspergillus laciniosus* inhibited 50-88.9% mycelial growth of *A. brassicicola, P. palmivora* and *P. aphanidermatum. Aspergillus pualistensis* provided of 50.6-80.6% growth inhibition of *A. brassicicola, Curvularia lunata, P. palmivora* and *P. aphanidermatum. Aspergillus spinosus* inhibited 56.1- 97.8% mycelial growth of *P. palmivora P. aphanidermatum* and *Rhizoctonia solani. Aspergillus takakii* provided of 54.4-81.1% growth inhibition of *P. Palmivora, P. aphanidermatum* and *Rhizoctonia solani.* However, eight *Aspergillus* spp. failed to inhibit *Sclerotium rolfsii.*

The efficacy of eight *Aspergillus* spp. crude extracts at 10,000 ppm yield completely suppressed 100% mycelia growth nine plant pathogenic fungi, whereas 1,000 ppm concentration suppressed 100% mycelial growth of *P. palmivora* and *P. aphanidermatum.*

In addition, several interesting secondary metabolites and bioactive compounds were recorded from our strains of *A. laciniosus* (KUFC 7896) *A.*

paulistensis (KUFC 7897) and *A. takakii* (KUFC 7898). The crude extracts of these three strains possess antimicrobial and anticancer activities in human colon carcinoma and breast adenocarcinoma. The bioactivity of these extracts suggests a potential for biotechnological applications and the possibility for further investigations on anticancer activities of the marine derived *Aspergillus* spp. beside the mycelial growth inhibition of plant pathogenic fungi.

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