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In vitro antibacterial activity of marine-derived fungi isolated from Pulau Redang and Pulau Payar Marine Parks, Malaysia against selected food-borne pathogens

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Abstract

Marine fungi are potential source of bioactive compounds as indicated by the increasing statistic of research findings. However similar research in Malaysia is still lacking. Hence, this study is undertaken to determine the antibacterial activity of four marine fungal isolates (PR1T4, PP2L4, PR3T13 and PR5T4) from Pulau Redang and Pulau Payar Marine Parks, Malaysia against *Salmonella* Typhi, *Listeria monocytogenes, Staphylococcus aureus*, and *Escherichia coli*. Fungal isolates were first macroscopically and microscopically characterized and later molecularly identified as *Penicillium citrinum, Sacroladium strictum, Aspergillus sydowii* and *Aspergillus* sp. respectively. Solid and broth fermentation of fungi were carried out to produce crude extracts and these extracts were screened for antibacterial activity. In general, solid fermentation extracts (SFE) showed significantly higher antibacterial activity ($p < 0.05$) against all four pathogens compared to broth fermentation extracts (BFE) as the largest inhibitory activity of SFE and BFE was (32.17±0.67 mm) and (27.57±0.81 mm) respectively both on *S. aureus* by *S. strictum*. Highest antibacterial activity against *L. monocytogenes* (27.95±0.81 mm) was exhibited by SFE of *A. sydowii* while SFE of *S. strictum* showed highest activity against *S. aureus* (32.17±0.67 mm) and *E. coli* (23.53±0.57 mm) and SFE of *Aspergillus* sp. showed largest inhibition towards *S.* Typhi (29.30±0.33 mm). These prominent results suggest that all four isolates have potential to be explored as new source of antibacterial agents against food-borne pathogens.

Introduction

Emergence of multiple drug resistant bacteria triggers high demand for new antibiotics and bioactive compounds. Resistant towards antimicrobial compounds caused difficulty in killing bacteria and lead to escalating number of food illness cases for example Salmonellosis by Salmonella Typhi (OzFoodNet, 2010). Findings of new compounds active against pathogenic bacteria are highly desired. For past few decades, terrestrial plants have been explored for bioactive compounds. In order to increase the probability of getting unique metabolites, researchers found marine organism as promising source (Bugni and Ireland, 2004). A large number of new bioactive metabolites have been isolated and identified and their biological activities have been tested (Blunt, 2007; 2008; 2009; 2010).

Marine-derived fungi have proven to be an alternative source of antibacterial, anticancer, anti-inflammatory, antiviral agents and antiplasmodial (Abdel-Lateff *et al.*, 2003; Bugni and Ireland, 2004). *Acremonium* sp. isolated from marine sediment of South East Coast of Tamilnadu, India has antibacterial activity against *E. coli* and the extract was reported to constitute flavonoids, phenols, amino acids, alkaloids and sterols (Samuel *et al*., 2011). Anthraquinone-citrinin isolated from sea fan-derived fungus Penicillium citrinum PSU-F514 displayed moderate antibacterial activity against both *S. aureus* and methicillin-resistant *S. aureus* with equal MIC values of 16 mg/mL (Nanthapong *et al*., 2012). *Fusarium chlamydosporum* producing fusaperazines A and B, was isolated from red alga *Carpopeltis affinis* (Asolkar *et al*., 2002). Gai *et al.* (2007) reported that *Fusarium* sp. (strain 05JANF165) produced antibiotic and antifungal metabolites chemically known as Fusarielin E which is active

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against *Pyricularia oryzae*.

These findings suggested that marine derived fungi could be reservoir of bioactive compounds. However, there is very limited report and literature on the isolation, characterization and bioactivity of marine derived fungi from Malaysia. Hence, this study is carried out to identify four marine fungi isolates from Pulau Payar and Pulau Redang Marine Parks and to screen their antibacterial activities against four foodborne pathogens; *S. aureus, L. monocytogenes, E. coli* and *S.* Typhi.

Materials and Methods

Source of marine fungi

178 fungal strains were isolated from marine organism; soft coral tree, nudibranch, sea fan, tunicate, sea whip, soft sponge, barrel sponge, brittle star, sea anemone, and yellow sponge at Pulau Payar and Pulau Redang Marine Parks. The isolates were cultured and kept in glycerol stock at Bacteriology Laboratory of Fisheries Research Institute (FRI), Batu Maung in Pulau Pinang. Only four isolates; PR1T4, PP2L4, PR3T13 and PR5T4 have been selected for further study. PR1T4 was isolated from tunicate *(Didemnum* sp.) while PP2L4 and PR3T13 were isolated from soft coral tree (*Dendronepthythya* sp.) and PR5T4 was isolated from sea fan. All isolates were sub-cultured from glycerol stock onto fresh Malt Extract agar (MEA) (Merck, Darmstadt, Germany) incubated at 25^{o}C for 7 days.

Marine fungi identification

Identification was carried out through macroscopic and microscopic observation followed by molecular identification. The macroscopic identification was done by observing the characteristics of 7 days old fungi colony on MEA plate. Microscopic identification of fungi was done by dropping a few drops of Lactophenol Cotton Blue stain (LPCB) on a slide and putting a small cut of fungal culture on it before being observed under compound microscope (400 X magnification) and the images of fungi were captured for record. To confirm the species of the fungi, molecular identification was carried out by extracting DNA of the selected fungi using conventional method following Gonzalez-Mendoza *et al*., (2010), DNA extract of marine fungi were subjected to Polymerase Chain Reaction (PCR) for amplification. Primers used were ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCCGCTTATTGATATGC-3' (White et. al, 1990). ITS1 and ITS4 primers amplified the ITS1-5.8S-ITS2 region (~550 base pairs) by PCR and subsequently sequenced. PCR was carried out under the following profile with initial denaturation for 10 minutes at 95°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C. Final step of elongation was carried out at for 10 minutes at 72°C. The reaction mixture was carried out in volume of 50 µl per tube containing 1 × buffer (PROMEGA), 2 mM $MgCl_2$, 200 pmol of each primers (ITS4 and ITS1), 4.0 µl of DNA template, 0.75 Unit of Taq Polymerase (PROMEGA) and 0.08 mM of dNTP. Gel electrophoresis was carried out 100 V for 35 minutes with 1% agarose in 0.5× TBE buffer. PCR products were subjected to gel electrophoresis, followed by sequencing which was carried out by external provider (First Base Sdn Bhd, Malaysia) and the sequence was compared to the reported sequence in database using Basic Local Alignment Search Tool (BLAST) (NCBI).

Preparation of extracts

Marine fungi that have been fermented for 30 days using both solid (MEA) and broth (MEB) medium and later were extracted using ethyl acetate (EtOAc) (3 x 200 ml). EtOAc and water (H_2O) phases were separated using a separation funnel and the aqueous phase is extracted twice with EtOAc. A rotary evaporator was used to dry the EtoAc extract under vacuum at 40°C to give oily residue. The dried extract is stored in the deep freezer prior to use. For antibacterial activity, crude extract was dissolved in methanol (1 mg/ml).

Test microorganisms

Four foodborne pathogens were used in this study Listeria monocytogenes (ATCC 19155), *Staphylococcus aureus, Escherichia coli, Salmonella* Typhi (ATCC 14028), were obtained from the Microbiology Laboratory, Faculty of Food Science and Technology, University of Putra Malaysia culture collection. Stock culture was prepared on Tryptic Soy Agar (TSA) slant (Merck, Darmstadt, Germany) were sub-cultured on every two weeks interval and stored in a chiller (4^{o}C). Bacterial suspensions were prepared by suspending a loopful of cultures into 10 ml of Tryptic soy broth (TSB) (Merck, Darmstadt, Germany) and incubated at 37^{o}C for 24 h.

Antibacterial activity determination

Antibacterial activity was done using to agar diffusion assay (Bennett *et al*., 1966). Food-borne pathogens were sub-cultured in TSB and incubated at 37^{o}C for 24 hours. Bacterial suspensions were prepared in 0.85% saline solution and the

concentration of bacterial inoculums was determined according to McFarland standard. The final concentration of each suspension was 10^8 CFU/ml (CLSI, 2006). A volume of 100 µl of bacterial suspension was spread over the surface of Mueller Hinton agar (MHA) (Merck, Darmstadt, Germany) using sterile cotton swab. A sterile cork borer (6mm in diameter) was used to punch hole and agar plug was removed. 100 µl (1mg/ml) of the crude extract was loaded into well. All assays were performed in triplicates. Chloramphenicol (30 µg) disc and 30 ul of methanol were used as control. The antibacterial activity was determined by measuring clear inhibition zone (in mm) around the well after 24 h of incubation at 37°C by using digital calliper (Fisher Scientific).

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts was determined using micro-dilution method according to the National Committee for Clinical Laboratory Standards (CLSI, 2006). MIC is the lowest concentration of crude extract that gave a reduction in growth compared with control while MBC is the lowest concentration yielding negative subcultures or only one colony on plating solid media.

Statistical analysis

The significance differences was determined by one-way analysis of variance (ANOVA) and followed by Duncan's multiple range tests (DMRT) for means comparison. All the statistical analysis was performed by using IBM SPSS Statistics (Statistical Product and Service Solution) Version 16.0 for Windows (SPSS Inc; Chichago III). Values of $p<0.05$ were considered statistically significant.

Results and Discussion

Macroscopic and microscopic identifications

The macroscopic and microscopic features of four fungi strains after 7 days of growth are tabulated in Table 1. PR1T4 is a fast growing fungus. The colonies are filamentous which is velvety in texture. The colonies are initially white for first few days and become dark green in time due to the production of dusty and dark green spores in enormous quantities. The colony is white at the periphery and has circle shape with undulate margin. The elevation of colony is crateriform which is thin at the center of colony and raised towards the edge of colony while the colour intensity fillowed the same pattern. Yellowish colour can be observed from the back of agar plate as the colony produced yellow soluble pigments. Microscopic identification showed hyphae of PR1T4 is one staged branched (biverticillate) appeared

Table 1. Macroscopic and microscopic identification of fungi isolates

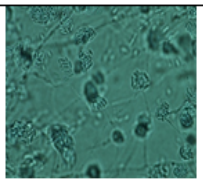
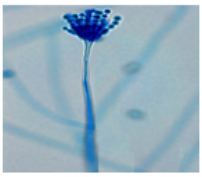
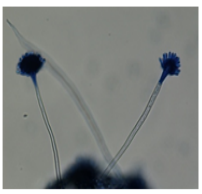
No	Isolates		Characteristic
1.	PR1T4		-Opaque -Single colony has circle shape and white at periphery. -Crateriform elevation -Margin is undulate -Wrinkled and velvety texture -Green from front view and brownish yellow from back view -Dark green spores in enormous quantity and dusty -Yellow soluble pigments
		 60X magnification	-One stage branched (biverticillate) -Hyaline and septate hyphae -Vacuolated hyphae -Flask-shaped phialides -Phialides form brush-like clusters which are also referred to as "penicilli". -Conidia are round, unicellular, and visualized as unbranching chains at the tips of the phialides.
2.	PP2L4		-Opaque -Single colony has circle shape -Umbonate elevation -Margin is entire -Surface is smooth and texture is fluffy -Whitish salmonaceous from front view and pinkish orange from back view. -White spores
		 60X magnification	-Hyphae is hyaline, aseptate -Hyphae is extremely delicate and septate with unbranched, delicate phialides extend at right angles from hyphae. -Conidia are oblong, septate and resemble clustered grains of rice known as mucoid heads. -Conidia are easily disrupted and naked phialides and scattered conidia can be identified.
3.	PR3T13		-Opaque -Single colony has irregular shape -Umbonate elevation -Margin is undulate -Wrinkled and rough -Green from front view and reddish brown from back view -Green spores -Spores are in enormous quantity and dusty -Dark brown soluble pigments - Texture is velutinous (dense, silky hairy).
		 100X magnification	-The long, septate abd smooth-walled stipes which bear the conidiophores are hyaline or slightly brownish. -The conidial heads are effuse/ radiate (spread out) -Conidiogenous cells are biseriate. -Produces rather long conidiophore stipes which gives the colonies that woolly or hairy appearance.
4.	PR5T4		-Opaque -Single colony has irregular shape with white periphery -Flat elevation -Margin is undulate -Wrinkled and rough -Green from front view and brown from back view -Green spores -Spores are in enormous quantity and dusty -Light brown soluble pigments
		 100X magnification	-Hyaline and septate hyphae -Conidiogenous cells are biseriate with globose shape vesicle. -The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. -Flask-shaped phialides. The phialides are biseriate. -Over the phialides are the round conidia forming radial chains.

Table 2. Inhibition zones of solid fermentation and broth fermentation extracts of four marine-derived fungi against *S. aureus, L. monocytogenes, E. coli* and *S.* Typhi

Fungal Extracts		Inhibition zones diameter (mm)			
		S. aureus	*L. monocytogenes*	*E. coli*	*S.* Typhi
P. citrinum	1	19.41±0.56^{b}	23.77±1.77^{d}	18.06±0.16^{c}	26.08±0.19^{e}
	2	16.36±0.35^{a}	21.45±0.21^{bc}	15.75±0.71^{b}	18.80±0.65^{c}
S. strictum	1	32.17±0.67^{g}	25.53±0.85^{e}	23.53±0.57^{f}	19.86±0.48^{c}
	2	27.57±0.81^{e}	22.30±0.78^{cd}	16.52±0.47^{b}	14.95±0.31^{a}
A. sydowii	1	29.37±0.97^{f}	27.95±0.81^{f}	20.30±0.64^{e}	24.8±0.85^{d}
	2	22.60±0.62^{d}	21.3±0.5^{bc}	19.3±0.31^{d}	16.8±0.40^{b}
***Aspergillus* sp.**	1	20.20±0.81^{bc}	21.80±0.65^{c}	18.30±0.39^{c}	29.30±0.33^{f}
	2	15.82±0.49^{a}	17.27±0.38^{a}	13.30±0.92^{a}	24.30±1.27^{d}
Chloramphenicol		20.77±0.3^{c}	20.00±0.6^{b}	25.30±0.5^{g}	30.70±0.9^{g}

*[1]- extract from solid fermentation
*[2]-extract from broth fermentation
*Values are mean ± S.D (mm) (n=3)
Different letters column indicate significantly ($p < 0.05$) different between fungal extracts
*Positive control: Chloramphenicol 30µg/disc

hyaline, septate and vacuolated. Conidia and phialides could be observed and directly attached to branch as metulae is absent. Phialides are flask-shaped and form brush-like clusters which are also known as "penicilli". Unbranching conidia come with round shape and unicellular at the tips of phialide in chain arrangement. All these characteristics matched with previous reports by Larone (1995) and St-Germain and Summerbell (1996).

PP2L4 is a slow growing fungus. The colonies were initially hyaline at first and become whitish salmonaceous after 3 days. Colonies of PP2L4 have a smooth, yeast-like appearance (slimy-gabrose) for first 2 days and changed to to fluffy, smooth and floccose on the next day. The elevation of colony is umbonate which is thick at the center of colony and become thinner towards the edge of colony. Single colony of PP2L4 has circle shape with entire margin. The colonies are whitish salmonaceous from the front view due to production of whitish spores whilst the reverse dominated by pinkish orange. Microscopic view of PP2L4 showed that the isolate has conidiogenous cells with adelophialides or reduced phialides where conidia attached directly at hyphae at right angles. Hyphae are septate and extremely delicate. The shape of conidia is oblong and look-like clustered grains of rice commonly known as mucoid heads. The cluster of conidia is easily disrupted where naked phialides and scattered conidia can be identified. These findings matched with previous study by Gams and Hawksworth (1976) and this isolate can be pre-assumed as belong to genus *Acremonium*.

PR3T13 is a moderate growing fungus. Single colony of PR3T13 is wrinkled and has irregular shape with undulate margin. Texture of colony is velutinous which is dense and silky hairy. The elevation of colony is umbonate which is thick at the center of colony and become thinner towards the edge of colony. The colonies are green with white periphery from upper part of agar plate whilst the reverse is reddish brown. Green spores were produced in enormous quantity and dusty. The culture produces dark brown soluble pigments. Microscopic view of PR3T13 showed the isolate has long, septate and smooth-walled stipes which gives the colonies woolly or hairy appearance. Conidiophores are slightly brownish. Conidial heads are radiate or spread out. Conidiogenous cells are uniseriate where presence of phialides and globose vesicle can be observed. The spores are in chain-like arrangement (radial).

PR5T4 is a moderate growing fungus. Single colony of PR5T4 is wrinkled and has irregular shape with undulate margin. Texture of colony is velutinous which is dense and silky hairy. The elevation of colony is flat. Culture on solid media is pale green on the upper part of plate whilst the reverse is light brown due to production of soluble pigments. The colony is white at the periphery. The spores are green in colour, enormous quantities and appear dusty. Observation under compound microscope revealed septate and hyaline hyphae. Conidial heads are radiate or spread out. Conidiogenous cells are uniseriate where presence of flask-shaped phialides and globose vesicle can be observed. Round conidia form over the tip of phialides in radial chain arrangement. All these characteristics matched with previous report by St-Germain and Campbell (1996) and Robert *et al.* (2004).

Molecular identification

Results from gel electrophoresis showed a single

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Marine fungi extracts (broth and solid fermentation) against food-borne bacteria

Extracts		Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (mg/ml)							
		S. aureus		*L. monocytogenes*		*E. coli*		*S.* Typhi	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
P. citrinum	[1]	9.375	18.75	18.75	18.75	18.75	18.75	9.375	18.75
18.75	[2]	18.75	18.75	18.75	18.75	18.75	15.00	18.75	37.50
S. strictum	[1]	9.375	18.75	9.375	18.75	18.75	18.75	18.75	18.75
	[2]	18.75	18.75	9.375	18.75	37.50	37.50	37.50	37.50
A. sydowii	[1]	18.75	18.75	18.75	18.75	18.75	18.75	18.75	18.75
	[2]	18.75	37.50	37.50	37.50	37.50	37.50	37.50	37.50
18.75*Aspergillus* sp.	[1]	18.75	18.75	18.75	18.75	18.75	37.50	18.75	37.50
	[2]	18.75	18.75	18.75	37.50	37.50	37.50	18.75	37.50
Chloramphenicol		0.02	0.08	0.008	0.02	0.08	0.20	0.003	0.04

[*1]- extract from solid fermentation
[*2]-extract from broth fermentation
[*]Positive control: Chloramphenicol 30µg/disc

band observed with the primer pair of ITS1/ITS4 at approximately 500 bp region. Sequences resulted from sequencing process have been compared with existed sequence in database using BLAST software and all strain showed more than 90% in similarity. PR1T4, PP2L4, PR3T13 and PR5T4 have been identified as *Penicillium citrinum, Sarocladium strictum, Aspergillus sydowii* and *Aspergillus* spp., respectively.

Antibacterial activity of crude fungal extracts

In vitro antibacterial activity of crude extracts of four selected marine derived fungi; PR1T4 *(Penicillium citrinum)*, PP2L4 *(Sarocladium strictum)*, PR3T13 *(Aspergillus sydowii)* and PR5T4 (*Aspergillus* spp.) is tabulated in Table 2. Solid fermentation extracts (SFE) displayed significantly higher inhibition zones against all tested microorganisms (gram positive and gram negative) compared to broth fermentation extract (BFE). The results suggested that the antimicrobial compound (s) of the extract is intracellular rather than excreted out or extracellular.

SFE of *P. citrinum* (PR1T4) exhibited significantly highest ($p<0.05$) antibacterial activity against *S.* Typhi with diameter of inhibition zones of 26.08±0.19 mm followed by *L. monocytogenes* (23.77±1.77 mm) and *S. aureus* (19.41±0.56 mm). The least (SFE) antibacterial activity was demonstrated against gram negative bacteria, *E. coli* with diameter of inhibition zones of 18.06±0.16mm. BFE of *P. citrinum* (PR1T4) had significantly highest ($p<0.05$) antibacterial activity against *L. monocytogenes* (21.45±0.21 mm), followed *S.* Typhi (18.80±0.65 mm), *S. aureus* (16.36±0.35 mm) and *E. coli* (15.75±0.71 mm).

On the other hand, both SFE and BFE of *S. strictum* (PP2L4) showed significantly highest ($p<0.05$) antibacterial activity against *S. aureus* (32.17±0.67 mm) and (27.57±0.81 mm) compared to other bacteria tested and the significantly lowest ($p<0.05$) antibacterial activity was on *S.* typhi (19.86±0.48 mm) and (14.95±0.31 mm). SFE of *A. sydowii* (PR3T13) had significantly highest ($p<0.05$) antibacterial activity against *S. aureus* (29.37±0.97mm) followed by *L. monocytogenes* (27.95±0.81 mm), *S.* Typhi (24.8±0.85 mm) and the significantly lowest activity ($p<0.05$) was against *E. coli* (20.30±0.64 mm).

For last isolate *Aspergillus* sp. (PR5T4), both SFE and BFE had significantly highest ($p<0.05$) antibacterial activity against *S.* Typhi with inhibition zone of 29.30±0.33 mm and 24.30±1.27 mm respectively. Both SFE and BFE extract had significantly lowest ($p<0.05$) antibacterial activity against *E. coli* (18.30±0.39 mm) and (13.30±0.92 mm). *P. citrinum* and *Aspergillus* sp. were most active against gram negative bacteria (*S.* typhi) while *S. strictum* and *A. sydowii* were most active against gram positive bacteria *(S. aureus)*.

Inhibition against gram positive bacteria was better compared to gram negative bacteria as highest inhibition zone on gram positive *(S. aureus)* was (32.17±0.67 mm) by *S. strictum* and highest inhibition zone on gram negative (*S.* Typhi) was (29.30±0.33 mm) by *Aspergillus* sp. *S. strictum* has the best antibacterial activity on both gram positive and negative bacteria while *P. citrinum* has least antibacterial activity compared to other extracts. *E. coli* and *S.* Typhi are classified as Gram negative food-borne pathogens and least activity of SFE and BFE of fungal isolates against these bacteria was due to the presence of multilayered cell wall of peptidoglycan

and outer lipopolysaccharide membrane of Gram negative bacteria which contain cell envelope while Gram positive bacteria consist of thick monolayer of peptidoglycan cell wall only (Bagamboula *et al*. 2004; Weerakkody *et al*., 2010). This cell envelope has made Gram negative of bacteria became more resistant towards inhibitory agents.

The results of MIC and MBC of the marine fungi against four foodborne pathogens are tabulated in Table 3. SFE of *P. citrinum* demonstrated lowest MIC and MBC (9.375 mg/ml) on *S. aureus* and *S.* Typhi while the BFE of *P. citrinum* show same value for both MIC and MBC (18.75 mg/ml) against all tested bacteria except for both *E. coli* and *S*. Typhi where the MIC was 18.75 mg/ml and MBC was 37.50 mg/ml. SFE of *S*. strictum had lowest MIC (9.375 mg/ml) against both *S. aureus* and *L. monocytogenes* and for other two bacteria, the value was 18.75 mg/ml. All four bacteria share the same value for MBC (18.75 mg/ml). On the other hand, the lowest MIC for BFE of *S. strictum* was against *L. monocytogenes* (9.375 mg/ml), followed by *S. aureus* (18.75 mg/ml) and 37.50 mg/ml for both *E. coli* and *S.* Typhi. The lowest MBC for BFE of *S. strictum* was 18.75 mg/ml on *S. aureus* and *L. monocytogenes* while for *E. coli* and *S.* Typhi the MBC was 37.50 mg/ml.

SFE of *A. sydowii* had same MIC and MBC value against all four bacteria (18.75mg/ml). For BFE of *A. sydowii*, the MIC was 37.50 mg/ml for all tested bacteria except *S. aureus* (18.75 mg/ml) and the all tested bacteria were completely killed (MBC) at concentration of 37.50 mg/ml. The MIC for SFE of *Aspergillus* sp. was 18.75 mg/ml for all four bacteria while the MBC was 18.75 mg/ml for *S. aureus* and *L. monocytogene*s and 37.50 mg/ml for *E. coli* and *S.* Typhi. According to differences between MIC and MBC, where MBC was higher compared to MIC with the ratio of 2:1, we can conclude that all these extracts had bactericidal effect against all tested bacteria. None of extracts have same or greater MIC and MBC value compared to Chloramphenicol.

Most of extracts showed comparable and in some cases better antibacterial activity than Chloramphenicol. For instance, both SFE and BFE of *S. strictum* and *A. sydowii* show greater inhibition against *S. aureus* compared to Chloramphenicol. Similarly for *L. monocytogenes*, all SFE and BFE of fungal isolates (except *Aspergillus* sp.) had higher antibacterial activity compared to control. This finding can be considered very promising since the ethyl acetate extracts were crude and produced by non-optimized fermentation which normally had poor yield of active compounds (Takahashi *et al.*, 2008).

From other marine sources have been reported, for example methanolic extracts of *Sarocladium* sp. isolated from South East Coast of Tamilnadu, India had been reported with low antibacterial activity against *E. coli* (11.00 mm) (Samuel *et al*., 2011). Comparatively our findings indicated higher inhibition by *S. strictum* against *E. coli* which is 23.53± 0.57 mm. Similarly, fermentation broth of the marine-derived fungus *Aspergillus* sp., isolated from the sponge *Xestospongia testudinaria* (Petrosiidae) collected from the South China Sea, yielded (Z)-5-(hydroxymethyl)-2-(6′-methylhept-2′-en-2′-yl) phenol have strong antibacterial activity against *E. coli* (10.00 mm) (Li *et al*., 2012) support result exhibited by this study; *E. coli* (18.30±0.39 mm). SFE of *A. sydowii* had greater inhibition zone (29.37±0.97 mm) compared to *A. sydowii* strain W4-2 isolated from the sponge *Agelas* sp. (27 mm) (Abd El-Hady *et al.*, 2014). Penicillanthranins A isolated from the sea fan-derived fungus *Penicillium citrinum* PSU-F51 displayed moderate antibacterial activity against *Staphylococcus aureus* with MIC of 16 µg/ml (Khamthong *et al*., 2012).

Ethyl acetate was used as extraction solvent due to the ability of this solvent in extracting many types of water-soluble organic compounds; alkaloids, polyketides, sugars, shikimates, amino acids, polyhydroxysteroids and saponins, thus, there will be higher possibility of getting bioactive compounds (Duarte *et al*., 2012). Furthermore, most present alkaloids, shikimates and polyketides known with antibacterial activity which is main target for this study. All these findings supported the fact that all four isolates might consist of antibacterial compounds and have great potential to be explored as inhibitory agents against food-borne pathogens.

Conclusion

PR1T4, PP2L4, PR3T13 and PR5T4 have been successfully identified as *P. citrinum, S. strictum, A. sydowii* and *Aspergillus* sp. respectively. Generally, most solid fermentation extracts in the present study have better antibacterial activity against the pathogens tested compared to broth fermentation extracts and comparable to commercial antibiotic. These findings suggested that these fungi have potential to be further explored and further study must be done to identify the active constituents contribute for antibacterial activity. Optimization of fermentation, purification and identification of pure compounds are also recommended for future study.

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