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Isolation and characterization of pigmented bacteria showing antimicrobial activity from Malaysian marine environment

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ABSTRACT

Aims: Natural products play a prominent role in the discovery of leads for the development of drugs in the treatment of human diseases. Much of nature remains to be explored, especially marine and microbial environments.
Methodology and results: Fifty-five pigmented marine bacteria were isolated from sponges, seawater, mangrove sediment, sea cucumber and mussel from different coastal area of Malaysia. The antimicrobial activities of these bacteria were investigated by disk diffusion method against pathogenic bacteria. Out of 55 isolates, 18 isolates exhibited antimicrobial activity, which based on morphological characterization, 53% of them were Gram positive and 47% were Gram negative. All active isolates were able to tolerate more than 4% NaCl in the nutrient agar medium that indicated they were autochthonous to marine environment and moderate salt tolerant in nature. Molecular identification of isolates by the strong antimicrobial activities indicates that isolates WPRA3 (JX020764) and SM11-3j belong to genus *Serratia* and isolate SDPM1 (JQ083392) belongs to genus *Zooshikella*.
Conclusion, significance and impact of study: The results of present study revealed that the active isolates are potential producer of antimicrobial secondary metabolites and might be utilized as drug candidate.

Keywords: marine bacteria, pigmented bacteria, antimicrobial activity, secondary metabolite

INTRODUCTION

Oceans that cover three-quarters of the earth surface are the largest ecosystem in the world. Oceans may serve as the most essential and continuously sources to the discovery of novel microbes and their associated bioactive compounds. The tremendous environment in the ocean can be distributed on the basis of habitats which are at low temperatures (psychrophiles), high salinity (halophiles) and under high pressure (barophiles) (Soliev *et al.*, 2011). These extreme marine environments can be stressful to resident organisms including microbes. Adaptation to these stress factors could involve metabolic changes in the production or secretion of chemical substances some of which could be bioactive. This could result into marine microbes being metabolically different in respect to their terrestrial counterparts.

Nowadays facing multiple drug resistant pathogenic microbes, such as methicillin-resistance *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) is a serious issue. This has led to the investigation and discovery of novel antibiotic from time to time to overcome this problem. The diversity of marine organisms' species and the complex living circumstance surrounding these microbes have resulted into production of novel and unique secondary metabolite with much stronger bioactivities compared with terrestrial organisms (Carte, 1996; Rinehart, 2000; Schwartsmann *et al*., 2001). Hence, there has been an ongoing purposeful search for new potential drugs from the sea for more than two decades (Anand *et al*., 2006). However, in this study, the search for antimicrobial as drug candidate is still in the early stage, likewise screening for antimicrobial activity of marine bacteria around the coastal region of Malaysia. In order to screen for active substance, especially for new compounds, several research studies are currently oriented towards the isolation of new species especially from extreme marine habitats. As for this, in Malaysia, effort to obtain commercialized antibiotic substances, from laboratory to market product requires strong collaboration between researchers from different disciplines such as pharmacology, toxicology, biotechnology and chemistry.

Recently, microbial pigments have been proven to contain active compounds such as *Streptomyces*, an actinomycete that mainly produce red color in their

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colony. Pigment like prodigiosin (red), violacein (violet), and pyocianin (blue-green) are known to have active compounds such as anitimicrobial activity, antiviral, antitumor, antiprotozoa, antioxidant, anticancer and much more (Ferreira *et al.*, 2004; Matz *et al.*, 2004; Deorukhkar *et al.*, 2007; Kim *et al.*, 2010). It is crucial to pin-point the adherence of pigment in bacteria, which is for industrial applications. Pigmented marine natural products can contribute to a variety of applications, from health, cosmetic up to the flavours of food additives, paint industry, fabric dye, and ink manufacture, making it the interesting subject of this study.

The marine environment in Malaysia constitutes a large reservoir of untapped resources for the discovery of bioactive compounds. Malaysia is a maritime country with unique treasures such as the ecosystem of mangroves, mudflats, coral reefs, seaweeds, lagoons and estuaries. Unfortunately, there is less research done on the discovery of biological diversity in these area, particularly in marine bacteria. Owing to this, a study has been carried out to isolate and characterize potential marine bacteria from Malaysian marine environment with potential antimicrobial compounds.

MATERIALS AND METHODS

Isolation of pigmented marine bacteria

Bacterial strains were isolated from different species of marine sponges and sea cucumber from Tinggi Island, mussel from Sungai Merbok (Kedah), mangrove sediment from Morib Beach and Sungai Melayu (Johor), and seawater of Port Dickson and Tinggi Island. Collected samples were rinsed three times with sterile sea water in order to remove the non-attached bacteria. Ten grams of each sample was placed into 100 mL marine broth (MB; Difco, USA) in 250 mL Erlenmeyer flask. All flasks were then incubated on a rotary shaker at 200 rpm for 24 hours at 28 °C. A 10-fold serial dilution of each flask was prepared while 100 µL of each dilution was plated onto marine agar (MA; Difco, USA) and incubated at 28 °C for 7 days. Isolation of pigmented bacteria with different colony characteristics was carried out from third day onwards up to the seventh day. The isolated colonies were repeatedly streaked to obtain pure cultures and maintained on marine agar slant at 4 °C for further studies.

Test microorganisms

The following Gram positive bacteria *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 11632 and methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram negative bacteria (*Aeromonas hydrophila* Ctt6, *Escherichia coli* ATCC 10536*, Pseudomonas aeruginosa* ATCC 27853 and *Vibrio parahaemolyticus*), yeast *Candida albicans* and fungi *Aspergillus fumigatus* were used in this study. The strains were obtained from the Universiti Kebangsaan Malaysia microbial culture collection. They were maintained on nutrient agar (NA; Difco, USA) slants at 4 °C.

Antimicrobial activity

Preliminary screening of antimicrobial activity

Primary screening of antimicrobial activity of all the pigmented bacteria were determined by disk diffusion method described by Bauer *et al.* (1966) with some modifications. In brief, except for SDPM1 isolate, 20 µL of each isolated bacteria grown in MB for 24 h was inoculated into standard paper disk and placed onto Mueller Hinton Agar (MHA; Difco, USA) plates containing the following pathogenic microorganisms *B. subtilis*, *E. coli* and *C. albicans.* As for SDPM1, a single colony of an overnight culture in nutrient agar supplemented with 3% NaCl was inoculated onto standard paper disk and placed onto Mueller Hinton Agar (MHA; Difco, USA) plates containing pathogenic microorganisms, *B. subtilis, E. coli* and *C. albicans.* All the plates were incubated at 37 °C for 24 h. The absence of growth around the disks indicated the sensitivity of the reference microorganisms. Secondary screening for antimicrobial activity was conducted for all positive isolates. All of the experiments were performed in triplicates.

Preparation of crude extract

In order to extract the bioactive compounds, 10 mL of 24 hours old culture of positive pigmented bacteria were prepared by incubating each bacterium into 50 mL MB in 250 mL Erlenmeyer flask at 28 °C in a rotary shaker (150 rpm). Then, 10 mL of enriched culture were transferred as seed into other 250 mL Erlenmeyer flasks containing 100 mL MB and incubated at 28 °C in a rotary shaker (150 rpm) for three days. To isolate the bioactive metabolites, the cell masses were separated by centrifugation at 4,000 rpm for 20 min and the supernatants were extracted with equal volume of ethyl acetate (EA). The solvent layers were collected and then evaporated in a rotary evaporator (Buchi rotavapor R-124) to obtain crude required for second antimicrobial screening.

In addition, because isolate SDPM1 exhibited greater inhibition zone when cultured in solid medium compared to that of from liquid medium, its bioactive compounds was harvested from the agar culture plate as described by Abraham (2004) with some modifications. Approximately 100 mL of nutrient agar was used in this study. In a single plate, the isolate SDPM1 was inoculated onto nutrient agar supplemented with 3% NaCl and incubated for 6 days at 29 °C. The cells were then gently scraped off and washed with sterile seawater by centrifugation at 10,000 rpm for 15 min at 4 °C. The cells pellet were fully mixed with 20 mL EA, protected from direct light with aluminium foil until they were completely bleached. The cells were removed by centrifugation and the EA extract was then evaporated in a rotary evaporator (Buchi rotavapor R-124)

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to obtain crude extracts for using in second antimicrobial activity screening.

Secondary screening of antimicrobial activity

Antimicrobial activity assay of the extracts was done using the disk diffusion method performed on MHA according to Bauer *et al*. (1966). The sensitivity of the test microorganism species to the EA crude extracts of the isolates was determined by measuring the sizes of inhibitory zones on the agar surface around the disks. All of the experiments were performed in triplicates. The results are reported as the average of three experiments. All positive pigmented isolates were subjected to preliminary characterization.

Morphological and physiological characterization of the positive pigmented isolates

Morphological and physiological characterization of active isolates was determined according to Bergey's Manual using isolates' colony observation on MA. Colony morphology of active pigmented bacteria was noted and this was followed by Gram staining. Overnight pure cultures of isolates growing on MA were used for Gram staining. Motility in SIM medium was also tested. Respiratory enzyme tests include oxidase and catalase test were investigated using standard methods described by Kovacs (1956) and Vera and Power (1980) respectively. The NA plates supplemented with different amount of NaCl in the range of 1% to 11% NaCl were used for salt tolerance assay.

Molecular identification of isolates with strong antimicrobial activity

Isolates which exhibited the strong antibacterial activity were chosen for molecular identification. PCR amplification of the 16S rRNA gene was performed using two oligonucleotide primers, forward 5'-CTCCTACGGGAGGCAGCAG-3′ and reverse 5'-WATTACCGCGGCKGCTG-3'. The PCR programme was set using heat thermal minicycler (MJ Research, USA) as follows: initial denaturation was carried out for 5 min at 94 °C. It was followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 1.5 min with a further 10 min extension at 72 °C, using UniversAll™ tissue PCR kit. For SDPM1 and SMII-3j isolates, DNA amplification was performed using primer pair forward 5-'AGAGTTTGATCCTGGCTCAG-3' and reverse 5'-GGTTACCTTGTTACGACTT-3' (Thiel and Imhoff, 2003). The PCR programme was set using heat thermal minicycler (MJ Research, USA) as follows: 95 °C for 2 min (initial denaturation), 50 °C for 30 s (annealing), and 72 °C for 45 s (DNA synthesis). This was followed by 22 cycles of 95 °C for 30 s, 50 °C and 72 °C for 2 min, with a further 10 min at 72 °C for final extension. All the PCR products were purified using QIAquick PCR purification kit (Qiagen, Germany) based on company instruction and were sent to FirstBase Laboratory for nucleotide sequencing.

Sequence comparison and phylogenetic analysis

Nucleotide sequences were analysed by using BioEdit Sequence Alignment Editor. The 16S rRNA gene sequences of the selected isolates were compared with available 16S rRNA gene sequences in the EMBL, GenBank and DDBJ databases using the BLASTn search facility at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using the MEGA version 4.0.2 program (Tamura *et al.*, 2007) for assignment of close relationships at species level.

RESULTS

Isolation of pigmented marine bacteria

A total of 55 pigmented bacteria were randomly isolated from sponge, seawater, mangrove sediment, sea cucumber and mussel around the coast of Malaysia which exhibited 41% of orange colony, 31% yellow, 11% red, 11% pink, 4% blue-green while 2% red and/or violet in colony (Figure 1).

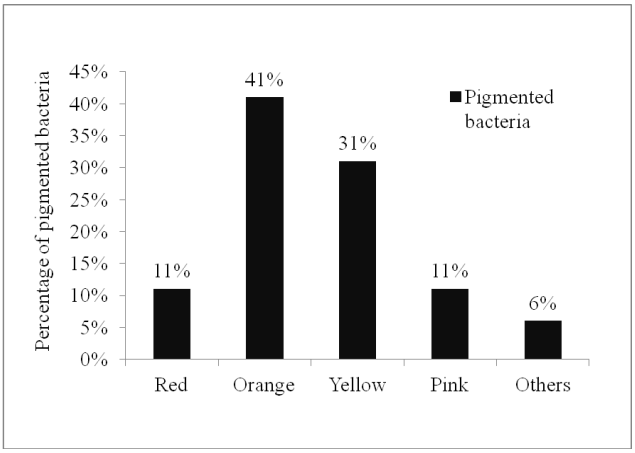

Figure 1: Percentage of the pigmented marine bacteria isolated from marine sources.

Antimicrobial activity

Preliminary screening of antimicrobial activity

Out of the 55 pigmented isolates subjected to preliminary screening for antimicrobial activity, 18 isolates exhibited antimicrobial activity against tested pathogens, that 52% against *E. coli*, 68% against *B. subtilis* and 37% against *C. albicans* (Table 1). From these positive antimicrobial isolates, 44% were isolated from six species of sponges, 22% from seawater, 22% from mangrove sediment, 6% sea cucumber and 6% from mussel (Figure 2).

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Table 1: Primary screening for antimicrobial activity of marine pigmented bacteria.

Isolates/ pathogens	BS	EC	CA
SDPM1	+	+	++
WTG22	++	-	++
SCTGRCW	-	++	++
SM11-3j	++	+	+
SPTOD1	-	+++	+++
SPTYF	-	++	-
SPTOF3	++	++	-
SPTYH4	+	++	-
SPTYI1	+	-	-
SPTYI2	+	++	-
SPTYJ1	++	+	-
SPTOL4	-	+	++
WPRA3	+++	-	-
WPRB4	++	-	-
WPRB6	-	-	++
SDR1	++	++	-
SDGB2	-	+	-
SDON	+++	-	++

+ 5.5-9.9 mm
++ 10.0-14.9 mm
+++ > 15.0 mm,
- : no inhibition zone; BS: *Bacillus subtilis*; EC: *Escherichia coli*; CA: *Candida albicans*

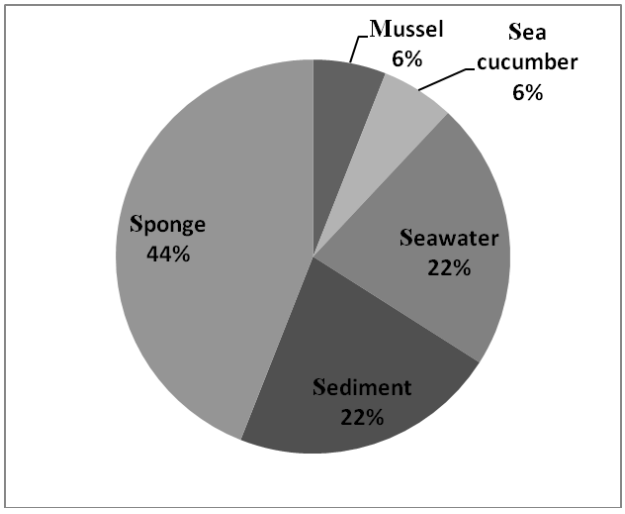

Figure 2: Percentage of the isolated pigmented bacteria with antimicrobial activity from different sources of marine environment.

Secondary screening of antimicrobial activity

A total of 9 test microbes were chosen for secondary screening of antimicrobial activity using EA extract of previous positive isolates. Of the 18 isolates, five isolates namely SDPM1, SM11-3j, SPTOD1, WPRA3 and WPRB4 exhibited antimicrobial activity against more than three test pathogenic microbes while eight isolates (SPTOF3, SPTYH4, SPTYI2, SPTYJ1, SPTOL4, SDR1, SDGB2 and SDON) showed activity against two test microbes. In addition, isolate SPTYF showed specific antimicrobial activity against *E. coli*, while SPTYI1 and WPRB6 specifically inhibited *B. subtilis* and *C. albicans*, respectively (Table 2). Remarkably, all of five isolates that exhibited higher antimicrobial activity were Gram negative. Example of crude extracts inhibitory effect of isolates WPRA3, SM11-3j and SDPM1 against *A. fumigatus, A. hydrophila and B. subtilis* are shown in Figure 3.

Morphological and physiological characterization

Out of the 18 active isolated bacteria, 44% were Gram negative and 56% were Gram positive. Colony morphology of active isolates such as colour and shape of colony was obtained on MA after 24 h. The isolates SPTOD1, SPTOF3, SDON and SPTOL4 were orange in colony, SPTYF, SPTYH4, SPTYI1, SPTYI2 and SPTYJ1 were yellow, SM11-3j, WPRA3, WPRB4, SDR1 and WPRB6 were red, WTG22 was pink, SDGB2 was green while SDPM1 was red (with metallic green sheen) and/ or violet. Cell morphology of the isolates was obtained by microscopic observation. Isolates SPTOD1, WTG22 and SPTOF3 were coccobacilli, isolate SPTYF was coccus, isolate SPTOL4 was streptococcus and the rest were rod in shape. The results of motility and respiratory enzyme tests indicated that 61% of positive isolates were motile, 22% were oxidase positive and 94% were catalase positive. All active isolates were able to tolerate more than 4% NaCl in the NA medium that indicated they were autochthonous to marine environment and moderate salt tolerance in nature. Result of morphological and physiological characterization of the isolates as shown in Table 3.

Molecular characterization

Isolates SDPM1, SM11-3j and WPRA3 which exhibited strong and broad spectrum antimicrobial activity were selected for molecular characterization. The size of PCR products was ~1.5 kb, which were detected with 1% agarose gel electrophoresis. The length of the partial 16S rRNA gene sequence of the isolates SDPM1, SM11-3j and WPRA3 were 1301 bp, 1445 bp and 1477 bp respectively. Comparative 16S rRNA gene sequence analysis showed that the isolate WPRA3 (GenBank accession number JX020764) shared 97% similarities with *Serratia nematodiphila* strain DZ0503SBS1 (EU914257) and *Serratia marcescens* subsp. sakuensi (AB061685), isolate SDPM1 (GenBank accession number

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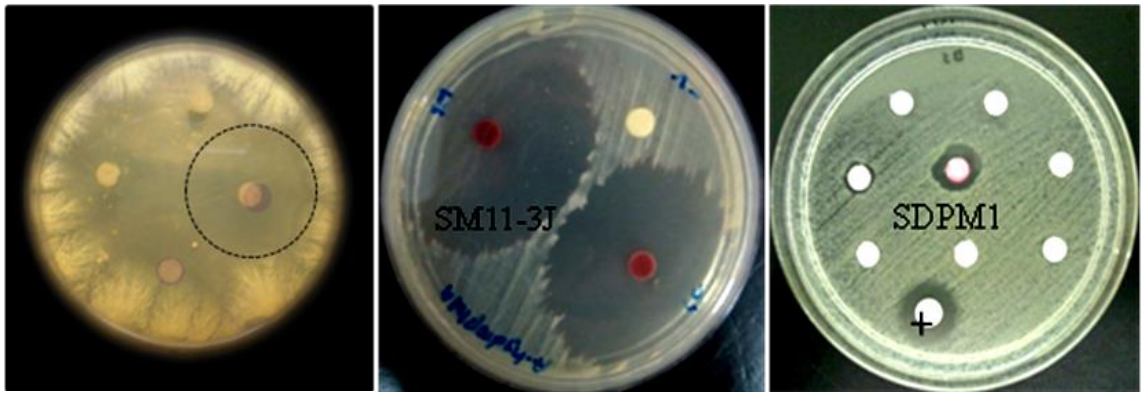
Table 2: Secondary screening of antimicrobial activity from ethyl acetate crude extracts of marine pigmented bacteria.

Pigmented isolates	Inhibition zone (mm) Test microorganisms								
	EC	PA	VP	AH	BS	SA	MRSA	CA	AF
SDPM1	-	-	9	-	9	7.5	9	11.5	-
WTG22	-	-	NT	-	5.5	-	-	-	NT
SCTGRCW	-	-	NT	-	-	-	-	-	NT
SM11-3j	-	NT	-	51	35	15	20	30	NT
SPTOD1	30	-	-	-	-	14	-	28	10
SPTYF	10	-	-	-	-	-	-	-	-
SPTOF3	14	-	-	-	10	-	-	-	-
SPTYH4	11	-	-	-	8	-	-	-	-
SPTYI1	-	-	-	-	8	-	-	-	-
SPTYI2	12	-	-	-	8	-	-	-	-
SPTYJ1	8	-	-	-	10	-	-	-	-
SPTOL4	8	-	-	-	-	-	-	10	-
WPRA3	-	15	35	-	45	25	30	-	32
WPRB4	-	-	20	-	10	10	10	-	-
WPRB6	-	-	-	-	-	-	-	12	-
SDR1	12	-	-	-	10	-	-	-	-
SDGB2	8	-	-	10	-	-	-	-	-
SDON	-	-	-	-	15	-	-	10	-

- : no inhibition zone
NT: not tested; EC: *Escherichia coli*; PA: *Pseudomonas aeruginosa*; VP: *Vibrio parahaemolyticus*; AH: *Aeromonas hydrophila* BS: *Bacillus subtilis*; SA: *Staphylococcus aureus*; MRSA: methicillin-resistant *S. aureus*; CA: *Candida albicans*; AF: *Aspergillus fumigatus*



A B C

Figure 3: Ethyl acetate crude extracts inhibitory effect of the isolate WPRA3 against *A. fumigatus* (A), isolate SM11-3j against *A. hydrophila* (B) and isolate SDPM1 against *B. subtilis* (C).

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Table 3: Morphological and physiological characterization of the positive pigmented isolates.

Isolates	Source	Origin	Gram	Cell morphology	Pigment	OX	CT	% NaCl	M
SDPM1	Sediment	M.B	-	Rod	Red / violet	+	+	6	+
WTG22	Seawater	T.I	-	Coccobacillus	Pink	-	+	6	+
SCTGRCW	Sea cucumber	T.I	-	Coccobacillus	Red	-	+	6	+
SM11-3j	Mussels	S.M	-	Rod	Red	-	+	6	+
SPTOD1	Sponge	T.I	-	Coccobacillus	Orange	-	+	8	+
SPTYF	Sponge	T.I	+	Coccus	Yellow	-	+	6	-
SPTOF3	Sponge	T.I	+	Coccobacillus	Orange	-	+	10	-
SPTYH4	Sponge	T.I	+	Rod	Yellow	-	+	8	-
SPTYI1	Sponge	T.I	+	Rod	Yellow	+	-	8	+
SPTYI2	Sponge	T.I	-	Rod	Yellow	-	+	8	-
SPTYJ1	Sponge	T.I	+	Rod	Yellow	-	+	8	+
SPTOL4	Sponge	T.I	+	Staphylococcus	Orange	+	+	10	+
WPRA3	Seawater	P.D	-	Rod	Red	-	+	6	+
WPRB4	Seawater	P.D	-	Rod	Red	-	+	6	+
WPRB6	Seawater	P.D	+	Rod	Red	-	+	8	+
SDR1	Sediment	S.M.J	+	Rod	Red	-	+	6	-
SDGB2	Sediment	S.M.J	+	Rod	Green	-	+	4	-
SDON	Sediment	S.M.J	+	Rod	Orange	+	+	4	-

T.I: Tinggi Island; P.D: Port Dickson; S.M: Sungai Merbok, Kedah; M.B: Morib Beach; S.M.J: Sungai Melayu, Johor
OX: oxidase; CT: catalase; M: motility

JQ083392) shared 98% similarities with *Zooshikella ganghwensis* strain JC2044 (AY130994) and isolate SM11-3j shared 96% similarities with *Serratia nematodiphila* strain DZ0503SBS1 (EU914257), *Serratia marcescens* subsp. sakuensi (AB061685) and *Serratia marcescens* subsp. marcescens ATCC 13880.

Phylogenetic analysis

Figure 4 shows the tree topology generated using the neighbour-joining method. There were two clades formed, clade 1 consists of the family Enterobacteriaceae and clade 2 consists of the family Hahellaceae with bootstrap values of 100. There were two subclades in clade 1, which are subclade 1a and subclade 1b. Subclade 1a with bootstrap value of 93 consists of the genus *Serratia* and form two clusters, while subclade 1b consists of the genus *Enterobacter* and *Klebsiella*. Clade 2 consists of two subclades, which are subclade 2a and subclade 2b. Subclade 2a consists of the genus *Hahella* while subclade 2b consists of the genus *Zooshikella* with bootstrap values of 100 and form two clusters. *Alteromonas macleodii* strain DSM 6062 (Y18228) was selected as an outgroup.

DISCUSSION

Bacterial isolation led to variety of pigmented isolates, the most were orange in color, followed by yellow, red, pink, blue-green and violet. Pigmentation is widespread among bacteria and pigments found in marine heterotrophic bacteria consist of carotenoid, flexirubin, xanthomonadine, and prodigiosin (Kim *et al.*, 2007). The carotenoids are considered to be the main and most

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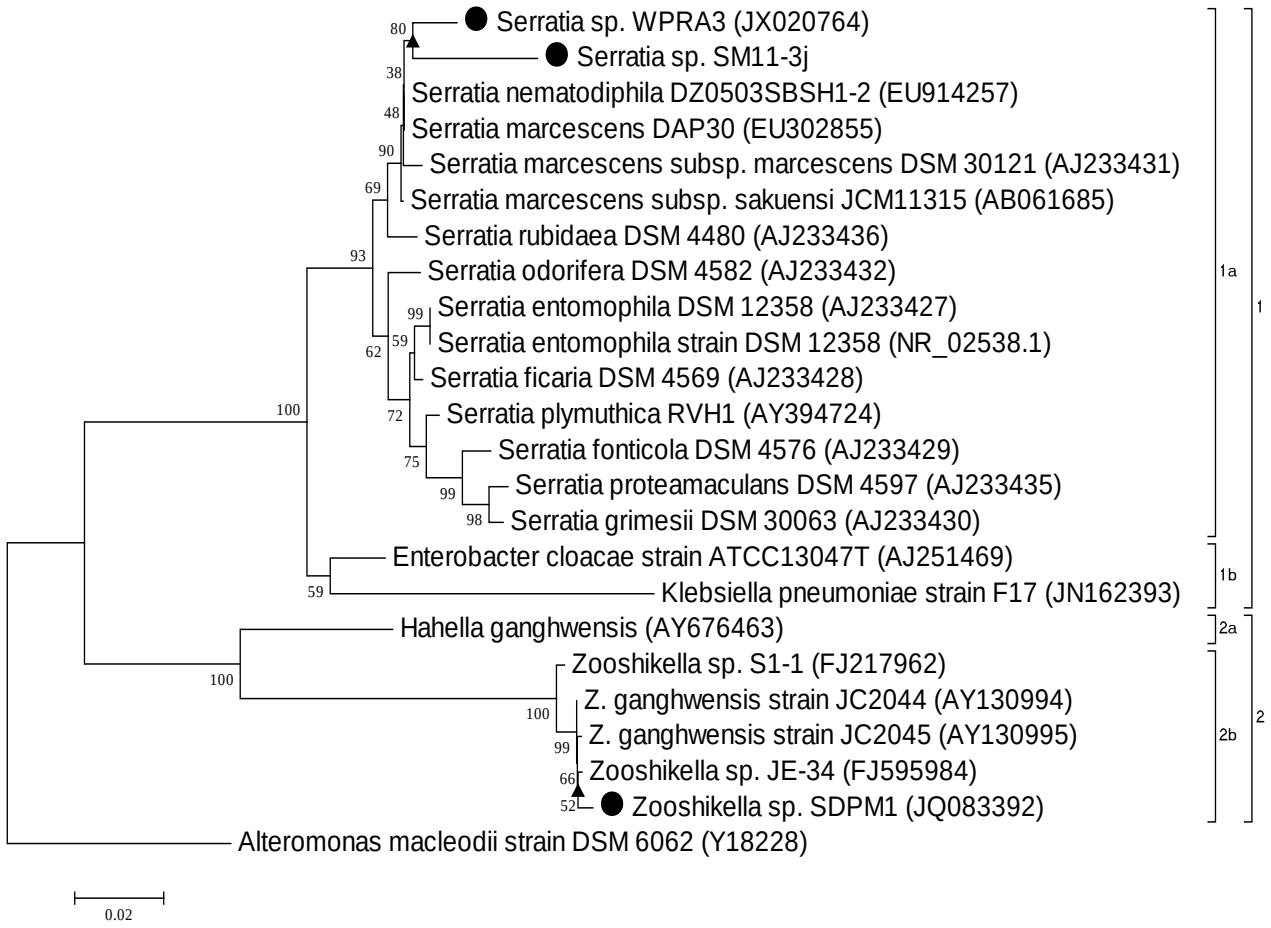

Figure 4: Phylogenetic relationship of *Serratia* sp. WPRA3 (JX020764), *Serratia* sp. SM11-3j and *Zooshikella* sp. SDPM1 (JQ083392) to related bacteria based on neighbour-joining tree analysis of 16S rRNA gene sequence data. Bootstrap values (expressed as percentage of 1000 replications) are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position. *Alteromonas macleodii* strain DSM 6062 (Y18228) was selected as an outgroup.

plentiful pigment groups, which appear orange, yellow or red (Marit *et al.*, 2010). Antimicrobial activity of marine bacteria is a renowned phenomenon and indicated in a number of studies (Lemos *et al.*, 1985; Dopazo *et al.*, 1988; McCarthy *et al.*, 1994). Results showed that bacteria isolated from sponges exhibited the highest antimicrobial activity compared to other sources. Marine sponges are rich sources of natural compounds, which exhibit wide variety of biological activity (De Rosa *et al.*, 2003). Hence, novel microorganisms with potential biological activity were isolated from marine sponges (Hentschel *et al.*, 2001). Moreover, the numbers of bacteria associated with marine sponges are two or three times more than seawater because of the specific surface and internal environmental niche of sponges (Friedrich *et al.*, 2001). In this study Gram negative isolates showed higher antimicrobial activity rather than Gram positive isolates. This could be ascribed to the cell wall differences between these bacteria. A Gram negative cell wall consists of lipopolysaccharide that can act as toxin to protect bacteria against predator (Barnett 1992). The component of lipopolysaccharide can also makes the cell wall impermeable to lipophilic solutes (Pandey *et al.*, 2004), hence gives more protection in Gram negative bacteria. A Gram-positive bacteria cell wall has a thick layer of peptidoglycan and should more susceptible because not an effective permeability barrier (Barnett, 1992; Pandey *et al.*, 2004). Our result also suggested that pigment could involve in antimicrobial activity of isolates. Isolates that produce red pigment have higher antimicrobial activity followed by orange, yellow and green. Pigment that produces by marine bacteria is one of the bioactive compounds that can be isolate. Marine bacteria like *Streptomyces*, *Pseudomonas* and *Vibrio* are able to produce indole derivatives (quinines and violacein) and alkaloids (prodiginines and tambajamines). The red pigmented prodigiosin compounds were first isolated from the ubiquitous bacteria which are *Serratia marcescens*

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and identified as secondary metabolites (Soliev *et al*., 2011). However, further research is suggested to confirm this hypothesis.

Based on 16S rRNA sequences, isolate WPRA3 displayed 97% homology while isolate SMII-3j displayed 96% homology with that of their nearest neighbours, *Serratia nematodiphila* DZ0503SBSH1 (EU914257) and *Serratia marcescens* subsp. sakuensi (AB061685) respectively. The isolate WPRA3 and SMII-3j were rod shaped bacteria and motile. They produced a colony on an agar plate in which the color was red. Grimont and Grimont (1991) reported that the three species of the genus *Serratia*, *S. marcescens*, *S. rubidaea* and *S. plymuthica* able to produce a reddish pigment called prodigiosin under specific conditions. Furthermore, both isolates, WPRA3 and SMII-3j were Gram negative bacteria and demonstrated oxidase negative and catalase positive, the basic characteristics of genus *Serratia.* The phylogenetic tree based on the 16S rRNA sequences as shown in Figure 4 indicates that both isolates, WPRA3 and SMII-3j have close relationship with the genus *Serratia.* These results suggested that isolates WPRA3 and SMII-3j can be classified in the genus *Serratia.* The isolate SDPM1 was rod shaped bacterium that demonstrated oxidase and catalase activities and produce red and/ or violet colony on an agar plate. Based on 16S rRNA sequences, isolate SDPM1 shared 98% similarities with its nearest neighbours, *Zooshikella ganghwensis* strain JC2044 (AY130994). Although isolate SDPM1 showed close relationship to the genus *Zooshikella* sp. JE-34 (FJ595984) in the phylogenetic tree (Figure 4), this isolate was suggested as a member of a new species in the genus *Zooshikella*. This was mainly based on the pigment production which recently known that *Zooshikella* able to produce red and yellowish-red but not violet pigment (Yi *et al*., 2003; Lan *et al*., 2008; Kim *et al*., 2010). Further identification of SDPM1 isolate is undergoing in order to confirm this suggestion.

CONCLUSION

A total of 55 marine pigmented bacteria had been isolated and were deposited in the university's culture collection. 18 isolates having antimicrobial activities and five of them were strongly active against pathogens tested and were suggested as a potentially drug candidate. Marine environment in Malaysia have good potential to serve as a source for the discovery of natural product. The understanding of biological properties of microbial pigment from environment in Malaysia not only make us curious about the pigment but also think that pigmented bacteria can provide a good bioactive compound or therapy for the other pathogens. The study about these pigmented bacteria also can contribute to medical or industrial uses like as a colorant or drugs. Further isolation or characterization of this bioactive compound from these pigmented marine bacteria will proceed and investigate.

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