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Marine actinomycetes from Malaysia marine environments with antagonistic potential

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ABSTRACT

Marine actinomycetes have garnered the attention of researchers worldwide due to their ability to produce unique and novel bioactive compounds. This study aimed at assessing the diversity and antagonistic potential of marine actinomycetes isolated from Langkawi Island, Kedah and Tioman Island, Johor. In a total of 215 isolates were successfully recovered of which 52% were obtained from Starch–yeast Extract Agar (SYE), 31.4% from Marine Agar (MA) and 16.3% from Actinomycetes Isolation Agar. Fourteen isolates were found to be positive of Polyketide Synthase Type I (PKS-I) and/or Non-ribosomal Peptide Synthetases (NRPS) genes and these isolates were then subjected to antimicrobial evaluation test against 3 Gram-positive bacteria – *Staphylococcus aureus*, Methicillin-resistant *S. aureus*, *Bacillus subtilis*; 4 Gram-negative bacteria – *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens*; and 2 fungi – *Candida albicans*, *Candida parapsilosis*. Most isolates were able to inhibit at least one test microorganism. There is, however, no direct correlation between the presence of the biosynthetic genes and antagonistic potential as demonstrated by the lack of antimicrobial activity though PKS-I and/or NRPS genes were present. Molecular identification using the 16S rRNA gene revealed 10 isolates belonging to the genus *Streptomyces* and each representative of the genera *Actinomadura*, *Rhodococcus*, *Gordonia* and *Salinispora*. Among the isolates, *Streptomyces* sp. T55 is a potent antibacterial actinomycete while *Streptomyces* sp. T109 is a good antifungal agent. Further characterization of marine actinomycetes was conducted using scanning electron microscopy, culture characteristics on several media (SYE, MA, Potato Dextrose Agar, ISP2, ISP3, ISP4, Czapek Agar), NaCl tolerance and carbon sources utilization profile using BIOLOG. Findings from this study demonstrate the potential of Malaysia's marine environment as a new resource of actinomycetes with biosynthetic capabilities which can be further explored for the development of the natural product.

Keywords: Marine; Actinomycetes; Diversity; Antagonistic

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1. Introduction

Actinomycetes are gram-positive bacteria with high G + C content. They have a long history in antibiotics production due to their extraordinary capabilities which makes them an absolutely important group of bacteria. Actinomycete's ability to produce compounds with biologically active properties precede other groups of bacteria which make them desirable for researchers to exploit. Actinomycetes are common soil inhabitants, however, over-exploitation of terrestrial counterpart had led to exhaustive cultivars and re-discovery of same compounds. Thus, new resources of actinomycetes are vital as a solution to this problem. Among the locations include sand dunes, mangroves and oceans.

The ocean is the largest ecosystem available for many organisms including microbes. Once thought to be devoid of microbes, the ocean is actually a rainforest of diversity containing more than 10 million species of which more than 60% are unknown [1]. Marine microbes can thrive in different depths of the ocean ranging from surface waters of the sea to the lower and abyssal depths, and from coral reefs to thermal vents at the seafloor. They also can be recovered from marine organisms such as corals, fishes, sponges, seaweeds and many more. In the marine ecosystem, marine microbes play a crucial role in decomposing and recycling organic matter as well as being a part of the food chain. Marine environments are relatively underexplored resources of actinomycetes. Though, many have proposed that actinomycetes present in the marine environments are probably originated from the terrestrial which has adapted to the marine environments, the discovery of the new obligate marine actinomycete belonging to the new genus *Salinispora* [2] demonstrated that true indigenous marine actinomycetes do exist in the ocean. Moreover, continuous isolation of new genera of marine actinomycetes such as *Spongiactinospora* [3], *Koreibacter* [4], *Marinactinospora* [5], *Sciscionella* [6], *Ponticoccus* [7] and *Serinicoccus* [8]; and new marine-derived species which include members of the genera *Actinoplanes* [9], *Saccharopolyspora* [10], *Streptomyces* [11], and *Williamsia* [12] further supported the existence of indigenous marine actinomycetes. The discovery of new taxa and new marine-derived species, together with their ability to produce unique metabolically active compounds demonstrate the actinomycete's active involvement in the marine microbial communities. Their persistence and stable populations in various habitats such as marine sediment and marine organisms, clearly indicate that indigenous marine actinomycetes indeed exist in the oceans.

Marine actinomycetes are able to adapt to a variety of living conditions in the marine environments such as high salinity, different range of temperature, pressure and pH. These living conditions have an influence on the genetic capabilities of the marine actinomycetes, thus, giving them the ability to produce different sets of secondary metabolites compared to the terrestrial ones. Many studies demonstrated the capabilities of marine actinomycetes producing new and unique compounds with wide applications. Examples include Fridamycins H and I from sponge-derived *Actinokineospora* [13], Bagremycins F and G from marine-derived *Streptomyces* [14], Fradiamine A from deep-sea *Streptomyces* [15] and salinaphthoquinones from *Salinispora arenicola* [16].

Being close proximity to the ocean, Malaysia is truly blessed with highly diverse marine life present in the marine environment. This presents a wide opportunity to fully capitalized the marine environment for exploration and exploitation as a new resource of marine actinomycetes. Nonetheless, information on the diversity and biosynthetic capabilities of marine actinomycetes from the Malaysian marine environment is still lacking although there have been several studies conducted on this particular topic [17–19]. Thus, this present study attempts to assess the diversity and the biosynthetic potential of marine actinomycetes isolated from sediment collected in two locations – Langkawi Island, Kedah and Tioman Island, Pahang and screening their antagonistic potential against several test organisms. Findings from this study will provide an insight into the status of marine actinomycetes in Malaysia's marine environment and can be further utilized in natural product discovery.

2. Materials and methods

2.1. Collection of sediment samples

Marine sediments were collected by SCUBA diving at depths of 5–10 m from two locations: Langkawi Island, Kedah and Tioman Island, Pahang. Sediment samples were collected using sterile 50 mL tubes and processed immediately within 6 h of collection.

2.2. Isolation of marine actinomycetes

One gram of sediment was mixed thoroughly with 10 ml of saline and was heated at 55°C for 10 min. This was followed by dilution (10^{-2} – 10^{-5}) and plating on Actinomycetes Isolation Agar (AIA), Marine Agar (MA) and Starch–yeast Extract Agar (SYE). The SYE agar was prepared using seawater to maximize the recovery of salt-requiring actinomycetes. All plates were then incubated at 28°C–30°C for 2–3 weeks. Actinomycete colonies on the isolation plates were picked out based on morphological bases, streaked and purified on the original media.

2.3. Detection of Polyketide Synthase Type I and Non-ribosomal Peptide Synthetases Genes

Genomic DNA from the isolates were subjected to a polymerase chain reaction (PCR) to detect the presence of Polyketide Synthase Type I (PKS-I) and Non-ribosomal Peptide Synthetases (NRPS) as described by Ayuso-Sacido and Genilloud [20]. Reactions were performed in a final volume of 50 μL containing 10% of template DNA, 0.4 μM of each primer, 0.2 μM of each dNTPs, 1U *Taq* DNA polymerase (Promega) with its appropriate buffer and 10% DMSO. The following were the specific primers for PKS-I gene sequence KIF: 5′-GCSTACSYSATSTACACSTCSGG-3′ and M6R: 5′-CGCAGGTTSCSGTACCAGTA-3′. The amplification protocols were 95°C for 5 min, 35 cycles of 95°C for the 30s, 55°C for 2 min, 72°C for 4 min, followed by 72°C for 10 min. The specific primers for NRPS adenylation sequences using A3: 5′-GCSTACSYSATSTCACSTCSGG-3′ and A7R: 5'-SASGTCVCCSGTGTA-3′. The PCR temperature

profile 95°C for 5 min, 35 cycles of 95°C for the 30s, 59°C for 2 min, 72°C for 4 min, followed by 72°C for 10 min. Amplification products were analyzed by electrophoresis in 1% (w/v) agarose gels.

2.4. Screening of antagonistic activity

Evaluation of antimicrobial activity was performed for isolates that exhibited the presence of both PKS-I and NRPS genes, and NRPS gene only. These isolates were challenged against 5 Gram-negative bacteria – *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Serratia marcescens*; 3 Gram-positive bacteria – *Staphylococcus aureus* (*S. aureus*), Methicillin-resistant *S. aureus* (MRSA), *Bacillus subtilis* and *Candida parapsilosis*. The antimicrobial test was conducted by placing the isolates on trypticase soy agar which had been inoculated with the test organisms. This test was conducted in triplicates and the plates were incubated at 37°C for 24 h. The antimicrobial activity was determined as a clear zone of inhibition surrounding the isolate with the diameter expressed in mm. Zone of inhibition was measured and recorded in the following manner: weak activity (+) (5–9 mm), moderate activity (++) (10–20 mm) and good activity (+++) (>20 mm).

2.5. Molecular identification of marine actinomycetes

PCR amplification of the 16S rRNA gene was conducted using two primers: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-GGTTACCTTGTTACGACTT-3′. The reaction mixture was prepared in a total volume of 50 μL containing 10% of the extracted DNA, 0.4 μM of each primer, 0.2 mM of each dNTPs, 0.2 μl BSA, 1 U *Taq* DNA polymerase (Promega) with its recommended reaction buffer. The PCR temperature profile used was 94°C for 5 min, 30 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 2 min 30 s and finally an extension step at 72°C for 10 min. Amplification products were analyzed by electrophoresis in 1% (w/v) agarose gels stained with ethidium bromide. Purified PCR product was sent for sequencing (1st Base Laboratory, Malaysia). Nucleotide sequences were analyzed and edited by using BioEdit Sequence Alignment Editor. BLAST analysis on partial 16S rRNA sequences of the isolates was carried out via GenBank BLASTn (http://www.ncbi.nlm.nih.gov) search tool.

2.6. Characteristic analyses of marine actinomycetes

2.6.1. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed on 7 d cultures grown on SYE agar to assess the morphological characteristics. Colonies on the plate were cut into 1 cm × 1 cm and fixed in 0.1% glutaraldehyde overnight. Serial dehydration from 30% to 100% was performed followed by critical point drying. Before viewing, samples were sputtered with gold and viewed with SEM Philips XL 300.

2.6.2. Growth characteristics of the isolates on various media

The isolates were grown in a variety of media to assess their cultural characteristics: color of aerial and substrate mycelia, the presence/absence of diffusible pigment and growth. Growth of the isolates on various media was assessed and assigned to no growth (NG), poor (+): very few colonies with poor sporulation, moderate (++): moderate number of colonies and nearly 75% formed spores or good (+++): high number of colonies with nearly 100% sporulation. The following media were used in this experiment: SYE, MA, Potato Dextrose Agar, ISP2, ISP3, ISP4 and Czapek Agar (CA). The plates were incubated at 30°C for 2–3 weeks.

2.6.3. Salt tolerance

The medium used for this purpose was SYEdH_2O with the addition of different amount of NaCl in the range of 1%–12%. The plates were incubated at 30°C for 2–3 weeks. Growth was be assigned as previously.

2.6.4. Carbon sources utilization

The Biolog GP2 MicroPlate was used in this experiment according to the manufacturer's instructions. The spores were collected and resuspended in a GP inoculating fluid and the turbidity was adjusted to 20%. Thioglycollate solution was then added to the solution to prevent the cells from clumping together. About 150 μL of the cells suspension was pipetted into each well of GP2 MicroPlate. The MicroPlate was incubated at 30°C for 2 d and was read using Biolog MicroStation™.

3. Results and discussion

In total of 215 isolates were recovered of which 125 isolates from marine sediment of Tioman Island and 90 isolates from Langkawi Island. The majority (52.3%) of the isolates were recovered from SYE while 31.4% from MA and the remainder 16.3% from AIA. Wet heat pretreatment and the presence of seawater in SYE and MA produced a higher yield of recovering actinomycetes from marine sediment. Actinomycete colonies were preliminarily recognized by having morphology such as the formation of tough, leathery colonies that adhered to the agar surface, the formation of aerial and substrate mycelia, the color of spore mass and production of diffusible pigment as illustrated in Fig. 1. Detection of PKS-I and NRPS genes was conducted using PCR on 40 actinomycete isolates which were chosen randomly based on colony morphology. PKS-I and NRPS are involved in the synthesis of a large number of important bioactive compounds produced by microorganisms. Detection of PKS-I and NRPS genes helps to assess the biosynthetic capability of actinomycetes [19,21], other bacterial taxa [22,23] and fungi [24,25] in producing biologically active compounds. Only 14 actinomycetes were found to show positive results for both PKS-I and NRPS genes or NRPS genes only. Evaluation of antagonistic activity against several test microorganisms was then conducted on these 14 actinomycetes to determine if the presence of PKS-I and NRPS genes correlates with antagonistic potential. Among the actinomycetes tested, isolate T55 showed antibacterial activity against most of the bacteria tested ranging from poor to good antibacterial activity. In fact, T55 was the only actinomycete displaying antibacterial activity against *Proteus mirabilis* and

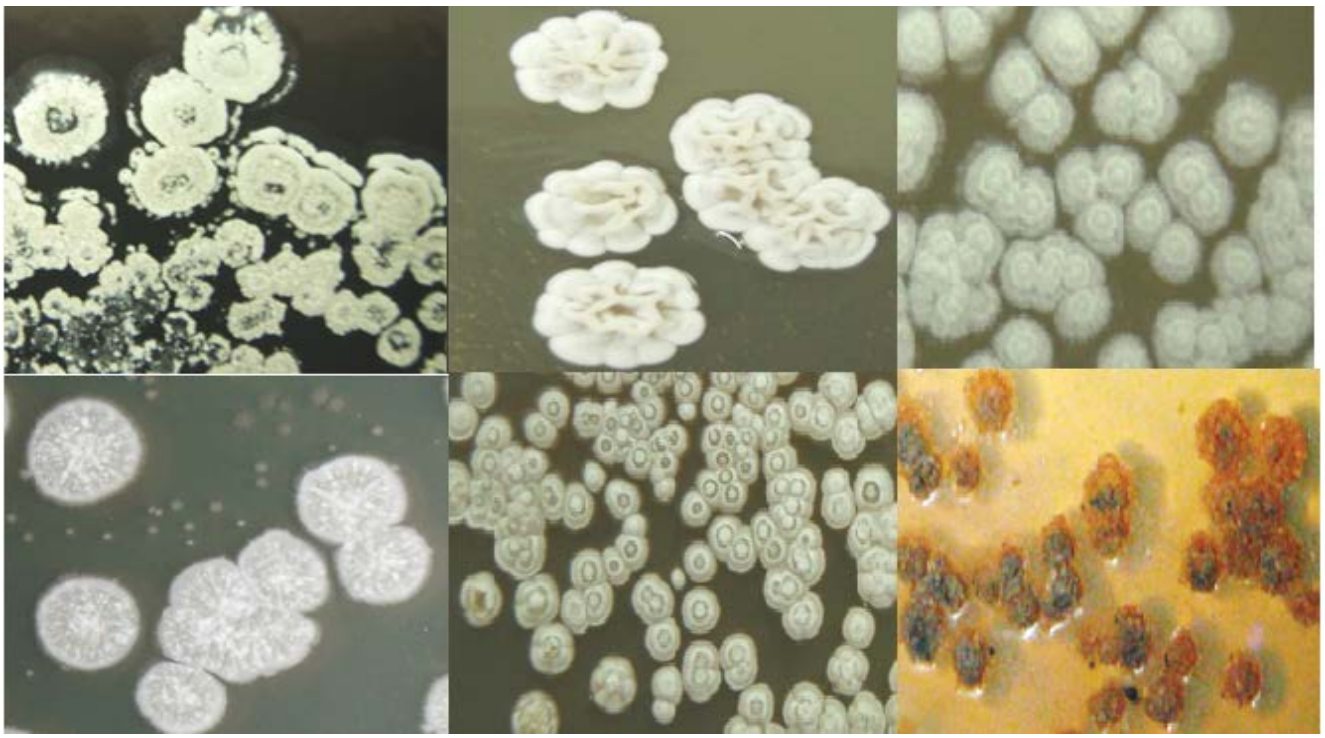

Fig. 1. Colony morphology of selected marine actinomycetes.

Serratia marcescens. Isolates T107, T109 and T110 exhibited good antifungal activity against both *Candida albicans* and *Candida parapsilosis* as well as antibacterial activity against *S. aureus* and *Bacillus subtilis*. Comparison of partial 16S rRNA gene sequences revealed that T55, T107, T109 and T110 belonged to the genus *Streptomyces* (Table 2). Indeed, the genus *Streptomyces* is known to be prolific producers of biologically important compounds which include antibiotics and contributed to 70% of the antibiotics available in the market. Therefore, it was anticipated that this genus would be the dominant group in demonstrating high antimicrobial activities as well as in the detection of PKS-I and NRPS genes. T112 showed 99% similarity with *Salinispora arenicola* NPS-14320. Both PKS-I and NRPS were detected in this isolate and T112 also displayed good antibacterial activities against *S. aureus* and MRSA. Genus *Salinispora* was the first obligate marine actinomycete described by Maldonado et al. [2] and their distribution is restricted to tropical and subtropical latitudes. Sabaratnam et al. [26] reported the occurrence of *Salinispora* spp. alongside with other genera of actinomycetes isolated from marine sponges collected in Tioman Island. In a more recent study by Ng and Tan [27] on marine sediment collected from the Pirate Reef, Tioman Island, about 23.58% of the actinomycetes recovered belonged to *Salinispora* spp. The findings of this study further confirmed the worldwide distribution of *Salinispora* spp. Moreover, Tioman Island can be considered as a hotspot for *Salinispora* strains.

Isolate L29 was closely related to genus *Actinomadura*, showed poor antimicrobial activity against *Bacillus subtilis* whereas T71, BP5, BP33 and L28 did not show any antimicrobial activities at all when challenged with the test organisms although both PKS-I and NRPS genes were detected in them. Based on the 16S rRNA sequence, both T71 and L28 belonged to the genus *Streptomyces*, BP5 - *Rhodococcus* and BP33 – *Gordonia*. Similar incidence was also reported by Zainal Abidin et al. [19], El Samak et al. [23] and Zhao et al. [28], thus, further demonstrates that there is no direct correlation between the presence of these genes and the antagonistic potential of these actinomycetes. Probable explanations for this occurrence include that the isolates were probably effective against other test organisms than the ones used in this study, the antimicrobial compound secreted by these isolates were probably not in sufficient amount to inhibit the growth of test organisms, the silencing of these genes in the isolates, and special conditions might be required for the isolates to trigger antimicrobial activity. Interestingly, several actinomycetes (T107, T109, T110 and T112) exhibited antibacterial activity against MRSA which makes them potential candidates for antibiotic development against antibiotic-resistant pathogen.

Fig. 2 depicts SEM images of selected marine actinomycetes. *Streptomyces* sp. T55 showed extensive branching of the mycelia. *Streptomyces* sp. L30 had straight spore chains with smooth-surfaced spores while *Streptomyces* sp. T83 had more compact and smooth spores which formed spiral spore chains. *Salinispora* sp. T112 spore surfaces were smooth and they were arranged in clusters. SEM of *Rhodococcus* sp. BP5 and *Gordonia* sp. BP33 revealed that their cells were in the shape of short rods. Table 3 illustrates the cultural characteristics of selected actinomycetes on several media. All isolates were found to be able to grow well on both with or without the presence of seawater with the exception of *Salinispora* sp. T112 which

Table 1
Distribution of PKS-I and NRPS genes with antagonistic activity of the marine actinomycetes

Isolate	Presence of PKS-I and NRPS genes	Gram-negative bacteria					Gram-positive bacteria			Fungi	
		Escherichia coli	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Serratia marcescens*	*Staphylococcus aureus*	MRSA	*Bacillus subtilis*	*Candida albicans*	*Candida parapsilosis*
T55	PKS-I, NRPS	+	–	++	+++	++	+++	+	–	–	–
T71	NRPS	–	–	–	–	–	–	–	–	–	–
T83	NRPS	–	–	–	–	–	++	–	–	–	–
T107	PKS–I, NRPS	+	–	–	–	+	++	+	++	+++	++
T109	PKS–I, NRPS	–	–	–	–	–	++	+	++	+++	+++
T110	PKS–I, NRPS	–	+	–	–	–	+	+	++	+++	++
T112	PKS–I, NRPS	–	–	–	–	–	+++	++	–	–	–
BP5	PKS–I, NRPS	–	–	–	–	–	–	–	–	–	–
BP33	NRPS	–	–	–	–	–	–	–	–	–	–
L28	NRPS	–	–	–	–	–	–	–	–	–	
L29	PKS–I, NRPS	–	–	–	–	–	–	–	+	–	–
L30	NRPS	–	–	–	–	–	++	–	+	–	–
L31	NRPS	–	+	–	–	–	++	–	+	–	++
L32	NRPS	–	+	+	–	–	++	–	++	–	–

–: no activity; +: weak; ++: moderate; +++: good.
MRSA – Methicillin-resistant *S. aureus*; NRPS – Non-ribosomal Peptide Synthetases; PKS-I – Polyketide Synthase Type I.

Table 2
Molecular identification of marine actinomycetes based on their partial sequence of 16S rRNA gene

Isolate	Closest sequence similarity	Accession no.	% Identity
T55	*Streptomyces mutabilis* 174465	AB184359	97
T71	*Streptomyces* sp. Sd-10	JF508416	98
T83	*Streptomyces* sp. x21	JF495018	99
T107	*Streptomyces* sp. 020101	FJ842611	98
T109	*Streptomycetaceae bacterium* CNQ857	AY464545	97
T110	*Streptomyces* sp. 020101	FJ842611	98
T112	*Salinispora arenicola* NPS-14320	FJ52866	99
BP5	*Rhodococcus ruber*	JF895525	98
BP33	*Gordonia* sp. CNJ786 PL04	DQ448772	97
L28	*Streptomyces avicenniae* MCCC 1A01535	EU399234	97
L29	*Actinomadura meyerae* 6147074	HQ291073	99
L30	*Streptomyces* sp. 13650C	EU741156	97
L31	*Streptomyces thermolilacinus*	AB184585	99
L32	*Streptomyces thermolilacinus*	AB184585	97

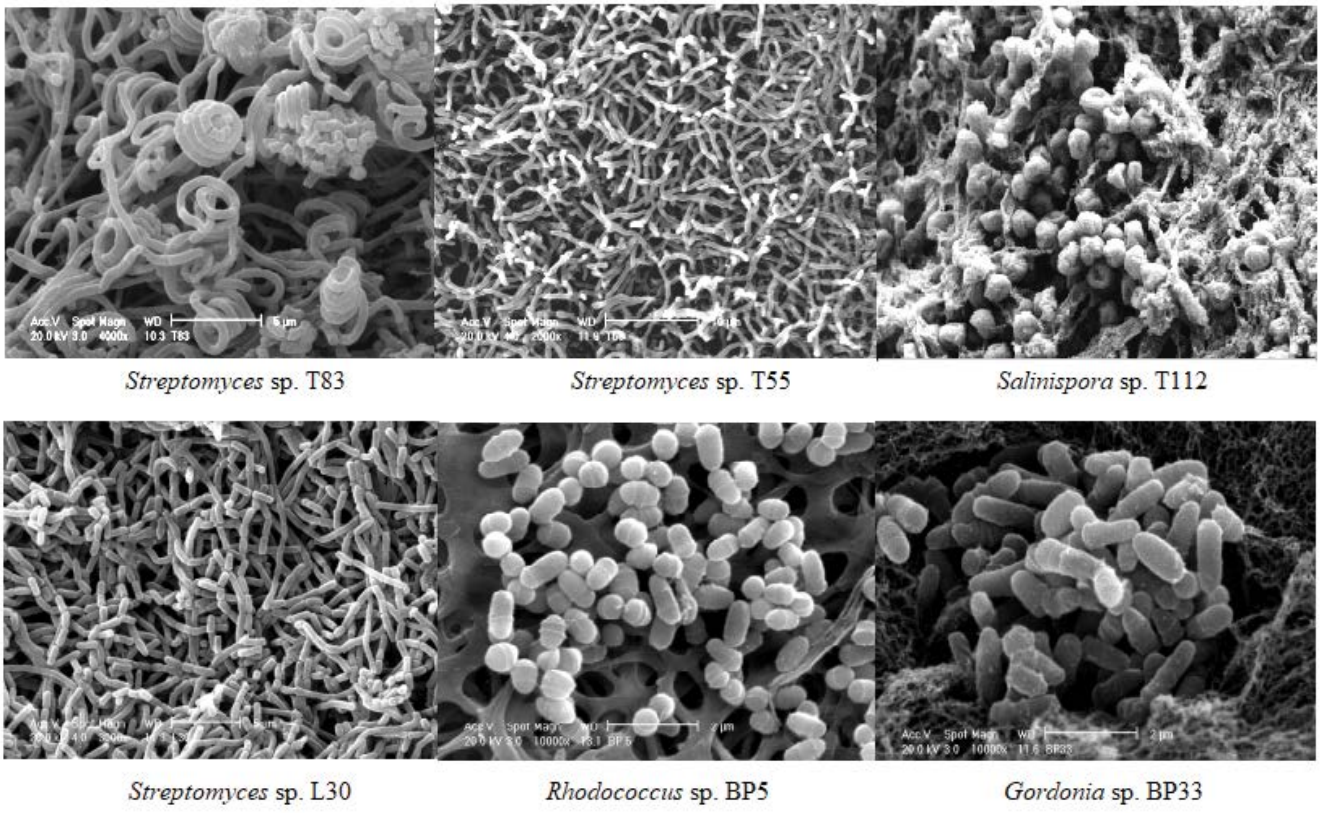

Fig. 2. SEM images of selected marine actinomycetes.

could only grow well on SYE and MA. This behavior further confirmed the absolute requirement of *Salinispora* sp. T112 for seawater/salt in order to grow. *Streptomyces* sp. T55 and *Streptomyces* sp. T109 was found to produce black/brown diffusible pigment into the medium which is related to melanin production. Melanin or melanoid production in actinomycetes is considered to be a useful criterion for taxonomical studies. Table 4 sums up NaCl tolerance, a number of carbon sources utilized (BIOLOG) besides the presence of PKS-I and NRPS and the antagonistic potential of selected actinomycetes. *Streptomyces* sp. T55 was the most potent actinomycetes that could be further explored as an antibacterial agent producer. This isolate demonstrated tolerance up to 10% NaCl but was able to utilize only 6 carbon

Table 3
Culture characteristics of selected marine actinomycetes

Isolate	Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment	Isolate	Media	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
T55 (*Streptomyces* sp.)	SYE	+++	White	Pale yellow	Absent	L29 (*Actinomadura* sp)	SYE	+++	White	White	Absent
	MA	+++	White	Pale yellow	Black		MA	+++	Pale yellow	Pale yellow	
	PDA	+++	White	Pale yellow	Brown		PDA	++	Pale yellow	Pale yellow	
	ISP2	+++	White	Pale yellow	Absent		ISP2	+++	White	White	
	ISP3	+++	Grey	White	Absent		ISP3	++	White	White	
	ISP4	+++	White	White	Absent		ISP4	+++	White	White	
	CA	+++	Beige	Beige	Absent		CA	++	White	Pale yellow	
									Colony colour		
T109 (*Streptomyces* sp.)	SYE	+++	White	Pale yellow	Absent	BP5 (*Rhodococcus* sp.)	SYE	+++	Red-orange		Absent
	MA	++	White	White	Absent		MA	+++	Orange		
	PDA	+++	White	White	Brown		PDA	+++	Peach		
	ISP2	+++	White	White	Black		ISP2	+++	Peach		
	ISP3	++	White	White	Brown		ISP3	++	Peach		
	ISP4	+++	White	White	Absent		ISP4	+	Peach		
	CA	NG					CA	+++	Peach		
T112 (*Salinispora* sp.)	SYE	+++	Black-orange	Orange	Absent	BP33 (*Gordonia* sp.)	SYE	+++	Red-orange		Absent
	MA	+++	Orange	Orange	Absent		MA	+++	Light orange		
	PDA	NG					PDA	+++	Peach		
	ISP2						ISP2	+++	Peach		
	ISP3						ISP3	++	Peach		
	ISP4						ISP4	+	Peach		
	CA						CA	+++	Peach		

SYE – Starch–yeast Extract Agar; MA – Marine Agar; PKS-I – Polyketide Synthase Type I; NRPS – Non-ribosomal Peptide Synthetases; PDA – Potato Dextrose Agar; CA – Czapek agar.

Table 4
Summary of presence of PKS-I and NRPS genes, antagonistic potential, NaCl tolerance and carbon source utilization profile of selected marine actinomycetes

Isolate	Presence of PKS-I and NRPS	Antimicrobial activity	NaCl (%) tolerance	Number of carbon source utilisation
T55 (*Streptomyces* sp.)	PKS-I, NRPS	*Escherichia coli* (+), *Pseudomonas aeruginosa* (++), *Serratia marcescens* (++), *S.aureus* (+++), MRSA (+)	1–10	6
T109 (*Streptomyces* sp.)	PKS-I, NRPS	*aureus* (++), MRSA (+), *Bacillus subtilis* (++), *Candida albicans* (+++), *Candida parapsilosis* (+++)	1–4	47
BP5 (*Rhodococcus* sp.)	PKS-I, NRPS	No antimicrobial activity detected	1–9	13
BP33 (*Gordonia* sp.)	NRPS	No antimicrobial activity detected	1–9	33
L29 (*Actinomadura* sp.)	PKS-I, NRPS	*Bacillus subtilis* (+)	1–7	11

+: weak; ++: moderate; +++: good.
NRPS – Non-ribosomal Peptide Synthetases; PKS-I – Polyketide Synthase Type I.

sources. *Streptomyces* sp. T109 showed strong antifungal activity as well as antibacterial activity against *S. aureus*, MRSA and *Bacillus subtilis*. This isolate was found to be able to utilize more than 40 types of carbon sources out of 96 carbon sources available on the Biolog GP2 MicroPlate but could only tolerate up to 4% NaCl. Although *Rhodococcus* sp. BP5 and *Gordonia* sp. BP33 did not demonstrate any antagonistic potential but the presence of both PKS-I and NRPS may indicate their hidden potential that warranted to be investigated further. Both isolates could tolerate up to 9% NaCl and *Rhodococcus* sp. BP5 was able to utilize 13 carbon sources while 33 carbon sources for *Gordonia* sp. BP33. Lastly, *Actinomadura* sp. L29 displayed weak antibacterial activity, could tolerate up to 7% NaCl and was able to utilize 11 carbon sources.

4. Conclusion

Findings from this study exemplified the potential of Malaysia's marine environment as a new resource of actinomycetes that is worth to be explored as indicated by the diversity of marine actinomycetes recovered. Moreover, the antagonistic potential of these marine actinomycetes could be applied and developed in a natural product discovery program in search of new antibiotics.

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