ANTIMICROBIAL ACTIVITIES OF SOME MARINE ACTINOMYCETES ISOLATED FROM MALAYSIAN WATERS

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INTRODUCTION

Actinomycetes are present in various ecological habitats such as soil, fresh water, lake, compost, sewage and marine environment. Actinomycetes have provided many important bioactive compounds such as antibiotics and other therapeutically useful compounds with diverse biological activities.The marine environments offers a potential natural resource of actinomycetes worth explored in search of new actinomycetes with biopharmaceutical importance. Actinomycetes from the marine environments have created a keen interest among researchers due to the species diversity and their ability to produce unique metabolites. Marine actinomycetes are distributed throughout the marine environment from shallow to deep sea sediments, marine water, marine plants and marine animals. They are efficient producers of new secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, insecticidal and enzyme inhibition. This study investigates the antimicrobial potential of some actinomycetes which were isolated from marine sediment and water samples from Malaysian waters.

MATERIALS AND METHODS

Genomic DNA extraction

Genomic DNA extraction was conducted using Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer instructions.

PCR Amplification

PCR amplification to detect the presence of polyketide synthase type I (PKS-I) and non-ribosomal peptide syntethases (NRPS) genes was carried out as described by Ayuso-Sacido & Genilloud (2005). The following degenerate oligonucleotides were used : (a) K1F: 5'TSAAGTCSAACATCGGBCA3', M6R: 5'CGCAGGTTSCSGTACCAGTA3' to detect PKS-1; (b) A3: 5'GCSTACSYSATSTACACSTCSGG3', A7R: 5'SASGTCVCCSGTSCGGTAS3' to detect NRPS. Amplifications were then performed according to the following profile: 5 min at 95ºC and 35 cycles of 30 s at 95ºC, 2 min at 55ºC for K1F/M6R, 59ºC for A3F/A7R or 58ºC and 4 min at 72ºC, followed by 10 min at 72ºC. Amplification products were analyzed by electrophoresis in 1% (w/v) agarose gels stained with ethidium bromide.

The 16S rRNA sequences were amplified using the following primers: F1: 5'AGAGTTTGATCCTGGCTCAG3' and R1: 5'GGTTACCTTGTTACGACTT3'. Amplification profile used was: 3 min 95ºC and 30 cycles of 30s at 95ºC, 1 min at 58ºC and 2 min 30s at 72ºC, followed by 10 min at 72ºC.The purified PCR products were then sent for sequencing. The sequence data were aligned by the BLAST algorithm at the NCBI website, and homologous sequences were retrieved from the GenBank database for analysis. A phylogenetic tree based on 16S rRNA gene sequences was constructed using MEGA version 4 (Kumar et al. 2007)

Antimicrobial activity

Antimicrobial activity was determined by the placing cells on TSA plates inoculated with test organisms. Inhibition zones were expressed as diameter and measured after incubation at 37ºC for 24 h for bacteria and yeasts, and at 28 for 48–72 h for fungus. The target organisms used were: *E.coli*, *P. vulgaris P. aeruginosa* , *P. mirabilis* and *S. marcescens*, *S. aureus,* Methicillin-resistant *S. aureus* (MRSA), *B.subtilis*, *Candida parapsilosis, A. niger* and *Candida albican.* The antimicrobial activities were expressed as the diameter of the growth inhibition zone (mm).

RESULTS AND DISCUSSION

The detection of PKS-1 and NRPS genes in the isolates was indicated by the presence of the corresponding fragment size range: 1200-1400bp for PKS-1 gene and 700-800bp for NRPS (**Figure 1**) when analyzed on gel electrophoresis. The presence of both PKS-1 and NRPS genes were detected on 8 isolates while another 2 isolates exhibited the presence of NRPS gene only (**Table 1**). Phylogenetic analyses of the 16S rRNA sequences of all ten isolates showed that 7 isolates belonged to the *Streptomyces* strains and another 3 isolates, each belonged to the *Rhodococcus, Salinispora* and *Actinomadura* strains respectively (**Figure 2**).

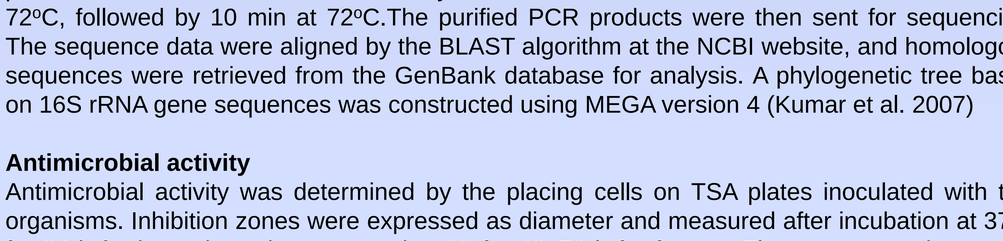


Figure 1 Agarose gel electrophoresis of PCR products
(a) Lane 1: 100 bp DNA ladder, 2: UKMCC_BP5, 3: UKMCC_L31, 4: UKMCC_PT6 , 5: UKMCC_PT7, 6: UKMCC_PT14, 7: UKMCC_T55, 8:UKMCC_T109, 9: UKMCC_T112, 10: UKMCC_L31
(b) (a) Lane 1: 100 bp DNA ladder, 2: UKMCC_BP5, 3: UKMCC_L31, 4: UKMCC_PT6 , 5: UKMCC_PT7

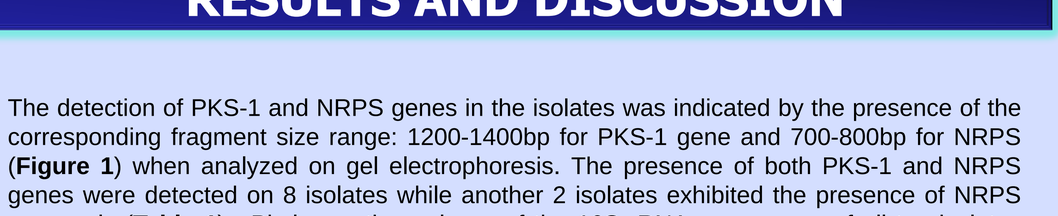


Table 1 Detection of PKS-I and NRPS genes

Isolate	PKS-I	NRPS
UKMCC_T55	+	+
UKMCC_T83	-	+
UKMCC_T109	+	+
UKMCC_T112	+	+
UKMCC_L31	-	+
UKMCC_BP5	+	+
UKMCC_PT6	+	+
UKMCC_PT7	+	+
UKMCC_PT14	+	+
UKMCC_PT15	+	+

Five isolates, mostly *Streptomyces* species demonstrated high antimicrobial activity against the test organisms which could be related to the presence of PKS-I and NRPS genes. However, 2 isolates (UKMCC_BP5 and UKMCC_PT14) displayed no antimicrobial activity at all even though both genes were detected in them. The presence PKS-I and NRPS genes might not necessarily result in antimicrobial activities since the function of polyketides is not confined to antimicrobial activities but may have other useful properties such as antihelmintics, anticancer or immunosuppressive agents (Zhao et al. 2009). Nevertheless, early evaluation of these isolates based on the presence of both genes would allow to focus the screening of isolates that possessed high metabolic potential.

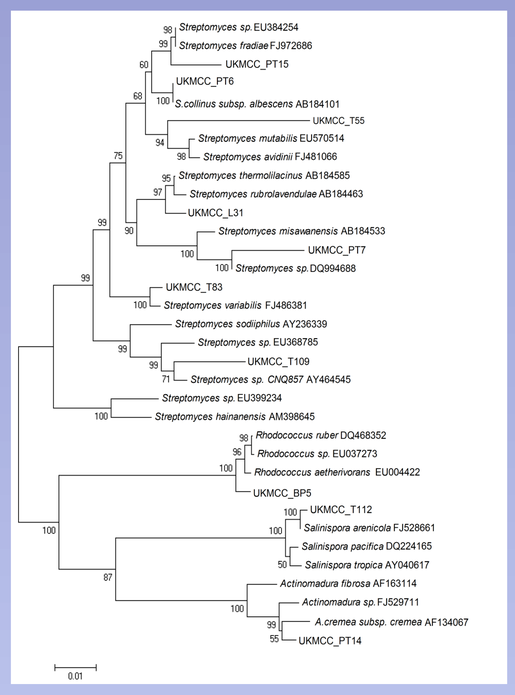
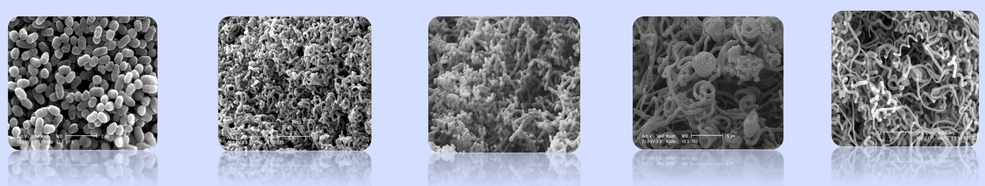


Fig. 2 Phylogenetic tree of the 16S rRNA sequences of the 10 selected strains. Phylogenetic analysis was performed based on the16S rRNA sequences of the selected strains, bootstrap values calculated from 1,000 re-samples using neighbor-joining (only values above 50% are shown).
Bar: 0.01 substitutions per nucleotide position

Table 2 Antimicrobial activities of the 10 isolates

Isolate	*E.coli*	*P. vulgaris*	*P. aeruginosa*	*P. mirabilis*	*S. marcescens*	*S. aureus*	MRSA	*B. subtilis*	*A. niger*	*C. albicans*	*C. parapsilosis*
UKMCC_T55	21	-	10	25	20	20	10	20	20	-	-
UKMCC_T83	-	-	-	-	-	16	12	-	15	-	-
UKMCC_T109	-	-	-	-	10	12	10	16	32	23	25
UKMCC_T112	-	-	-	8	-	28	10	-	-	-	-
UKMCC_L31	-	15	10	-	-	18	-	15	20	-	15
UKMCC_BP5	-	-	-	-	-	-	-	-	-	-	-
UKMCC_PT6	-	12	10	-	-		10	10	15	-	-
UKMCC_PT7	-	-	12	8	-	30	10	19	10	-	-
UKMCC_PT14	-	-	-	-	-	-	-	-	-	-	-
UKMCC_PT15	-	-	-	10	-	28	-	-	-	-	-

The antimicrobial activities were expressed as diameter of the inhibition zone (mm)
–, activity not detectable



CONCLUSION

In conclusion, Malaysian waters have the potential to become one of the important natural resources for exploration of marine actinomycetes that possess the ability to produce a relatively high rate of new antimicrobial bioactive agents.

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