Journal of Microbial World
Volume 13, No. 1, April 2020

Original Article

Antimicrobial potentiality, type-I polyketide synthase and nonribosomal peptide synthetase biosynthetic genes from some marine *Actinomycetes*

Fatemeh Shayesteh[1], Nur Syuhana Binti Zakaria[2], Gires Usup[3], Asmat Ahmad[4]

[1]Assistant professor, Department of Fisheries, Faculty of Marine Science and Technology, University of Hormozgan, Bandar Abbas, Iran.
[2]Msc student, School of Bioscience and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia.
[3]Professor, School of Environmental Science and Natural Resources, Faculty of Science and Technology, University Kebangsaan Malaysia.
[4]Associate professor, School of Bioscience and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia.

Abstract

Background & Objectives *Actinomycetes* are commonly known as exceptionally prolific source of secondary metabolites with diverse biological activities. The aim of this study was to identify some *Actinomycete* isolates from Malaysia marine environment and evaluate for type-I polyketide synthase (PKS-I) and nonribosomal peptide synthetase (NRPS) genes as well as antimicrobial activity.
Materials and Methods: Selected isolates were identified based on their morphology and molecular properties. PKS-I and NRPS genes were detected using specific primers and the potential of their antimicrobial activity was investigated by disc diffusion method.
Results: The isolates varied morphologically on the basis of colony morphology, spore chain shape, aerial and substrate mycelium formation. Based on 16S rRNA gene sequences analysis, isolates Sdstm3k, Sdtm108 and Sdts4 were highly similar to *Streptomyces* sp. (95%), whereas isolates Bvpd17e and SctgJI demonstrated highest similarity to *Micrococcus* sp. M2-19 (99%) and *Micrococcus leteus* (95%) respectively. While isolate Sdsb2k1a and Sdts46 were unidentified. The detection of PKS-I and NRPS genes revealed that only isolates SctgJI and Sdsb2k1a had both genes. Isolates *Streptomyces* sp. Sdst3k1 and *Streptomyces* sp. Sdts4 demonstrated the strongest and broadest spectrum of antimicrobial activity against 10 human pathogens tested.
Conclusion: The present study indicated that *Actinomycetes* isolated from marine environment in Malaysia can be a good source of the discovery of new bioactive compounds.
Keywords: marine bacteria, Actinomycetes, PKS-I and NRPS genes, antimicrobial activity.
Received: January 2020 Accepted: March 2020

Correspondence to: Fatemeh Shayesteh
Tel: +98 9175497100
E-mail: Shayesteh_fatemeh@yahoo.com
Journal of Microbial World 2020, 13(1): 77-86.

Original Article

مجله دنیای میکروب‌ها
سال سیزدهم شماره اول (پیاپی ٤٢) بهار ١٣٩٩
صفحات ٨٦-٧٧

پتانسیل ضد میکروبی، بیوسنتز ژن های پلی کتاید سنتتاز تیپ I (PKS-I) و پپتید سنتتاز غیر ریبوزومی (NRPS) در برخی از *اکتینومیست های* دریایی

فاطمه شایسته*[1]، نور سوهانا ذکریا[2]، گیرس یوسف[3]، اسمت احمد[4]

[1]استادیار، دانشگاه هرمزگان، دانشکده علوم و فنون دریایی، گروه شیلات، [2]کارشناس ارشد، دانشگاه ملی کشور مالزی، دانشکده علوم و فناوری، گروه علوم زیستی و زیست فناوری، [3]استاد، دانشگاه ملی کشور مالزی، دانشکده علوم و فناوری، گروه علوم زیست محیطی و منابع طبیعی، [4]دانشیار، دانشگاه ملی کشور مالزی، دانشکده علم و فناوری، گروه علوم زیستی و زیست فناوری.

چکیده

سابقه و هدف: *اکتینومیست* ها به عنوان منابع متابولیت های ثانویه با فعالیت های بیولوژیکی متنوع شناخته می شوند. این مطالعه با هدف شناسایی برخی از *اکتینومیست* های جدا سازی شده از محیط دریایی در مالزی، بررسی ژن های پلی کتاید سنتتاز تیپ I (PKS-I) و پپتید سنتتاز غیر ریبوزومی (NRPS) و همچنین بررسی ویژگی های ضد میکروبی آنها انجام گرفت.

مواد و روش ها: *اکتینومیست* های انتخاب شده به وسیله روش های مورفولوژیکی و مولکولی شناسایی شدند. وجود ژن های NRPS و PKS-I به وسیله پرایمرهای اختصاصی بررسی گردید و همچنین فعالیت ضد میکروبی سویه های انتخاب شده به روش دیسک گذاری بررسی شد.

یافته ها: جدایه های انتخاب شده بر اساس خصوصیات مورفولوژیکی کلنی، شکل زنجیره اسپورها و نوع میسیلیوم های رویشی و هوایی متفاوت بودند. بر اساس تجزیه و تحلیل ژن 16S sRNA سویه های Sdstm3k، Sdtm108 و Sdts4 شباهت زیادی به *Streptomyces* sp. نشان دادند (٩٥٪) در صورتی که سویه های Bvpd17e و SctgJI به ترتیب بیشترین شباهت را با *Micrococcus* sp. M2-19 (٩٩٪) و *Micrococcus leteus* (٩٥٪) نشان دادند. در حالی که سویه های Sdsb2k1a و Sdts46 ناشناس بودند. با بررسی ژن های NRPS و PKS-I مشخص گردید که فقط سویه های SctgJI و Sdsb2k1a دارای هر دو ژن می باشند. سویه های Sdst3k1 و Sdts4 قوی ترین و وسیع ترین فعالیت ضد میکروبی را در برابر ١٠ پاتوژن انسانی مورد بررسی نشان داد.

نتیجه گیری: بر اساس مطالعه حاضر مشخص گردید که *اکتینومیست* های جداسازی شده از محیط زیست دریایی در مالزی می تواند به عنوان یکی از منابع مناسب کشف ترکیبات زیست فعال جدید باشد.

واژگان کلیدی: باکتری دریایی، *اکتینومیست*، ژن های NRPS و PKS-I، خاصیت ضد میکروبی.

دریافت مقاله: دی ماه ٩٨ پذیرش برای چاپ: اسفند ماه ٩٨

*) آدرس برای مکاتبه: دانشگاه هرمزگان، دانشکده علوم و فنون دریایی، گروه شیلات
تلفن: ٠٩١٧٥٤٩٧١٠٠ پست الکترونیک: Shayesteh_fatemeh@yahoo.com

Introduction

The incidence of antibiotic resistance pathogenic bacteria has increased dramatically all over the world (1). According to O'Neill's independent review (2) about 700 000 people around the world die annually due to drug-resistant infections. If current trends continue, such infections could entail the death of 10 million people a year by 2050 (3). In this context, the discovery of novel bioactive compounds, particularly those with new mechanism of action, is not only needed for modern medicine but absolutely required to avoid future pandemics. Marine sources are efficient producers of new natural products that show a range of biological activities including antibacterial, anticancer, antifungal, cytotoxic, antitumor, anti-parasitic, anti-inflammatory, antiviral, antioxidant, antimalarial, etc (3). Among natural products from marine microorganisms, *Actinomycetes are the most economically and biotechnologically valuable prokaryotes and are responsible for the* production of about half of the discovered secondary metabolites (4). Various antimicrobial substances from *Actinomycetes have been isolated and characterized including aminoglycosides, beta-lactams, glycopeptides,* macrolides, anthracyclines, nucleosides, peptides, polyesters, polyenes, polyketides, tetracyclines and actionomycins (4). NRPS and PKS-I genes are responsible for producing of secondary metabolites including well-known antibiotics, anticancer agents, siderophores, toxins, surfactants, immunosuppressants, and anti-inflammatorials (5-8). Recent studies have also shown that marine microorganisms can be an important source of new nonribosomal peptide metabolites (9, 10). Malaysian waters are one of the most spectacular coastal and marine environments in Malaysia has a rich biodiversity. According to best of our knowledge, little studies have been conducted to isolating and antimicrobial screening of *Actinomycetes from the Malaysia* marine environment (11, 12). As regards detection of PKS-I and NRPS genes help to assess the biosynthetic capability of *Actinomycetes* in production biologically active compounds (13,14), the present study was conducted to identify marine actinomycetes isolated from different marine environment in Malaysia based on their morphological and molecular characteristics and evaluate for PKS-I and NRPS genes. The potential of selected isolates for production of antimicrobial compound were also investigated.

Materials and Methods:

Marine Actinomycetes from culture collection

Seven isolates (Table 1) of marine microbial culture collection (Aquatic Microbiology Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, UKM), were selected. Selected isolates were cultured on starch peptone agar supplemented with sterile sea water. Nalidixic acid (25 µg / mL) and cycloheximide (25 µg / mL) were added to culture media for the inhibition of fungi and other bacteria, respectively. Plates were then incubated at 28 ˚C for 1-7 days. The isolates were maintained on plates at 4 ˚C for short-term storage. For long-term preservation, mycelial suspension in 20% glycerol were kept at -80 ˚C.

Morphological identification of selected Actinomycetes

Different characteristics of selected isolates such as colony, cell and spore morphology, gram-stain phenotype, spore chain morphology, aerial and substrate mycelia

Table 1. Source, location, morphological characterization and microscopic observation of marine *Actinomycetes*.

Isolates	Location	Source	Cell shape	Colony morphology	Spore	Spore chain	Number of spores in a chain
Bvpd17e	Port Dickson	Bivalve	Coccus	Circular, small, raised, opaque, sticky, non-spore forming, grow on agar surface	-	-	-
SctgJI	Pulau Tinggi	Sea cucumber	Coccus	Circular, small, raised, opaque, wet, non-spore forming, grow on agar surface	-	-	-
Sdsb2k1a	Sebatu	Sediment	Unbranched Hyphae filamentous	Tick powdery spores, have aerial and substrate mycelium, irregular shape, contoured, dry	Smooth	Curved	>10
Sdst3k1	Stulang Latu	Sediment	Unbranched Hyphae filamentous	Powdery spores, have aerial and substrate mycelium, irregular shape, dry	Smooth	Curved	3-10
Sdtm108	Tioman Island	Sediment	Branched hyphae filamentous	Powdery spores, have aerial and substrate mycelium, irregular shape, radial limitation, contoured, dry	Smooth	Curved	3-10
Sdts4	Sepangar Bay	Sediment	Branched hyphae filamentous	Thick powdery spores, have aerial and substrate mycelium, irregular shape, contoured, dry	Smooth	Curved	>10
Sdts46	Sepangar Bay	Sediment	Branched hyphae filamentous	Powdery spores, have aerial and substrate mycelium, irregular shape, contoured, dry	Smooth	Straight	>10

morphology was determined (15).

Molecular phylogeny of selected isolates based on 16S rRNA gene sequencing

The isolates were cultured in starch peptone broth for 24- h and 7-days for non-spore-forming and spore-forming isolates, respectively. Genomic DNA was extracted using a Wizard ® Genomic DNA Purification Kit (promega) according to the manufacturer's description. The 16S rRNA gene amplification was generated using 16S-F (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S-R (5'-GGTTACCTTGTTACGACTT-3') primers (16). The sequences obtained from selected *Actinomycetes*, was compared with the related sequences in the NCBI data base using Blast and aligned using CLUSTA-W (17). Evolutionary analysis was performed using the MEGA 4 software (18).

PCR amplification of PKS-I and NRPS genes

Genomic DNA from the isolates were subjected to polymerase chain reaction (PCR) to detect the presence of polyketide synthase type I (PKS-I) and non-ribosomal peptide syntetases (NRPS) as described by Ayuso-Sacido and Genilloud (19). The following were the specific primers for PKS-I gene sequence K1F: (5'-TSAAGTCSAACATCGGBCA3') and M6R: (5'CGCAGGTTSCSGTACCAGTA-3'). The specific primers for NRPS were: A3F (5'-GCSTACSYSATSTACACSTCSGG-3') and A7R (5'-SASGTCVCCSGTSCGGTAS-3'). Amplification products were analyzed by electrophoresis in 1% (w/v) agarose gel.

Determination of antimicrobial activity

The clinical bacteria and fungus pathogens were cultured on nutrient broth and potato dextrose agar, respectively. *Actinomycetes* were separately grown in marine broth for the production of secondary metabolites. Cultures were incubated on a rotary shaker (200 rpm) at 28 ˚C for 1 and 7 days for non-spore forming and spore forming bacteria, respectively. The antimicrobial activity of *Actinomycetes* was demonstrated by disc diffusion method according to Hamadan & Mikolajcik (20) and Apella et al., (21).

Results

Morphological identification of selected Actinomycetes

Based on morphological observation, two isolates (Bvpd17e and SctgJI) did not produce spore and able to grow only above the agar surface within 1-3 days. The other five isolates (Sdsb2k1a, Sdst3k1, Sdtm108, Sdts4 and Sdts46) possess typical morphology of *Streptomyces* which produce aerial hyphae spore chain which is segmented in spore chain. Additionally, they were able to grow after 3-5 days on agar surface and colonies found to penetrate into the agar. The light microscopic result for these five isolates illustrated that isolates Sdsb2k1a and Sdst3k1 had unbranched hyphae filaments while the remaining 3 bacteria (Sdtm108 and Sdts46) produce branched hyphae filaments. In addition, lactophenol staining demonstrated that only isolate Sdts46 generate straight spore chain whereas the rest (Sdsb2k1a, Sdst3k1, Sdtm108 and Sdts4) produce curved spore chain. Morphological characteristics of selected isolates is indicated in Table 1.

16S rRNA gene sequencing of selected Actinomycetes

The 16S rRNA gene of isolate Bvpd17e (628 bp) showed 99% identity to Micrococcus sp. m2-19. While isolates SctgJI (1484 bp), Sdst3k1 (1527 bp), Sdtm108 (1488 bp) and Sdts4 (1424 bp) demonstrated 95% similarity to *Micrococcus luteus*, *Streptomyces* sp. IMER-B3-25, *Streptomyces* sp. 32 and *Streptomyces* sp. C25, respectively. Moreover, isolates Sdsb2k1a (1540 bp) and Sdts46 (1477bp) were not distinguishable based on their 16S rRNA gene sequencing, however they demonstrated 89% and 95% identity to uncultured bacterium clone q48f312/pp124 and uncultured soil bacterium clone T3_4, respectively (Fig 1).

PCR amplification of PKS-I and NRPS genes

The presence of the PKS-I and NRPS genes, demonstrated a band with a size range of 1200-1400 bp and 700bp, respectively. It was demonstrated that isolate Bvpd17e showed the presence of PKS-I gene only while isolates Sdst3k1, Sdtm108, Sdts4 and Sdts46 displayed the presence of NRPS gene only. Interestingly, 2 isolates namely SctgJI and Sdsb2k1a showed the presence of both NRPS and PKS-I genes. Results from PKS-I and NRPS genes are presented in Fig 2 and 3, respectively.

Antimicrobial activity of Actinomycetes isolates

The results from antimicrobial activity indicated that two isolates Namely Bvpd17e and Sdsb2k1a did not show inhibitory activity against any of pathogens. Isolates Sdtm108 and Sdts46 was only active against *C. albicans.* Interestingly, secondary metabolites produced by isolates sdst3k1 and Sdst4 were able to inhibit all pathogens used in this study.

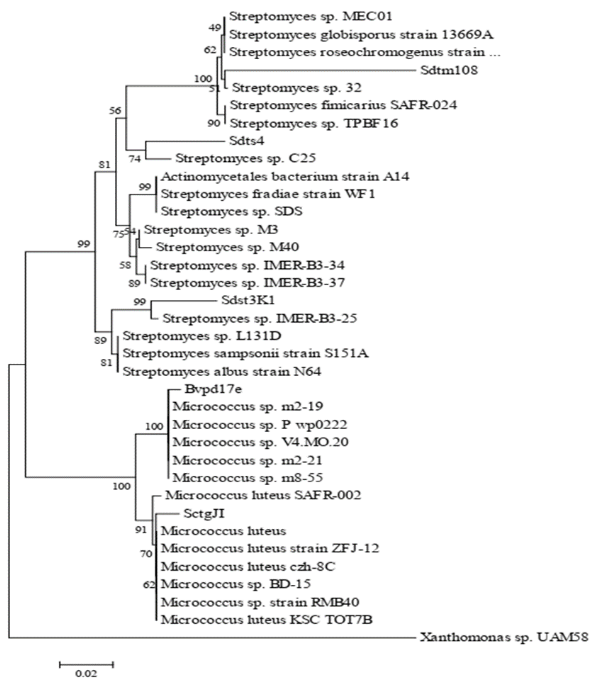

Fig 1. Phylogenetic relationship of selected isolates to related bacteria based on Neighbour-joining tree analysis of 16S rRNA gene sequence data. A sequence of *Xanthomonas* sp. UAM58 was used as the out-group.

Moreover, isolate SctgJI capable of inhibiting growth of three clinical pathogens namely MRSA, A. hydrophila and C. albicans (Table 2).

Discussion

Comparison of phenotypic characterization of isolates Bvpd17e and SctgJI with the literatures shown that these two isolates belong to *Micrococcus* species. Members of *Micrococcus* sp. are Gram-positive, coccus-shape bacteria which usually arranged in the tetrad form, cell diameter between 0.8-1.0 µm (22), aerobic, mesophilic, neutrophilic, not spore forming, catalase positive and no motility (23). In addition, the yellow pigment production is resemble to *Micrococcus luteus*, however the other five isolates namely Sdsb2k1a, Sdst3k1, Sdtm108, Sdts4 and Sdts46, showed similarity with family members of *Streptomycetaceae* which are Gram-positive bacteria with branched filaments, have aerial and substrate mycelium, segmented hyphae, produce spore chain at the top or as a side branch. Although the similarity percentage sequences of six marine *Actinomycetes* with the references strains were high (≥ 95 %), but most of them were identified only at the genus level. It is generally accepted that organisms displaying 16S rRNA sequence similarity value of 97% or less belong to different species (24). Even isolates with partial 16S rRNA sequences of 99% similarities could possibly be new species of *Streptomyces* as demonstrated by several studies (23, 25). Results of the 16S rRNA gene sequencing analysis showed that only isolate SctgJI was identi-

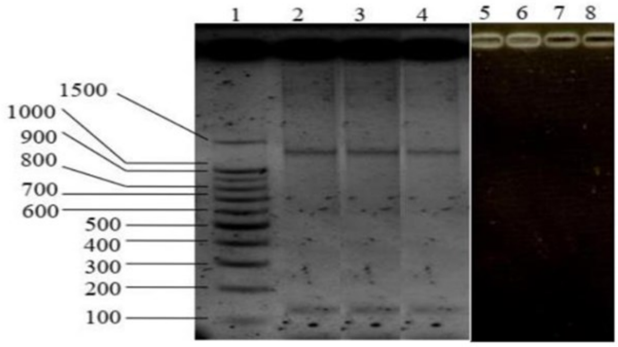

Fig 2. Visualization of PKS-I gene amplification of 7 marine *Actinomycetes* isolated by electrophoresis in 1% (w/v) agarose gel stained with ethidium bromide. Lanes 1: 100 bp DNA ladder, 2: Bvpd17e, 3: SctgJI, 4: Sdsb2k1a, 5: Sdst3k1, 6: Sdtm108, 7: Sdts4, 8: Sdts46

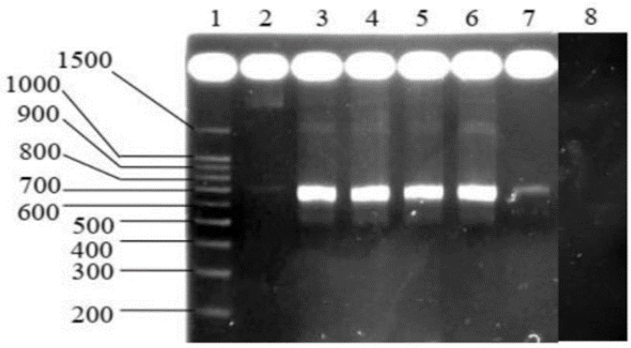

Fig 3. Visualization of NRPS gene amplification of 7 marine *Actinomycetes* isolated by electrophoresis in 1% (w/v) agarose gel stained with ethidium bromide. Lanes 1: 100 bp DNA ladder, 2: SctgJI, 3: Sdts108, 4: Sdts4, 5: Sdst46, 6: Sdst3k1, 7: Sdsb2k1a, 8: Bvpd17e.

Table 2. Antimicrobial activity pattern of marine *Actinomycetes* against various clinical pathogens.

Isolates	Pathogens									
	MRSA	SA	PA	VP	PM	SM	EC	AH	CA	AF
Bvpd17e	-	-	-	-	-	-	-	-	-	-
SctgJI	11	-	-	-	-	-	-	9	11	-
Sdsb2k1a	-	-	-	-	-	-	-	-	-	-
Sdst3k1	21	13	9	13	8	8	11	10	16	21
Sdtm108	-	-	-	-	-	-	-	-	8	-
Sdts4	16	11	8	11	11	8	10	9	13	20
Sdts46	-	-	-	-	-	-	-	-	24	-

fied up to species level (*Micrococcus luteus*) while two isolates namely Sdsb2k1a and Sdts46 did not show similarity with any genus or species of *Actinomycetes*, however based on morphological analysis indicated these isolates are belong to the genus *Streptomyces* (Table 1). Further analysis is required which include morphological, physiological, biochemical and DNA-DNA hybridization to identify these two isolates until species level. Although isolates Sdst3k1, Sdtm108 and Sdts4 were identified as *Streptomyces* sp. based on 16S rRNA sequencing (Fig 1). The fact that to obtain rare *Actinomycetes* (Micrococcus sp.) or unknown species other than genus *Streptomyces*, demonstrated the diversity and potential of Malaysian waters to be explored as new resources of *Actinomycetes*. Molecular identification using the sequence of 16S rRNA gene showed that isolates Sdst3k1 and Sdts4 which demonstrated broadest antimicrobial activities, are belong to the genus *Streptomyces* (Fig 1), indeed the genus *Streptomyces* are known to be prolific producers of biologically important compounds which include antibiotics and contributed to 70% of the antibiotics available in the market. Some important antibiotics from *Streptomyces* are kanamycin produced by *Streptomyces kanamyeticus* (26), Oxytetracycline produced by *S. rimosus* (27), tetracycline (28) and actinomycin C (29) from *S. aureofaciens* and *S. chyrsomallus*, respectively. Zainal Abedin et al., (11) have reported a broad spectrum of antimicrobial *Streptomyces* (*Streptomyces* sp. UKMCC-PT15) isolated from seawater collected from Pulau Tinggi, Malaysia. It is, therefore particularly interesting to note that isolates Sdtm108 and Sdts46 inhibited exclusively the growth of *C. albicans* but not any of the other pathogens. It has been underlined that specific antimicrobial agents that act against a certain pathogen may have advantages over broad spectrum agents, whereby resistance development and side effects could potentially be reduced (30). PKS-I and NRPS genes are involved in the synthesis of large number of important bioactive compounds produced by microorganisms. Detection of PKS-I and NRPS genes help to assess the biosynthetic capability of *Actinomycetes* (13,22), other bacteria taxa (31,22) and fungi (32) in production biologically active compounds. All isolates possess both or at least one genes. It is interesting to note that isolates Sdsb2k1a and Bvpd17e had no antimicrobial activity against all tested pathogenic microorganisms while isolate Sdsb2k1a possess both PKS-I / NRPS genes and isolate Bvpd17e possess PKS-I gene. Similar incidence was also reported by Zhao et al., (33) and Bredholt et al., (34). Plausible explanations for this phenomena include that 1) the isolates were probably effective against other pathogenic organisms than the ones used in this study, 2) the antimicrobial compounds secreted by these isolates were probably not in sufficient amount to inhibit the growth of test organisms, 3) they produce other possible active compounds such as antiviral, anticancer, or antiparasite, 4) the silence of these genes in the isolates, or 5) special conditions might be required for the isolates to demonstrate antimicrobial activities.

Conclusion

In conclusion, the less explored Malaysian marine ecosystem represents a rich resource for new *Actinomycetes* species and bioactive compounds. Further studies are required to purify, identify and validate the identity of antimicrobial compounds from these isolates.

Ethical Consideration

Authors of all ethics including non-plagiarism, Dual publishing has complied with data distortions and data making in this article.

Acknowledgement

This study was supported by The Ministry of Science, Technology and Innovation (MOSTI) and grant number 04-01-02-SF0144.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Okeke I, Laxminarayan R, Bhutta Z, Duse A, Jenkins P, O'Brien T, Pablos-Mendez A, Klugman K. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infecti Dis. 2005; 5 (8): 481-493.

2. O'Neill J. Tackling drug-resistant infections globally: final report and recommandations [Internet]. London (UK): The review on antimicrobial resistance. Available from https://amr review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf. 2016.

3. Jakubiec-Krzesniak K, Rajnisz-Mateusiak A, Guspiel A, Ziemska J, Solecka J. Secondary Metabolites of Actinomycetes and their Antibacterial, Antifungal and Antiviral Properties. Pol J Microbiol. 2018; 67 (3): 259-272.

4. Berdy J. Bioactive microbial metabolites. *J Antibiot 2005; 58:1-26.*

5. Cane DE, Walsh CT, Khosla C. Harnessing the biosynthetic code: combinations, permutations, and mutation. Science. 1998; 282 (5386): 63-68.

6. Cane DE, Walsh CT. The parallel and convergent universes of polyketide synthetases and nonribosomal peptide synthetases. Chem Biol. 1999; 6 (12): 319-325.

7. Crosa JH, Walsh CT. Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol R. 2002 *66 (2): 223-249.*

8. Weissman KJ. Introduction to polyketide biosynthesis. In: Hopwood DA, editor. Complex Enzymes in Microbial Natural Product Biosynthesis, *Part B:* Polyketides, Aminocoumarins and

Carbohydrates Methods in Enzymology. Academic Press; 2009. pp. 3-16.

9. Munro MH, Blunt JW, Dumdei EJ, Hickford SJH, Lill RE, Li S, Battershill CN, Duckworth AR. The discovery and development of marine compounds with pharmaceutical potential. J Biotechnol. 1999; 70 (1-3): 15-25.

10. Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep. 2008; 25 (1): 35-94.

11. Zainal Abidin ZA, Abdul Malek N, Zainuddin Z, Chowdhury AJK. Selective isolation and antagonistic activity of *actinomycetes* from mangrove forest of Pahang, Malaysia. Front Life Sci. 2016; 9 (1): 24-31.

12. Faja O, Sharad AA, Younis KM, Usup G, Ahmad A. Isolation, screening and antibiotic profiling of marine Actinomycetes extracts from the coastal of Peninsular Malaysia. Int J Chemtech Res. 2017; 10 (3): 212-224.

13. Schneemann I, Kajahn I, Ohlendorf B, Zinecker H, Erhard A, Nagel K, Wiese J, Imhoff JF. Mayamycin, a Cytotoxic Polyketide from a *Streptomyces* Strain Isolated from the Marine Sponge *Halichondria panacea.* J Nat Prod. 2010; 73 (7):1309-1312.

14. Kennedy J, Baker PW, Piper C, Cotter PD, Walsh M, Mooij MJ, Bourke MB, Rea MC, O'Connor PM, Ross RP, Hill C, O'Gara F, Marchesi JR, Dobson ADW. Isolation and analysis of bacteria with antimicrobial activities from the marine sponge *Haliclona simulans* collected from Irish waters. Mar Biotech. 2008; 11 (3): 384-396.

15. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Evol Microbiol. 1966; 16 (3): 313-340.

16. Piterina AV, Barlett J, Pembroke JT. Molecular analysis of bacterial community DNA in sludge undergoing autothermal thermophilic aerobic digestion (ATAD): pitfalls and improved methodology to enhance diversity recovery. *Divers*. 2010; 2 (4): 505-526.

17. Thompson JD, Higgins DG, Gibson TJ. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22 (22): 4673-4680.

18. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24 (8): 1596-1599.

19. Ayuso-Sacido A, Genilloud O. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb Ecol. 2005; *49 (1): 10-24.*

20. Hamadan J, Mikolajcik EM. Acidolin: an antibiotic produced by *Lactobacillus acidophilus. J Antibiot. 1974; 27 (8): 631-636.*

21. Apella MC, Gonzalez SN, Nader de Macias ME, Romero N, Oliver G. *In vitro studies on the inhibition* of the growth of *Shigella sonnei by Lactobacillus casei and Lactobacillus acidophilus. J Appl Bacteriol. 1992; 73 (6): 480-483.*

22. Zhao XQ, Jiao WC, Jiang B, Yuan WJ, Yang TH, Hao SH. Screening and identification of actinobacteria from marine sediments: investigation of potential producers for antimicrobial agents and type I polyketides. World J Microbiol Biotechnol. 2009; 25 (5): 859-866.

23. Chen HH, Zhao GZ, Park DJ, Zhang YQ, Xu LH, Lee JC, Kim CJ, Li WJ. *Micrococcus* *endophyticus sp. nov., isolated from surface-sterilized* Aquilaria sinensis roots. Int J Syst Evol Microbiol. 2009; 59: 1070-1075.

24. Stackebrandt E, Goebel BM. Taxonomic Note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol. 1994; 44 (4): 846-849.

25. Zhu H, Guo J, Yao Q, Yang S, Deng M, et al. *Streptomyces vietnamensis* sp. nov., a *streptomycete* with violet–blue diffusible pigment isolated from soil in Vietnam. Int J Syst Evol Microbiol. 2007; 57: 1770-1774.

26. Okami Y, Tazaki T, Katumata S, Honda K, Suzuki M, et al. Studies on *Streptomyces kanamyceticus,* producer of kanamycin. J Antibiot. 1959; 12: 252-256.

27. Petkovic H, Cullum J, Hranueli D, Hunter IS, Peric Concha N, Pigac J, Thamchaipenet A, Vujaklija D, Long PF. Genetics of *Streptomyces rimosus, the oxytetracycline producer. Microbiol Mol Biol Rev. 2006; 70 (3): 704-728*.

28. Ross A, Schugerl K. Tetracycline production by *Streptomyces aureofaciens: the time lag of production. Appl* Microbiol Biotechnol. 2005; 29 (2-3): 174-180.

29. Konoshenko GI, Avraleva IV, Anisova LN, Orlova TI. Biologically active substances by a number of strains of the actinomycin C producer *Streptomyces chyrsomallus.* Antibiot Khimioter. *1994; 39 (2-3): 22-25.*

30. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009; 325 (5944): 1089-1093.

31. Xin Y, Kanagasabhapathy M, Janussen D, Xue S, Zhang W. Phylogenetic diversity of Gram- positive bacteria cultured from Antarctic deep-sea sponges. Polar Biol. 2011; 34 (10): 1501-1512.

32. Zhou K, Zhang X, Zhang F, Li Z. Phylogenetically diverse cultivable fungal community and polyketide synthase (PKS), non-ribosomal peptide synthase (NRPS) genes associated with the South China Sea sponges. Microb Ecol. 2011; 62 (3): 644-654.

33. Zhao K, Penttinen P, Guan T, Xiao J, Chen Q, Xu J, Lindstrom K, Zhang L, Zhang X, Strobel GA. The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in *Panxi plateau*, China. Curr Microbiol. 2011; 62 (1): 182-190.

34. Bredholt H, Galatenko OA, Engelhardt K, Tjaervik E, Terekhova LP, Zotchev SB. Rare actinomycete bacteria from the shallow water sediments of the Trondheim fjord, Norway: isolation, diversity and biological activity. Environ Microbiol. 2007; 9 (11): 2756-2764.