Jurnal
Teknologi

Full Paper

# DIVERSITY AND ANTIMICROBIAL ACTIVITY OF MANGROVE SOIL ACTINOMYCETES ISOLATED FROM TANJUNG LUMPUR, KUANTAN

Nurfathiah Abdul Malek[a], Zarina Zainuddin[a], Ahmed Jalal Khan Chowdhury[b], Zaima Azira Zainal Abidin[b*]

[a]Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Malaysia
[b]Department of Marine Science and Technology, Kulliyyah of Science, International Islamic University Malaysia, Malaysia

**Article history**
Received
*26 June 2015*
Received in revised form
*18 September 2015*
Accepted
*22 October 2015*

*Corresponding author
zzaima@iium.edu.my

## Abstract

Actinomycetes are biotechnologically important for their unrivalled capacity to produce bioactive secondary metabolites. Although thousands of antibiotics have been discovered from actinomycetes, these represent only a small fraction of the entire reservoir. Thus, screening of actinomycetes from poorly studied environment is a valuable attempt. In the present study, an effort was made to isolate, identify and evaluate the antimicrobial activities of actinomycetes from Tanjung Lumpur Mangrove Forest of Pahang, Malaysia; an underexplored ecosystem. Out of 1366 actinomycetes that were successfully enumerated, a total of 40 representative isolates were selected for further evaluation. The antimicrobial activity of the representative isolates were first assessed using the cross streak method against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*. Twenty five isolates displayed antagonistic potential against at least one of the test organisms. Of this, 40% exhibited antibacterial activity, 24% exhibited antifungal activity and 36% displayed both. The cell–free supernatant of the active isolates were then further evaluated using the agar well diffusion method, in which only 4 isolates displayed inhibitory activity. A total of 13 representative isolates were identified and characterized using 16S rRNA gene partial sequencing. They were further classified into 7 genera namely *Streptomyces*, *Micromonospora*, *Rhodococcus*, *Gordonia*, *Pseudonocardia*, *Mycobacterium* and *Actinophytocola*. These findings suggested that mangrove of Tanjung Lumpur is a rich source of actinomycetes for the discovery of bioactive secondary metabolites.

*Keywords*: Diversity, mangrove, actinomycetes, Tanjung Lumpur, antimicrobial activity



## 1.0 INTRODUCTION

In recent years, the hunt for new bioactive compounds continues to rise in order to combat and reverse the rapid spread of life threatening diseases caused by multiple antibiotic resistant pathogens. Although considerable progress has been made within the field of chemical synthesis, high throughput screening and engineered biosynthesis of antimicrobial compounds; nature remains as the most versatile and prominent source [1], [2]. Secondary metabolites can be derived from all living things. However, among them, actinomycetes are of special interest; as they are known to produce chemically diverse compounds with a wide range of biological activities [3].

Actinomycetes are slow growing, aerobic, filamentous Gram–positive bacteria belonging to the class *Actinobacteria*. Actinomycetes are widely distributed in nature. They are primarily soil inhabitants, but have also been found in the atmosphere, aquatic ecosystem as well as extreme environment such as the cryophilic and thermophilic regions [1], [4], [5]. Actinomycetes are responsible for the production of various commercially available antibiotics such as tetracycline, rifampicin, chloramphenicol and erythromycin [4]. To date, approximately 70% of naturally occurring antibiotics originate from actinomycetes [5].

On the ground that poorly explored habitats provides better prospects for discovering new bioactive compounds, actinomycetes from such

ecosystems are currently being the centre of interest [1], [4], [6]. Little is known about the diversity of actinomycetes in mangrove ecosystem though the isolation of *Isoptericolla chiayiensis* [7], *Jishengella endophytica* [8], *Verrucosisspora wenchangensis* [9], *Micromonopora haikuoensis* [10] and *Actinoallumurus acanthiterraei* [11] demonstrates the view that mangrove has the potential of becoming new repertoire of highly diverse actinomycetes.

Mangrove ecosystem is unique for its nutritional versatility as its muddy alluvial soil is high in organic matter, nitrogen and sulphur content which able to support diverse group of microorganisms [12]. This habitat is under the influence of both terrestrial and marine ecosystem, forming a distinctive saline environment. This can be a determinant factor to genetic and metabolic adaptation, promoting the production of chemically diverse bioactive compounds. Tanjung Lumpur mangrove that is situated in Pahang, Malaysia is under the influence of Kuantan River which flows out to South China Sea. This study area lies in wet tropical zone with semidiurnal tides [13]. This location is an underexplored ecosystem, hence, it is foreseen to be a rich repertoire of bioactive actinomycetes. The primary aim of this study was to isolate, identify and evaluate the antimicrobial potential of actinomycetes isolated from mangrove forest of Tanjung Lumpur, Malaysia.

## 2.0 EXPERIMENTAL

### Isolation of Actinomycetes

Sediment samples were collected from 2 sampling sites of Tanjung Lumpur mangrove (N 03° 48.074' E 103° 19.682' and N 03° 48.068' E 103° 19.671'). At each location, 3 sediment core samples were collected at a depth of 0–30 cm. The collected samples were air dried at room temperature, ground and sieved to remove large organic matters prior pretreatment. Selective pretreatments include wet heat treatment in sterilized sea water (60 °C, 20 min) and dry heat treatment (120 °C, 60 min) [14]. The pretreated sediments were serially diluted 1:10 (v/v) to $10^{-6}$ and plated onto series of isolation media: starch–yeast extract agar (SYE), malt extract–yeast extract agar (ISP2), oatmeal agar (ISP3), inorganic salt–starch agar (ISP4), starch–casein agar (SCA), glucose asparagine agar (GAA), actinomycetes isolation agar (AIA) and marine agar (MA). The SYE was prepared using sea water to maximize the recovery of salt–requiring actinomycetes. All media were supplemented with nystatin (50 µg $ml^{-1}$) and incubated at 30°C for 14 days. Isolates were selected based on their typical actinomycete morphological appearances.

### Antagonistic Potential of Actinomycetes

The isolates were preliminary screened for their antagonistic activity using the conventional cross streak method against *Bacillus subtilis* IMR B 144/11 C, *Candida albicans* IMR C 523/11 A, *Escherichia coli* ATCC 25922, *Serratia marcescens* IMR 974/05 B and *Staphylococcus aureus* ATCC 25923. The individual isolate was streaked at the centre of nutrient agar plate and incubated at 30°C for 7 days. Fresh overnight culture of the test organisms were perpendicularly streaked to the isolates and incubated at 37°C (24 hr) and 30°C (48 hr) for bacteria and fungus, respectively. Control plates were prepared without inoculating the actinomycetes to assess the normal growth of the test organisms. Antagonistic potential was observed by measuring the zone of inhibition formed. Results are presented in the following manner: – no activity, + weak activity (< 25% inhibition), ++ moderate activity (25–50% inhibition) and +++ good activity (> 50% inhibition) [15].

### Antimicrobial Assay Using Agar Well Diffusion Method

Actinomycete isolates displaying promising antagonistic activity were inoculated into 100 ml of SYE broth and incubated for 7 days at 30°C with continuous shaking at 200 rpm. Cell–free supernatant of each isolate was obtained through centrifugation of the fermentation medium at 9000 rpm and filtration using 0.2 µm filter membrane. The same test organisms used during preliminary screening were employed in this assay. Wells (6 mm) were made using sterile borer on Mueller Hinton agar that was previously seeded with the test organisms. About 100 µl of cell–free supernatant was added to each well. The diameters of inhibition were observed after 24 hr (37°C) and 48 hr (30°C) of incubation for bacteria and fungus, respectively. The results were described in the following manner: weak activity (5–9 mm), moderate activity (10–20 mm) and good activity (> 20 mm) [16].

### Molecular Identification of Actinomycetes

Genomic DNA of representative isolates were extracted using MasterPure™ Gram Positive DNA Purification Kit (Epicentre, USA) using the manufacturer's protocol. The PCR amplification of 16S rRNA gene were done using the following set of primers: 27F (5'–AGAGTTTGATCCTGGCTCTCAG–3') and 1492R (5'–GGTTACCTTGTTACGACTT–3') [17]. The PCR reactions were performed in a final volume of 50 µl which consist of 200 ng DNA template, 25 µl of MyTaq™ Mix 2X (Bioline, UK) and 0.4 µM primers under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 60 s and 72 °C for 4 min; and extension step at 72°C for 10 min. The amplification products were confirmed using 1% agarose gel and sent to 1st Base Laboratory, Malaysia for purification and sequencing.

### Phylogenetic Analysis of 16S rRNA Gene Sequences

The resultant 16S rRNA gene sequences were manually verified and edited using BioEdit Sequence Alignment Editor. The partial nucleotide sequences analysis of the isolates were carried out via GenBank BLASTn (http://www.ncbi.mlm.nih.gov) search tool. This provided the closest phylogenetic neighbours of each isolates. Partial 16S rRNA gene sequences of the isolates were deposited to GenBank under the following accession numbers: K4-03 (KR902623), K4-07 (KR902624), K4-08 (KR902625), K4-13 (KR902626), K4-15 (KR902627), K4-16 (KR902628), K4-19 (KR902629), K5-03 (KR902630), K5-04 (KR902631), K5-11 (KR902632), K5-13 (KR902633), K5-14 (KR902634) and K5-19 (KR902635). Sequences were then aligned using ClustalW software and the phylogenetic tree was inferred by the neighbour joining algorithm using Molecular Evolutionary Genetic Analysis (MEGA version 6.0).

## 3.0 RESULTS AND DISCUSSION

### Selective Isolation

In total, 1366 actinomycete isolates have been successfully recovered from Tanjung Lumpur mangrove using various isolation media and pretreatment procedures. They were selected based on their dull, chalky, leathery appearances and earthly smells. The wet heat pretreatment was found to be more effective in enumerating actinomycetes from Tanjung Lumpur mangrove; 79.3% of the total isolates were successfully recovered using this mild heating regime. The ISP4 medium displayed the highest percentage of recovery (30.0%), followed by AIA (23.6%). However, the use of SCA and SYE resulted in greater recovery of wide variety of actinomycetes, based on their morphological characteristics. Both spore forming and non–spore forming actinomycetes had been isolated using these media. This indicates that presence of seawater in SYE and high carbon–to–nitrogen ratio in SCA promote the recovery of actinomycetes.

Forty representative isolates were selected based on their morphological characteristics and further categorized into 7 colour series based on the colour of their mature aerial mycelia namely black, grey, orange, pink, red, white and yellow. Members of white series (55.0%) represent the most predominant group, followed by orange (27.5%) and grey (12.5%). This finding is in agreement with a study conducted by Xi and colleagues (2011) which demonstrated the domination of these colour groupings from marine associated ecosystem in China [18]. Members of white and grey colour series tend to have unfragmented substrate mycelia and abundant aerial mycelia with long spore chains. While the orange colour series appeared as tiny and solid colonies with unusual hyphae.

### Antimicrobial Activity of the Actinomycetes

The antimicrobial activity of the isolates were first assessed using the cross streak method against 5 test organisms (2 Gram–positive bacteria, 2 Gram–negative bacteria and 1 fungus) as presented in Table 1. It was observed that 62.5 % (n=25) of the total representative isolates displayed antagonistic activity against at least one of the test organisms. Of the 25 isolates, 40% exhibited antibacterial activity, 24% exhibited antifungal activity and 36% displayed both. Most bioactive isolates displayed antagonistic potential against the Gram–positive bacteria than the Gram–negative ones. Gram positive bacteria lack of lipopolysaccharide outer membrane, thus, promoting the permeability of bioactive secondary metabolites produced by actinomycete isolates [19].

Evaluation on the antimicrobial activity of the cell–free supernatant of the isolates was demonstrated using the agar well diffusion method against the same test organisms during the preliminary screening. Three members of genus *Streptomyces* (K4–03, K4–13, and K5–04) and one of genus *Micromonospora* (K5–13) exhibited antimicrobial activity against more than one test organisms. Isolate K4-03 and K5-04 displayed broad spectrum of antimicrobial activity against all test organisms for both cross streak and agar well diffusion methods (Table 1). Both isolates were found to belong to genus *Streptomyces* based on the 16S rRNA partial sequence analysis, whereby isolate K4-03 and K5-04 having the closest relationship to *Streptomyces enissocaelis* NBRC100763 (99% similarity) and *Streptomyces albidoflavus* DSM40455T (98% similarity), respectively. The cell–free supernatant of isolate K4-03 displayed strong inhibitory activity against Gram positive bacteria (*B. subtilis* = 23.3 ± 0.6 mm and *S. aureus* = 21.0 ± 0.6 mm) and moderate activity against Gram negative bacteria and fungus (*E.coli* = 16.7 ± 1.0 mm, *S. marcescens* = 19.7 ± 0.6 mm and *C. albicans* = 17.7 ± 0.6 mm). Therefore, *Streptomyces* sp. K4–03 can be considered as the most promising actinomycetes isolated from mangrove of Tanjung Lumpur for microbial natural product discovery. The identification of *Streptomyces* as the most bioactive genus is in agreement with other previous studies [14]. To date, *Streptomyces* remains as the unrivalled microbial bioactive compounds producers [2], [3].

It was also remarkable to note that members of some rare genera displayed antimicrobial activity in the cross streak assay. Isolate K4–16, a member of genus *Pseudonocardia* demonstrated specific antifungal potential by exhibiting moderate activity against *C. albicans* as demonstrated by previous studies [16], [20]. Another rare actinomycete, isolate K4–08 which belongs to genus *Actinophytocola*

**Table 1** Antimicrobial activity of selected bioactive actinomycetes using cross streak and agar well diffusion method

| Isolate | *B. subtilis* | | *S. aureus* | | *E. coli* | | *S. marcescens* | | *C. albicans* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CS | AW | CS | AW | CS | AW | CS | AW | CS | AW |
| K4–02 | + | – | + | – | – | – | – | – | +++ | – |
| K4–03 | +++ | +++ | +++ | +++ | +++ | ++ | +++ | ++ | +++ | ++ |
| K4–04 | +++ | – | + | – | – | – | – | – | +++ | – |
| K4–05 | – | – | – | – | – | – | – | – | – | – |
| K4–06 | ++ | – | – | – | – | – | – | – | – | – |
| K4–08 | +++ | – | +++ | – | – | – | – | – | – | – |
| K4–09 | +++ | – | +++ | – | – | – | +++ | – | +++ | – |
| K4–10 | +++ | – | +++ | – | – | – | – | – | – | – |
| K4–11 | +++ | – | +++ | – | – | – | + | – | – | – |
| K4–13 | +++ | + | +++ | ++ | + | + | + | ++ | – | + |
| K4–14 | +++ | – | +++ | – | – | – | +++ | – | – | – |
| K4–15 | – | – | – | – | – | – | – | – | +++ | – |
| K4–16 | – | – | – | – | – | – | – | – | ++ | – |
| K4–18 | + | – | + | – | – | – | – | – | – | – |
| K4–19 | +++ | – | ++ | – | – | – | +++ | – | – | – |
| K5–02 | – | – | – | – | – | – | – | – | – | – |
| K5–03 | + | – | – | – | – | – | – | – | + | – |
| K5–04 | +++ | ++ | +++ | ++ | +++ | + | +++ | ++ | ++ | ++ |
| K5–09 | + | – | + | – | – | – | – | – | – | – |
| K5–10 | – | – | – | – | – | – | – | – | – | – |
| K5–12 | – | – | – | – | – | – | – | – | – | – |
| K5–13 | + | ++ | + | + | – | – | – | – | – | – |
| K5–18 | + | – | + | – | – | – | – | – | +++ | – |
| K5–19 | + | – | – | – | – | – | – | – | – | – |
| K5–20 | + | – | + | – | – | – | – | – | – | – |

*Note*. "CS"– cross streak assay; "AW"– agar well diffusion assay; "–" no activity; "+" weak activity; "++" moderate activity; "+++" good activity

demonstrated strong antibacterial activity against *B. subtilis* and *S. aureus*. Studies showed that screening on antimicrobial activity of members of this genus had not yet been reported. Even though isolate K4–08 and K4–16 displayed promising antimicrobial activity in the cross streak assay, their cell–free supernatant did not exhibit any positive activities. Nevertheless, on the premise that rare actinomycetes might produce novel bioactive compound, isolate K4–08 and K4–16, alongside isolate K4–03 are the most suitable candidates for microbial bioprospecting studies. Hence, optimization can be done to trigger the production of antimicrobial compounds into the fermentation medium. In this study, 10% of total isolates displayed antimicrobial activities, thus, providing an evidence that mangrove actinomycetes of Tanjung Lumpur are highly capable in production of bioactive metabolites.

## Phylogenetic Analysis and Diversity

A total of 13 actinomycetes were identified using molecular characterization. Three isolates were selected based on their promising antimicrobial activity, while others were chosen based on their morphological characteristics. The 16S rRNA gene of the isolates were compared with those retrieved from Genbank and used to construct phylogenetic tree as illustrated in Figure 1. In total, representative of 7 established actinomycetes genera belonging to 5 families which were *Streptomycetacea* (*Streptomyces*), *Micromonoporacea* (*Micromonospora*), *Mycobacteriacea* (*Mycobacterium*), *Nocardiacea* (*Gordonia, Rhodococcus*) and *Pseudonocardiacea* (*Actinophytocola*, *Pseudonocardia*) had been successfully isolated. This clearly displayed a wide taxonomic distribution of actinomycetes in Tanjung Lumpur mangrove ecosystem. Members of genus *Streptomyces* and *Micromonospora* were the most predominant observed with relative abundance value of 38.5% (*n*=5) and 21.3% (*n*=3), respectively.

It was interesting to note that single isolates belonging to the genera *Mycobacterium*, *Actinophytocola*, *Pseudonocardia* and *Gordonia* formed relatively distinct phylogenetic lines, with 97–100% similarity to their nearest neighbouring strains. These genera are categorized under the rare actinomycetes group; a classification made on actinomycetes that has much lower isolation frequency than that of *Streptomyces* [21]. Pairwise comparison of the 16S rRNA gene sequences of isolate K4–07 and K5–11 displayed similarity to *Mycobacterium peregrinum* ATCC14467 and *Gordonia terrae* NBRC100016 at 98% and 99%, respectively. These genera were classified under the order of *Corynebacteriales*, hence, they were clustered alongside *Rhodococcus* sp. K5-14 in the same descendent clade as seen in Figure 1. This provides further evidence about the presence of these genera in mangrove ecosystem as reported by previous studies. Hong and co-workers (2009) isolated *Gordonia* and *Rhodococcus* from mangrove of Hainan Province in China [14], while Guo and colleagues (2011) studied the population of

*Mycobacterium* sp. from mangrove wetland of Hong Kong [22].

Isolate K4–16 displayed relatively monophyletic cluster with strain *Pseudonocardia antitumoralis* SCSIO01299 (99% similarity), which was supported by a bootstrap value of 99% in the neighbour–joining tree. It also formed a distinct phyletic line with *P. kongjuensis* and *P. ammonioxydans*. Studies have shown that members of genus *Pseudonocardia* can be found in various habitats including mangrove as reported by Mangamuri and colleagues [16].

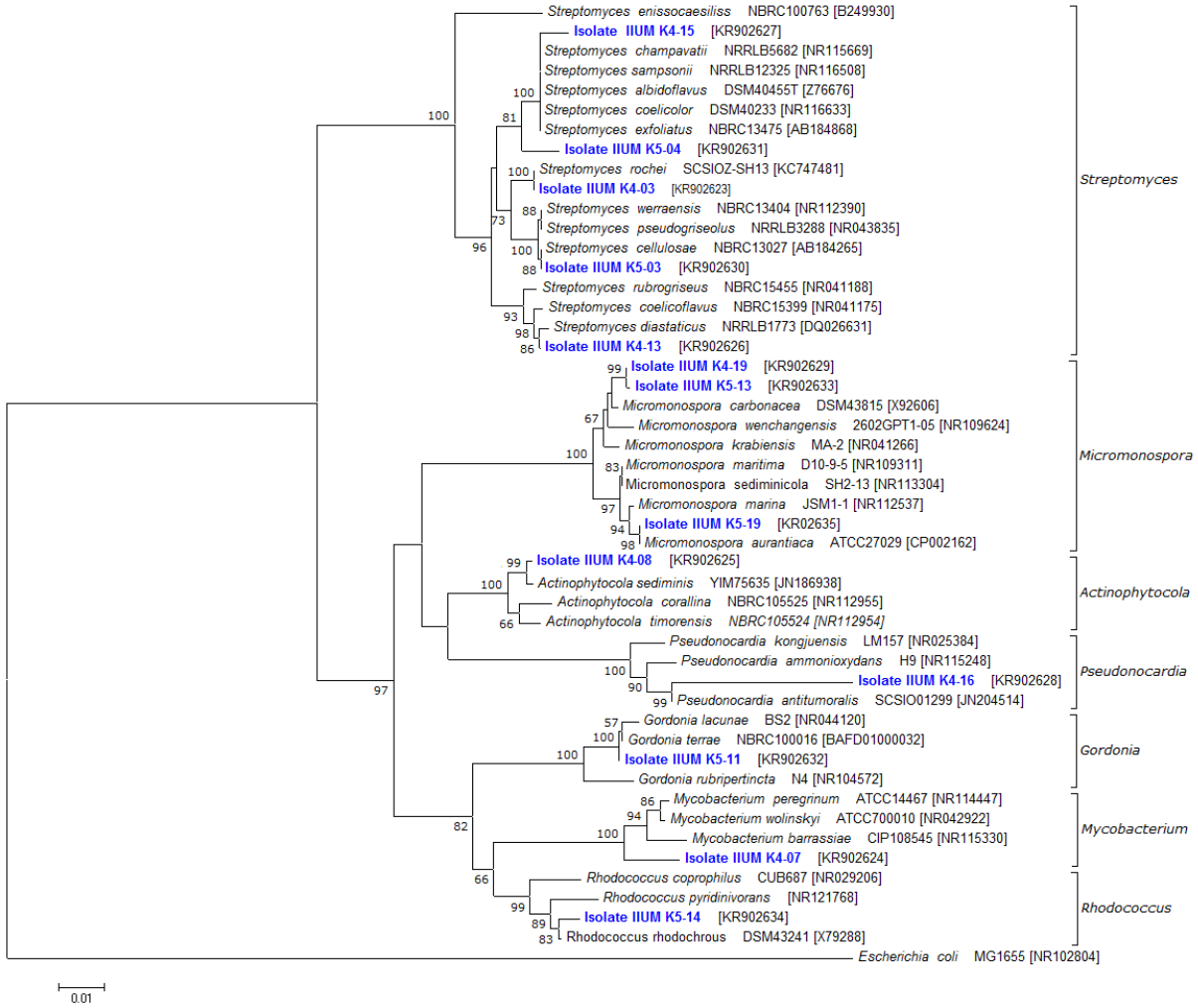


**Figure 1** Phylogenetic tree based on 16S rRNA sequences using neighbour–joining method showing relationships between the selected 13 isolates isolated from mangrove of Tanjung Lumpur and their closely related type strains. Values on the node indicate the bootstrap values based on 1000 resampled dataset. The scale bar represents 0.01 substitutions per nucleotide position

This study also demonstrates the isolation of genus *Actinophytocola;* a member of family *Pseudonocardiacea* that was not commonly reported in mangrove environment. Isolate K4–08 formed a distinct phylogenetic cluster supported by 99% bootstrap value with *A. sediminis* (99% similarity) that was previously found in the deep sea sediment of South China Sea [23]. This shows actinomycetes from mangrove ecosystem possess strong phylogenetic line with those originate from marine environment. Isolate K4–08 was obtained from sediment sample (N 03° 48.074' E 103° 19.682') pretreated using dry heat method and incubated on ISP4 medium. It formed unfragmented substrate mycelium on agar media and appeared as cream coloured colonies on both SYE and ISP4 media. Aerial mycelia were also observed with spore chain structures. To date, genus *Actinophytocola* only consist of 7 validly published species names isolated from various habitats. *A. oryzae* was recovered from the roots of Thai glutinous rice plants [24], while *A. burenkhanensis* [25], *A. corralina* [26], *A. timorensis* [26] and *A. xinjiangensis* [27] from terrestrial soils as well as *A. gilvus* [28] that was isolated from extreme desert environment. This genus has yet been reported in tidal–influenced ecosystem such as mangrove. Thereby, this is the first report to describe

the isolation of genus *Actinophytocola* from mangrove sediment, particularly in Malaysia.

## 4.0 CONCLUSION

In conclusion, large numbers of actinomycetes were successfully enumerated from mangrove of Tanjung Lumpur using various established isolation procedures. Actinomycetes present in this mangrove ecosystem is not only limited to *Streptomyces–Micromonospora* grouping but rich diversity of rare genera were also discovered. This study demonstrates the first association of genus *Actinophytocola* with mangrove ecosystem. The present findings not only provide further evidence on the existence of various taxonomic actinomycete groups, but also display the potential of this diversity for biotechnological exploitation. High percentage of bioactive actinomycetes isolated from this habitat indicates that mangrove of Tanjung Lumpur provide a valuable source of actinomycetes with interesting biosynthetic capabilities.

## Acknowledgement

We would like to acknowledge the Ministry of Higher Education, Malaysia for the financial support under grant FRGS13–039–0280.

## References

[1] Goodfellow, M. and Fiedler, H. P. 2010. A Guide to Successful Bioprospecting: Informed by Actinobacterial Systematics. *Antonie Van Leeuwenhoek*. 98(2): 119-142.
[2] Demain, A. L. and Sanchez, S. 2009. Microbial Drug Discovery: 80 Years of Progress. *Journal of Antibiotic*. 62(1): 5-16.
[3] Berdy, J. 2005. Bioactive Microbial Metabolites. *Journal of Antibiotics*. 58(1): 1-26.
[4] Chaudhary, H. S., Soni, B., Shrivastava, A. R. and Shrivastava, S. 2013. Diversity and Versatiliy of Actinomycetes and Its Role in Antibiotic Production. *Journal of Applied Pharmaceutical Science*. 3(8): S83-S94.
[5] Subramani, R. and Aalsberg, W. 2012. Marine Actinomycetes: An Ongoing Source of Novel Bioactive Metabolites. *Microbiological Research*. 167(10): 571-580.
[6] Subramani, R. and Aalsberg, W. 2013. Culturable rare Actinomycetes: Diversity, Isolation and Marine Natural Product Discovery. *Applied Microbiology and Biotechnology*. 97: 9291-9321.
[7] Tseng, M., Liao, C. H., Chiang, W. P. and Yuan, G. F. 2011. *Isoptericola chiayiensis* sp. nov. Isolated from Mangrove Soil. *International Journal of Systematic Evolutionary and Microbiology* 61(7): 1667-1670
[8] Xie, Q. Y., Wong C., Wang, R., Qu, Z., Lin, H. P., Goodfellow, M. and Hong, K. 2011. *Jishengilla endophytica* gen. nov., sp. nov., a New Member of the Family *Micromonosporaceae*. *International Journal of Syetematic Evolutionary and Microbiology*. 61(5): 1153-1159.
[9] Xie, Q., Lin, H., Li, L., Brown, R., Goodfellow, M., Deng, Z. and Hong, K. 2012. *Verrucosisspora wenchangensis* sp. nov., Isolated from Mangrove Soil. *Antonie Van Leeuwenhoek*. 102(1): 1-7.
[10] Xie, Q. Y., Qu, Z., Lin. H. P., Li, L. and Hong, K. 2012. *Micromonospora haikouensis* sp.nov., Isolated from Mangrove Soil. *Antonie van Leeuwenhoek*. 101(3): 649-655.
[11] Tang, Y. L., Lin, H. P., Xie, Q. Y., Li, L., Peng, F., Deng, Z. and Hong, K. 2013. *Actinoallomurus acanthiterrae* sp. nov., an Actinomycete Isolated fro Rhizosphere Soil of the Mangrove Plant *Achantus ilicifolius*. *Internationational Journal of Systematic Evolutionary and Microbiology*. 63(5): 1874-1879.
[12] Ara, I., Bakir, M. A, Hozzein, W. N. and Kudo, T. 2013. Population, Morphological and Chemotaxonomical Characterization of Diverse Rare Actinomycetes in the Mangrove and Medicinal Plant Rhizosphere. *African Journal of Microbiology Research*. 7(16): 1480-1488.
[13] Kamaruzzaman, Y., Nurul Nadia, M. Y., Noor Azhar, M. S., Ong, M. C., Shahbudin, S., Jalal, K. C. A. and Bidai, J. 2011. Heavy Metal Concentration in the Surface Sediment of Tanjung Lumpur. *Sains Malaysiana*. 40(2): 89-92.
[14] Hong, K., Gao, A. H. Xie, Q. Y., Gao, H., Zhuang, L., Lin, H. P., Yu, H. P. Li, J., Yao, X. S., Goodfellow, M. and Ruan, J. S. 2009. Actinomycetes for Marine Drug Discovery Isolated ffrom Mangrove Soils and Plants in China. *Marine Drugs*. 7(1): 24-44.
[15] Sweetline, C., Usha, R. and Palaniswamy, M. 2012. Antibacterial Activity of Actinomycetes from Pichavaram Mangrove of Tamil Nadu. *Applied Journal of Hygiene*. 1(2): 15-18.
[16] Mangamuri, U. K., Muvva, V., Poda, S. and Kamma, S. 2012. Isolation, Identification and Molcular Characterization of Rare Actinomycetes from Mangrove Ecosystem of Nizampatnam. *Malaysian Journal of Microbiology*. 8(2): 83-91.
[17] Wilson, K. H., Blitchington, R. B. and Greene, R. C. 1990. Amplification of Bacterial 16S Ribosomal DNA with Polymerase Chain Reeaction. *Journal of Clinical Microbiology*. 28(9): 1942-1946.
[18] Ruan, L. X. and Huan, Y. 2012. Diversity and Biosynthetic Potential of Culturable Actinomycetes Associated with Marine Sponges in the China Seas. *International Journal of Moecular Science*. 13(5): 5917-5932.
[19] Naikpatil, S. V. and Rathod, J. L. 2011. Selective Isolation and Antimicrobial Activity of Rare Actinomycetes from Mangrove Sediment of Karwar. *Journal of Ecobiotechnology*. 3(10): 48-53.
[20] Carr, G., Derbyshire, E. R., Caldera, E., Currie, C. R. and Clardy, J. 2012. Antibiotic and Antimalarial Quinones from Fungus–growing Ant–associated *Pseudonocardia* sp. *Journal of Natural Product*. 75(10): 1806-1809.
[21] Kurtboke, D. I. 2012. Biodiscovery from Rare Actinomycetes: An Eco–taxonomical Perspective. *Applied Microbiology and Biotechnology*. 93(5): 1843-1852.
[22] Guo, C., Ke, L., Dang, Z. and Tam, N. F. 2011. Temporal Changes in *Sphingomonas* and *Mycobacterium* Populations in Mangrove Sediments Contaminated with Different Concentration of Polycylic Aromatic Hydrocabons (PAHs). *Marine Pollution Bulletin*. 62(1): 133-139.
[23] Zhang, D. F., Zhang, Z., Zhang, X. M., Yang, L. L., Tian, X. P., Long, L. J., Zhang, S. and Li, W. J. 2014. *Actinophytocola sediminis* sp. nov., an Actinomycete Isolated from a Maride Sediment. *International Journal of Systematic Evolutionary and Microbiology*. 64(8): 2834-2840.
[24] Idananda, C., Matsumoto, A. Inhashi, Y., Takahashi, Y., Duangmal, K. and Thamchaipenet, A. 2010. *Actinophytocola oryzae* gen. nov., sp. nov., Isolated from the Roots of Thai Glutinous Rice Plants, a New Member of the Family *Pseudonocardiaceae*. *International Journal of Sytematic Evolutionary and Microbiology*. 60(5): 1141-1146.
[25] Ara, I., Tsetseg, B., Daram, D., Suto, M. and Ando, K. 2011. *Actinophytocola burenkhanensis* sp. nov., Isolated from

Mongolian Soil. *International Journal of Sytematic Evolutionary and Microbiology*. 61(5): 1033-1038.

[26] Otoguro, M., Yamamura, H., Tamura, T, Izaldi, R., Ratnakomala, S., Ridwan, R., Kartina, G., Triana, E., Nurkanto, A., Lestari, Y., Lisdiyanti, Y., Widyastuti, Y. and Ando, K. 2011. *Actinophytocola timorensis* sp. nov. and *Actinophytocola corralina* sp. nov. Isolated from Soil. *International Journal of Scientific Evolutionari and Microbiology*. 61(4): 834-838.

[27] Guo, X., Qiu, D., Ruan, J. and Huang, Y. 2011. *Actinophytocola xinjiangensis* sp. nov., Isolated from Virgin Forest Soil. *International Journal of Systematic Evolutionary and Mircobiology*. 61(12): 2928-2932.

[28] Sun, H. M., Zhang, T., Yu, L. Y., Mou, X. Z. and Zhang, Y. Q. 2014. *Actinophytocola gilvus* sp. nov., isoalted from desert soil crusts, and emended description of the genus *Actinophytocola* Indananda *et al*. 2010. *International Journal of Systematic Evolutionary and Mircobiology*. 64(9): 3120-3125.