



Water quality characterisation, antibacterial activity and metabolite profiling of Malaysian tropical mangrove-derived *Actinophytocola* sp. K4-08

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ABSTRACT

Mangrove ecosystems constitute a large portion of the coastline in the tropical and subtropical regions of Earth and are characterized by their salinity and tidal variation which results in frequent anaerobic conditions and a wide range of redox potential. Such conditions make mangroves hotspots for microbial diversity, and the microbial community plays essential roles in the functioning and maintenance of the ecosystem. The complex microbial communities that inhabit the sediment of mangroves plays a crucial role in the coupling of biogeochemical cycles between the land and ocean. Hence, the objectives of this work were to characterise *Actinophytocola* sp. K4-08, to assess the antibacterial ability of the crude extracts obtained from *Actinophytocola* sp. K4-08 growth culture and to determine potential compounds present in the extract through gas chromatography-mass spectrometry (GC-MS) profiling. *Actinophytocola* sp. K4-08, a rare actinomycete was previously isolated from mangrove forest sediment in Kuantan, Pahang, Malaysia. *Actinophytocola* sp. K4-08 colonies appeared in a round-irregular shape with formation of powdery white aerial mycelia spores around the colony and dense, white-creamed substrate hyphae in the middle. Scanning electron microscopy showed a regular round spore chain with short branching. This bacterium could tolerate up to 10% sodium chloride (NaCl) and able to utilise gentiobiose, D-raffinose, α -D-glucose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, inosine, D-galacturonic acid, citric acid, acetic acid and formic acid as carbon sources and resistance to minocycline and aztreonam antibiotics. PKS-I and NRPS genes, usually related to secondary metabolite ability were detected in this bacterium. Three crude extracts were prepared – methanol, ethyl acetate and acetone. Methanolic and ethyl acetate extracts exhibited strong antibacterial activity against *Bacillus subtilis* while acetone showed weak antibacterial activity. Further analysis was conducted on methanolic extract through Fourier-transform infrared spectroscopy and GC-MS. Fourier-transform infrared spectrum produced peaks at 3,327; 2,927; 1,636; 1,411 and 1,018 cm^{-1} which corresponded to O–H stretching bond in alcohol, stretching C–H in alkane group, stretching of C=C in alkene group, C–H bending in alkane group and stretching carbonyl group in primary alcohol respectively. GC-MS profiling identified 9 compounds and they were 3,4-dihydroxy-3,4-dimethylhexane-2,5-dione, 2,4-di-tert-butylphenol (2,4-DTBP), 1-docosene, hexadecanoic acid methyl ester, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, 1-nonadecene, linoleic acid methyl ester, oleic acid methyl ester, and stearic acid methyl ester. Linoleic acid methyl ester, oleic acid methyl ester and hexadecanoic acid methyl ester are usually linked to antioxidant activity whereas 2,4-DTBP is secondary metabolite that is associated with many biological potentials. However, the findings of this study indicate the promising potential of rare actinomycete *Actinophytocola* sp. K4-08 to be developed as antibacterial agent towards the applications in the medicinal and pharmaceutical industries.

Keywords: Mangrove rare actinomycete; *Actinophytocola* sp. K4-08; Antibacterial; Phenotypic characterisation; Metabolite profiling

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1. Introduction

Mangrove forests are coastal intertidal ecosystems, characterized by mangrove trees growing in slow moving saline waters, that constitute a large portion of the coastline in the tropical and subtropical regions. The dynamic water regime created by the tides results in different microhabitats in which microbial communities play an essential role in the functioning and maintenance of the mangrove ecosystem [1]. It was found that the most abundant microbial families in the sediments are associated with nutrient cycling consistent with the essential role of the microbiome in maintaining the health of the mangrove ecology. The recent lack of discovery of novel antibiotics and the increase of antibiotic-resistant microorganisms present significant problems in infectious disease therapy. The mangrove forest ecosystem is an important natural source of novel organisms that have high potential to produce bioactive compounds [2].

Actinomycetes are spore-forming gram-positive bacteria containing a high ratio of guanine-cytosine (>55 mol%) in their genome and classified under the phylum of Actinobacteria and order of *Actinomycetales*. They are characterized by aerial and substrate mycelium growth, and they are known for their superior ability in producing secondary metabolites. These microorganisms are ubiquitous and widely distributed in various ecosystems especially terrestrial soil and are an essential component of the bacterial community [3]. More than 140 genera of actinomycetes have been described, and 80% of them are *Streptomyces*, which account for a majority contribution in natural product novel compounds.

Actinomycetes can be categorized into two major groups, the dominant and the rare actinomycete group or 'non-streptomycete'. The most common isolated actinomycete genera are *Streptomyces* and *Micromonospora*. Rare actinomycetes such as *Pseudonocardia* sp., *Verrucosisspora* sp., *Actinophytocola* sp., *Nocardiosis* sp., *Saccharopolyspora* sp., *Actinocorallia* sp., *Nonomuraea* sp., *Nocardia* sp., *Rhodococcus* sp., *Streptosporangium* sp., *Actinomadura* sp. which have lower isolation rate and harder to cultivate due to their extremely slow growth. Rare actinomycetes are of particular interests as they may reveal novel and interesting secondary metabolites, assuming that species novelty would lead to chemical novelty.

Examples of novel compounds produced by rare actinomycetes are Krasilnikolides A and B and Detalosylkrasilnikolide A isolated from underexplored actinomycete *Krasilniko* [4], Catellatolactams A-C from *Catellatospora* [5], antifungal polyketide named Forazoline A from *Actinomadura* sp. [6] and *Nesterenkonia*, a novel cyclic ether isolated from the deep-sea derived *Nesterenkonia flava* [7].

To date, genus *Actinophytocola* represents only eleven species with validly published names. The first species was *Actinophytocola oryzae* reported by Indananda et al. [8] isolated from the roots of Thai glutinous rice plants. *Actinophytocola timorensis*, *Actinophytocola corallina* [9], *Actinophytocola burenkhanensis* [10], *Actinophytocola xinjiangensis* [11], all of which were isolated from soil, *Actinophytocola sediminis* from marine sediment [12], *Actinophytocola gilvus* [13] and *Actinophytocola algeriensis* [14] from desert soil, *Actinophytocola xanthii* [15] and *Actinophytocola glycyrrhizae* [16] from plant rhizosphere and the latest addition, *Actinophytocola*

gossypii [17] from rhizosphere soil. Actinomycetes exploration in Kuantan mangrove forests isolated many actinomycetes including rare actinomycetes [18,19]. One particular isolate, *Actinophytocola* sp. K4-08 has 98% identity with *Actinophytocola sediminis* and displayed antibacterial activity during preliminary screening [20].

Based on above contexts, the objectives of this research were to characterize *Actinophytocola* sp. K4-08, to assess the antibacterial ability of the crude extracts obtained from *Actinophytocola* sp. K4-08 growth culture and to determine potential compounds present in the extract through gas chromatography-mass spectrometry (GC-MS) profiling.

2. Materials and methods

2.1. Phenotypic characterisation of *Actinophytocola* sp. K4-08

Actinophytocola sp. K4-08 was previously isolated from Kuantan mangrove forest with Accession no. KR902625 [20]. Phenotypic characterisation of this bacterium involved scanning electron microscopy (SEM), salt tolerance test, culture characteristics and Biolog GEN III MicroPlate identification.

2.1.1. Scanning electron microscopy

Actinophytocola sp. K4-08 was cultured on starch yeast extract (SYE) agar prepared with filtered saltwater for 10 d at 30°C. About 5 mm × 5 mm of fresh growth colony was cut out and fixed in McDowell–Trump fixative overnight. Sample was washed with water, and post-fixed in osmium tetroxide (1%, w/v) for 1 h. The sample was washed twice with water and dehydrated in ascending ethanol (30%, 50%, 70%, 90%, and 100%) followed by critical point drying (CPD). Finally, sample was coated in gold and examined under SEM (Carl Zeiss AG – EVO® 50 Series, Germany) with magnification over a range of about six orders of magnitude from about 10 to 3,000,000 times.

2.1.2. Salt tolerance test

SYE agar was prepared using distilled water and the addition of sodium chloride (NaCl) in the range of 0%–10% (w/v) followed by incubation at 30°C and examined after 10 d. The growth of actinomycete on agar was observed by their aerial spore mass colour, substrate mycelial pigmentation, and the colour of any diffusible pigment.

2.1.3. Culture characteristics *Actinophytocola* sp. K4-08

Actinophytocola sp. K4-08 was inoculated onto a set of agar media, namely, inorganic salt–starch agar (ISP4), starch–casein agar (SCA), and nutrient agar (NA). The growth, aerial spore mass color, substrate mycelium pigmentation, and production of diffusible pigment were used to describe the morphological appearance of each isolate.

2.1.4. Phenotypic fingerprint: Biolog GEN III MicroPlate

Spores of *Actinophytocola* sp. K4-08 was scraped gently using a sterile cotton swab and suspended in a special

gelling inoculating fluid IF-A. The turbidity was adjusted to 92% T-95% T. Next, 100 μ L of cell suspension was inoculated into the GEN III MicroPlate (Biolog Inc., Hayward, CA, USA) and incubated at 30°C for 24 h. The MicroPlate was interpreted using Biolog's Microbial Identification System Software.

2.2. Polymerase chain reaction amplification of PKS-I and NRPS genes

Genomic DNA *Actinophytocola* sp. K4-08 was extracted using the MasterPure Gram Positive DNA Purification Kit (Epicentre, USA) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of non-ribosomal peptide synthetase (NRPS) and type I polyketide synthase (PKS-I) genes was done using the following sets of primers: A3F and A7R for NRPS and KIF and M6R for PKS-I [21]. The PCR was performed in a final volume of 50 μ L, which consisted of a 200-ng DNA template, 25 μ L of MyTaq Mix 2X (Bioline, UK), and 0.4–1 μ L primers under the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 58°C for 2 min, and 72°C for 4 min and the extension step at 72°C for 10 min. The detection of NRPS and PKS-I genes was indicated by the presence of the corresponding fragment size: 700–800 bp for NRPS and 1,200–1,400 bp for PKS-I.

2.3. Preparation of crude extracts

Actinophytocola sp. K4-08 was cultivated in a 100 mL SYE broth as a seed culture and incubated for 30°C in a rotary shaker at 150 rpm for 10 d. About 5 mL of the seed culture was added to 800 mL of SYE broth and incubated at 30°C for two weeks with continuous shaking at 150 rpm. Cells were harvested through centrifugation at 9,000 rpm at 4°C for 15 min while cell free supernatant was obtained by filtering the culture broth using a 0.2 μ m membrane filter. Methanolic extract was obtained by maceration of the cells in a double volume of methanol with continuous shaking overnight. The cell-free supernatant was extracted twice with ethyl acetate while the mother liquor recovered from ethyl acetate extraction was applied to amberlite XAD-2 resin column and eluted by acetone with 1–2 bed volumes per hour producing acetone extract. All extracts (methanol, ethyl acetate, acetone) were evaporated to dryness using rotary evaporator.

2.4. Antibacterial activity

Modified Kirby–Bauer disc diffusion method was used to evaluate antibacterial activity of the crude extracts of *Actinophytocola* sp. K4-08. Ethyl acetate, methanol, and acetone crude extracts were recuperated in dimethyl sulfoxide (DMSO) solvent to give a final concentration of 1.0 mg/mL. Blank antibiotic discs were impregnated with different volume of extract – 20, 40, 60, 80, and 100 μ L and left to dry. The discs were then placed on Mueller Hinton agar (MHA) previously inoculated with *Bacillus subtilis*. A standard antibiotic vancomycin (10 $^{\circ}$ μ g) disc served as a positive control while blank disc impregnated with 20 μ L of DMSO as a negative control. The antibacterial activity was determined by measuring the diameter of the inhibition zone (expressed

in mm) where the colonies are failed to grow. The test was conducted in triplicate, and results were expressed as mean \pm standard deviation (SD).

2.5. Fourier-transform infrared spectroscopy analysis

The functional group of methanol extract was analysed using Fourier-transform infrared spectroscopy (FTIR; PerkinElmer, USA) based on methods proposed by Rao et al. [22]. The crude atomic compounds were absorbed at the range of 600–4,000 cm^{-1} using the attenuated total reflectance (ATR) method. The infrared spectrums were analysed using PerkinElmer Spectrum Quant 10.4.00 Software. The spectra were plotted as peak intensity vs. wavenumber (cm^{-1}), and the presence of functional groups was interpreted based on universal infrared spectrum table chart frequency ranges.

2.6. GC-MS profiling

Secondary metabolites present in the methanol crude extract were analysed GC-MS using PerkinElmer ClarusTM 680/GC SQ8T/MS system (USA) with a fused silica Elite-5MS capillary column (30 m \times 0.25 mm I.D. \times 0.25 μ m). The extract was diluted with dichloromethane (DCM) and was carried by 99.99% helium gas as the carrier gas at a constant flow rate of 1.00 ml/min with an injection volume of 1.0 μ L. The oven temperature was programmed with the initial temperature at 30°C, then increased to 200°C with a rate of 15°C/min, and subsequently held for 2.00 min. The temperature of the injector was increased up to 230°C with increments at 5°C/min and maintained for 5.67 min. Mass spectra were taken at 70 eV with a scan range of 50–500 m/z. The total GC running time was 25 min. The interpretation of GC-MS mass spectra was performed using the National Institute of Standards and Technology (NIST) database, containing over 62,000 patterns. The unknown component's mass spectrum was compared to the spectrum of known components stored in the NIST library.

3. Results and discussion

3.1. Mangrove-derived *Actinophytocola* sp. K4-08

Actinophytocola sp. K4-08 colonies appeared in a round-irregular shape with formation of powdery white aerial mycelia spores around the colony and dense, white-creamed substrate hyphae in the middle (Fig. 1A). SEM inspection indicated *Actinophytocola* sp. K4-08 formed a regular round spore chain with short branching as depicted in Fig. 1B. This bacterium showed good growth on ISP4, SCA and NA and diffusible pigment was not produced in all media. Spores were observed in ISP4 and SCA but not NA. Salt tolerance experiments are mainly to determine the ability of the organism to survive in various NaCl concentrations since the mangrove ecosystem is an intertidal zone with a high salinity environment. *Actinophytocola* sp. K4-08 could tolerate up to 10% NaCl (Fig. 2). At 0% of NaCl this bacterium produced thin film colonies which could easily be picked up with no spore formation; unlike the usual actinomycete colony that strongly adheres on medium. Spores were detected at 5% until 10% of NaCl which might suggest that

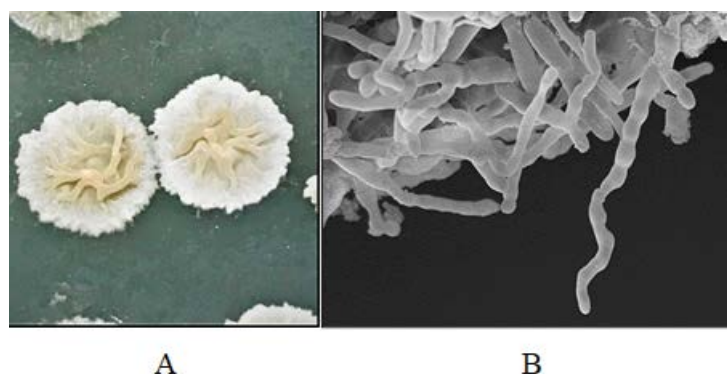


Fig. 1. (A) Colonies of *Actinophytocola* sp. K4-08. (B) Scanning electron microscopy image of *Actinophytocola* sp. K4-08.

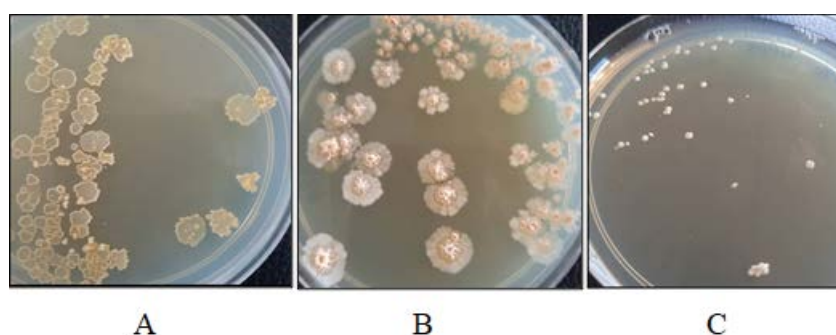


Fig. 2. *Actinophytocola* sp. K4-08 growth on starch yeast extract agar with different salt content. (A) 0% NaCl, (B) 5% NaCl, and (C) 10% NaCl.

the presence of salt might influence the formation of spores. Phenotypic fingerprint through Biolog GEN III MicroPlate revealed that *Actinophytocola* sp. K4-08 could utilise gentiobiose, D-raffinose, α -D-glucose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, inosine, D-Galacturonic acid, citric acid, acetic acid and formic acid as carbon sources, and resistance to minocycline and aztreonam antibiotics. Bioactive compounds are synthesized by two biosynthetic systems designated as NRPS and PKS-I [21] and detection of these genes may aid in gauging the possible potential present in actinomycetes. Both PKS-I and NRPS genes were detected in *Actinophytocola* sp. K4-08 suggesting the capability of producing secondary metabolites in this bacterium.

3.2. Antibacterial activity and metabolite profiling

All crude extracts (methanol, ethyl acetate and acetone) were subjected to antibacterial test against *B. subtilis* using disc diffusion method. All extracts demonstrated antibacterial activity against *B. subtilis* with methanolic extract showing the strongest followed by ethyl acetate and acetone extract showing the least antibacterial activity (Table 1). Inhibition zone of 12.0 ± 0.0 mm was produced by methanolic extract of 100 mg/disc while ethyl acetate extract gave inhibition zone of 11.0 ± 0.8 mm, and only 8.0 ± 0.0 mm of inhibition zone for acetone extract. Based on the antibacterial test, methanolic extract was selected for further investigation through FTIR and GC-MS. The FTIR transmittance spectrum of methanolic extract shows peaks at 3,327; 2,927;

Table 1
Antibacterial activity of crude extracts of *Actinophytocola* sp. K4-08 against *Bacillus subtilis*

Crude extract (1 mg/mL)	Volume (μ L)/disc	Inhibition zone diameter (mm)
Ethyl acetate	20	7.6 ± 0.5
	40	8.8 ± 0.0
	60	9.3 ± 0.5
	80	10.5 ± 1.4
	100	11.0 ± 0.8
Methanol	20	8.3 ± 0.6
	40	9.9 ± 0.0
	60	10.8 ± 0.9
	80	11.0 ± 0.0
	100	12.0 ± 0.0
Acetone	20	6.2 ± 0.3
	40	6.8 ± 0.2
	60	7.7 ± 0.5
	80	7.7 ± 0.5
	100	8.0 ± 0.0

1,636; 1,411 and 1,018 cm^{-1} (Fig. 3). The first broad and medium peak at $3,327 \text{ cm}^{-1}$ was attributed to O–H stretching of alcohol. The medium peak at $2,927 \text{ cm}^{-1}$ indicated alkane functional group with C–H stretching. The vibrational sharp

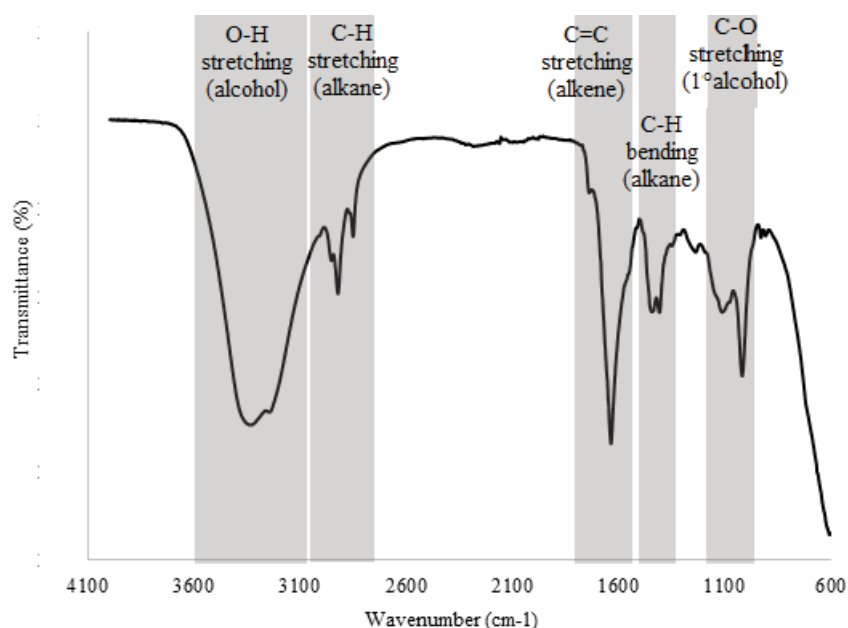


Fig. 3. Fourier-transform infrared spectroscopy spectrum of methanolic extract of *Actinophytocola* sp. K4-08 ranging from 600 to 4,000 cm^{-1} .

Table 2

Compounds from methanolic extract of *Actinophytocola* sp. K4-08 based on GC-MS chromatogram

Peak no.	Retention time (min)	Composition	Molecular formula	Molecular weight (MW)
1	4.924	3,4-Dihydroxy-3,4-dimethylhexane-2,5-dione	$\text{C}_8\text{H}_{14}\text{O}_4$	174.19
2	10.161	2,4-Di-tert-butylphenol (2,4-DTBP)	$\text{C}_{14}\text{H}_{22}\text{O}$	206.32
3	12.442	1-Docosene	$\text{C}_{22}\text{H}_{44}$	308.60
4	13.953	Hexadecanoic acid, methyl ester/methyl palmitate	$\text{C}_{17}\text{H}_{34}\text{O}_2$	270.50
5	14.058	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate	$\text{C}_{18}\text{H}_{28}\text{O}_3$	292.41
6	14.888	1-Nonadecene	$\text{C}_{19}\text{H}_{38}$	266.50
7	16.314	9,12-Octadecadienoic acid (Z,Z)-, methyl ester/linoleic acid, methyl ester	$\text{C}_{19}\text{H}_{34}\text{O}_2$	294.50
8	16.409	9-Octadecenoic acid (Z)-, methyl ester/methyl cis-9-octadecenoate/oleic acid, methyl ester	$\text{C}_{19}\text{H}_{36}\text{O}_2$	296.50
9	16.814	Methyl stearate/octadecanoic acid, methyl ester/stearic acid, methyl ester	$\text{C}_{19}\text{H}_{38}\text{O}_2$	298.50

peak appeared at $1,636\text{ cm}^{-1}$ denoted to the stretching of C=C of alkene bond. The peak positioned at $1,411\text{ cm}^{-1}$ indicated the covalent bond of C–H bending alkane, whereas the peak emerged at $1,018\text{ cm}^{-1}$ shows the presence of medium carbonyl group stretching at primary alcohol.

In total of 9 compounds were identified in GC-MS analysis and they were 3,4-dihydroxy-3,4-dimethylhexane-2,5-dione, 2,4-di-tert-butylphenol (2,4-DTBP), 1-docosene, hexadecanoic acid methyl ester, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, 1-nonadecene, linoleic acid methyl ester, oleic acid methyl ester, and stearic acid methyl ester (Table 2). Fatty acid methyl ester (FAME) derivatives – hexadecanoic acid methyl ester, linoleic acid methyl ester and oleic acid methyl ester are usually associated with antioxidant activity. Compound 2,4-DTBP is a common toxic secondary metabolite produced by various groups of

organisms. 2,4-DTBP was found in 16 species of bacteria in 10 families, such as nitrogen-fixing cyanobacteria, gram-positive bacteria in hot spring, soils, and gram-negative bacteria in soil and freshwater. Bioactivities of this compound include antimicrobial, cytotoxic activity and antioxidant. Purified 2,4-DTBP from *Streptomyces* sp. KCA1 exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and cytotoxic activity against breast cancer cell line (MCF7) [23]. A similar study reported that 2,4-DTBP from *Streptomyces bacillaris* ANS2 was found to have anti-tubercular (TB) and anti-cancer properties [24]. Purified 2,4-DTBP from *B. subtilis* isolated from seaweed surface demonstrated antibiofilm potential against Group A *Streptococcus* [25]. Whether 2,4-DTBP from *Actinophytocola* sp. K4-08 was linked to the antibacterial property is yet to be determined as this study used crude methanolic extracts. Purification attempts

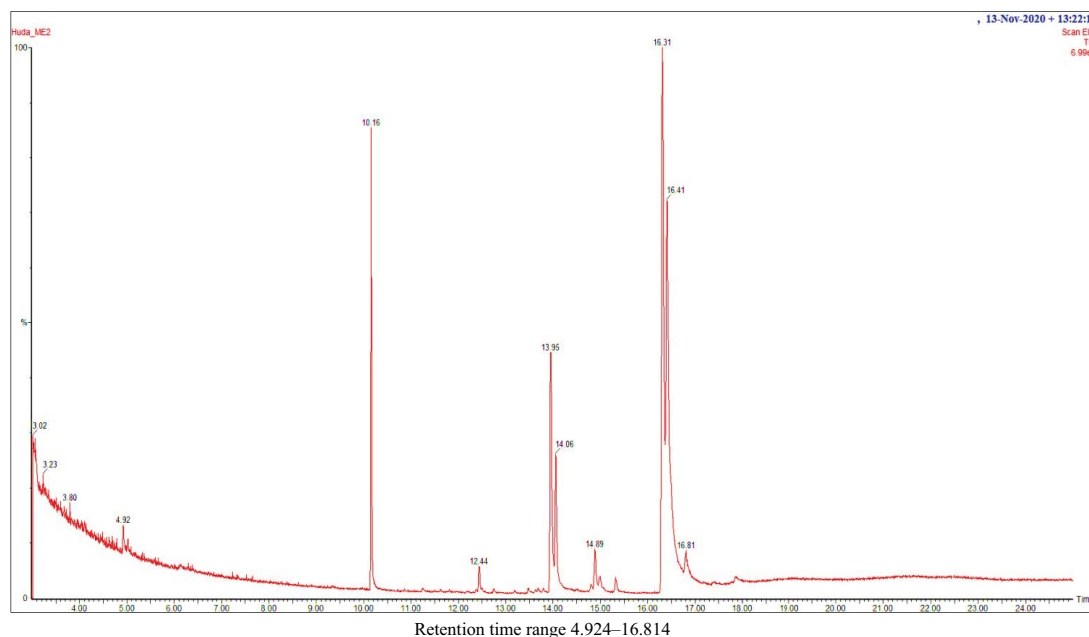


Fig. 4. GC-MS profile of methanolic extract of *Actinophytocola* sp. K4-08.

of bioactive compounds from *Actinophytocola* sp. K4-08 should be made to ascertain the identity of the responsible antibacterial compound.

Genus *Actinophytocola* are soil dwellers and a few studies highlighted the association of this genus with plant rhizosphere microbiome conferring beneficial properties to the plants such as disease resistance [26], plant growth and nutrition uptake [27] and adaptation to environmental stress [28] but no reports on antimicrobial activity or other biological activity by this genus. Nevertheless, findings from this study demonstrated the potential of *Actinophytocola* as secondary metabolite producer indicated by antibacterial activity and metabolite profiling. Optimization of culture conditions and growth parameters manipulation may assist in recovery of many bioactive compounds. Other avenues such as genome mining may reveal cryptic pathway that could be translated to novel compounds from *Actinophytocola*.

4. Conclusion

This study unveiled the biological potential of *Actinophytocola* K4-08, a mangrove-derived actinomycete as a promising antibacterial agent. Metabolite profiling provided a preview of secondary metabolites present in *Actinophytocola* K4-08 which warrants a further investigation on antibacterial compound.

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References

- [1] C.O. Santana, P. Spealman, V.M.M. Melo, D. Gresham, T.B. Jesus, F.A. Chinalia, Microbial community structure and ecology

- in sediments of a Pristine Mangrove Forest, Biogeosciences, (2019), doi: 10.1101/833814.
- [2] K. Kachanuban, P. Yan, P. Fu, W. Zhu, P. Wilaipun, Identification of antibiotic compounds from Thai mangrove soil-derived *Streptomyces iconiensis*, J. Fish. Environ., 46 (2022) 145–162.
- [3] A.A. Bhatti, S. Haq, R.A. Bhat, Actinomycetes benefaction role in soil and plant health, Microb. Pathogen., 111 (2017) 458–467.
- [4] S. Lu, T. Zhou, K. Fukaya, E. Harunari, N. Oku, D. Urabe, Y. Igarashi, Krasilnikolides A and B and Detalosylkrasilnikolide A, cytotoxic 20-membered macrolides from the genus *Krasilnikovia*: assignment of anomeric configuration by J-based configuration analysis, J. Nat. Prod., 85 (2022) 2796–2803.
- [5] C. Liu, Z. Zhang, K. Fukaya, D. Urabe, E. Harunari, N. Oku, Y. Igarashi, Catellatolactams A–C, plant growth-promoting Ansamacrolactams from a rare actinomycete of the genus *Catellatospora*, J. Nat. Prod., 85 (2022) 1993–1999.
- [6] F. Zhang, T.P. Wyche, Y. Zhu, D.R. Braun, J.-X. Yan, S. Chanana, Y. Ge, I. Guzei, M.G. Chevrette, C.R. Currie, M.G. Thomas, S.R. Rajski, T.S. Bugni, MS-derived isotopic fine structure reveals Forazoline A as a thioketone-containing marine-derived natural product, Org. Lett., 22 (2020) 1275–1279.
- [7] C.-L. Xie, Q. Liu, J.-M. Xia, Y. Gao, Q. Yang, Z.-Z. Shao, G. Liu, X.-W. Yang, Anti-allergic compounds from the deep-sea-derived actinomycete *Nesterenkonina flava* MCCC 1K00610, Mar. Drugs, 15 (2017) 71, doi: 10.3390/md15030071.
- [8] C. Indananda, A. Matsumoto, Y. Inahashi, Y. Takahashi, K. Duangmal, A. Thamchaipenet, *Actinophytocola oryzae* gen. nov., sp. nov., isolated from the roots of Thai glutinous rice plants, a new member of the family *Pseudonocardiaceae*, Int. J. Syst. Evol. Microbiol., 60 (2010) 1141–1146.
- [9] M. Otoguro, H. Yamamura, T. Tamura, R. Irzaldi, S. Ratnakomala, R. Ridwan, G. Kartina, E. Triana, A. Nurkanto, Y. Lestari, P. Lisdiyanti, Y. Widyastuti, K. Ando, *Actinophytocola timorensis* sp. nov. and *Actinophytocola corallina* sp. nov., isolated from soil, Int. J. Syst. Evol. Microbiol., 61 (2011) 834–838.
- [10] I. Ara, B. Tsetseg, D. Daram, M. Suto, K. Ando, *Actinophytocola burenkhanensis* sp. nov., isolated from Mongolian soil, Int. J. Syst. Evol. Microbiol., 61 (2011) 1033–1038.
- [11] X. Guo, D. Qiu, J. Ruan, Y. Huang, *Actinophytocola xinjiangensis* sp. nov., isolated from virgin forest soil, Int. J. Syst. Evol. Microbiol., 61 (2011) 2928–2932.
- [12] D.-F. Zhang, Z. Jiang, X.-M. Zhang, L.-L. Yang, X.-P. Tian, L.-J. Long, S. Zhang, W.-J. Li, *Actinophytocola sediminis* sp. nov.,

- an actinomycete isolated from a marine sediment, *Int. J. Syst. Evol. Microbiol.*, 64 (2014) 2834–2840.
- [13] H.M. Sun, T. Zhang, L.Y. Yu, X.X. Lu, X.Z. Mou, Y.Q. Zhang, *Actinophytocola gilvus* sp. nov., isolated from desert soil crusts, and emended description of the genus *Actinophytocola* Indananda et al. 2010, *Int. J. Syst. Evol. Microbiol.*, 64 (2014) 3120–3125.
- [14] K. Bouznada, N. Bouras, P. Schumann, C. Spröer, N. Sabaou, H. Klenk, *Actinophytocola algeriensis* sp. nov., an actinobacterium isolated from Saharan soil, *Int. J. Syst. Evol. Microbiol.*, 66 (2016) 2760–2765.
- [15] W. Wang, B. Wang, H. Meng, Z. Xing, Q. Lai, L. Yuan, *Actinophytocola xanthii* sp. nov., an actinomycete isolated from rhizosphere soil of the plant *Xanthium sibiricum*, *Int. J. Syst. Evol. Microbiol.*, 67 (2017) 1152–1157.
- [16] C. Cao, Y. Sun, B. Wu, S. Zhao, B. Yuan, S. Qin, J. Jiang, Y. Huang, *Actinophytocola glycyrrhizae* sp. nov. isolated from the rhizosphere of *Glycyrrhiza inflata*, *Int. J. Syst. Evol. Microbiol.*, 68 (2018) 2504–2508.
- [17] X. Ge, S. Yang, C. Zhen, W. Liu, *Actinophytocola gossypii* sp. nov. and *Streptomyces gossypii* sp. nov., two novel actinomycetes isolated from rhizosphere soil of cotton, *Int. J. Syst. Evol. Microbiol.*, 73 (2023), doi: 10.1099/ijsem.0.005832.
- [18] Z.A. Zainal Abidin, K.C.A. Jalal, N. Abdul Malek, Z. Zainuddin, Diversity, antimicrobial capabilities, and biosynthetic potential of mangrove actinomycetes from coastal waters in Pahang, Malaysia, *J. Coastal Res.*, 82 (2018) 174–179.
- [19] Z.A. Zainal Abidin, N. Abdul Malek, Z. Zainuddin, K.C.A. Jalal, Selective isolation and antagonistic activity of actinomycetes from mangrove forest of Pahang, Malaysia, *Front. Life Sci.*, 9 (2016) 24–31.
- [20] N. Abdul Malek, Z. Zainuddin, A.J.K. Chowdhury, Z.A. Zainal Abidin, Diversity and antimicrobial activity of mangrove soil actinomycetes isolated from Tanjung Lumpur, Kuantan, *Jurnal Teknologi*, 77 (2015) 37–43.
- [21] A. Ayuso-Sacido, O. Genilloud, 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.*, 49 (2005) 10–24.
- [22] K.V.R. Rao, P. Mani, B. Satyanarayana, T.R. Rao, Purification and structural elucidation of three bioactive compounds isolated from *Streptomyces coelicoflavus* BC 01 and their biological activity, *3 Biotech*, 7 (2017) 1–12.
- [23] A. Seenivasan, R. Manikkam, M. Kaari, A.M. Sahu, S.M. Said, S.G. Dastager, 2,4-Di-tert-butylphenol (2,4-DTBP) purified from *Streptomyces* sp. KCA1 from *Phyllanthus niruri*: isolation, characterization, antibacterial and anticancer properties, *J. King Saud Univ. Sci.*, 34 (2022) 102088, doi: 10.1016/j.jksus.2022.102088.
- [24] M. Kaari, J. Joseph, R. Manikkam, R. Kalyanasundaram, A. Sivaraj, S. Anbalmani, S. Murthy, A. Kumar Sahu, M. Said, S.G. Dastager, B. Ramasamy, A novel finding: 2,4-di-tert-butylphenol from *Streptomyces bacillaris* ANS₂ effective against *Mycobacterium tuberculosis* and cancer cell lines, *Appl. Biochem. Biotechnol.*, 195 (2023) 6572–6585.
- [25] D. Viswapriya, U. Prithika, S. Deebika, K. Balamurugan, S.K. Pandian, *In vitro* and *in vivo* antibiofilm potential of 2,4-di-tert-butylphenol from seaweed surface associated bacterium *Bacillus subtilis* against group A *Streptococcus*, *Microbiol. Res.*, 191 (2016) 19–31.
- [26] M. Duan, L. Wang, X. Song, Assessment of the rhizosphere fungi and bacteria recruited by sugarcane during smut invasion, *Braz. J. Microbiol.*, 54 (2023) 385–395.
- [27] Y. Zhou, Z. Pang, H. Jia, Z. Yuan, R. Ming, Responses of roots and rhizosphere of female papaya to the exogenous application of GA₃, *BMC Plant Biol.*, 23 (2023) 35, doi: 10.1186/s12870-022-04025-6.
- [28] A.J. Fernández-González, J.A. Ramírez-Tejero, M.P. Nevado-Berzosa, F. Luque, M. Fernández-López, J. Mercado-Blanco, Coupling the endophytic microbiome with the host transcriptome in olive roots, *Comput. Struct. Biotechnol. J.*, 19 (2021) 4777–4789.