

**BIOCHEMICAL SCREENING OF MARINE *MICROMONOSPORA MARINA* KPMS1 (MH036351) AND ITS ANTIBACTERIAL ACTIVITY AGAINST MULTIDRUG RESISTANT BACTERIA**

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Received: 20 September 2018, Revised and Accepted: 09 November 2018

**ABSTRACT**

**Objective:** The present investigation aimed at the screening of pharmaceutically potential antimicrobial metabolite isolated from marine *Micromonospora* sp. to combat the multidrug-resistant bacteria.

**Materials and Methods:** The Marine sediments were randomly collected for the isolation of *Micromonospora* sp., from Gulf of Mannar, East Coastal Region, located at Kayalpatnam, Tuticorin district, Tamil Nadu, India. The *Micromonospora* sp. was cultivated by serial dilution and crowded plating method on actinomycetes isolation agar. The isolated colonies were identified by morphological, cultural, and biochemical methods. The antibacterial study of *Micromonospora* sp. was performed on Mueller-Hinton agar medium against multidrug-resistant bacteria isolated from urinary tract infection. The antibacterial compound was separated and characterized by Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectrum.

**Results:** Based on the morphological, cultural, and biochemical studies, the isolated colonies were found to be the genera of *Micromonospora*. Among *Micromonospora* genera, *Micromonospora marina* KPMS1 strain showed potent antibacterial activity against multidrug-resistant *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. The FT-IR and NMR studies showed the structural elucidation of active compounds derived from *M. marina* KPMS1. The 16S rRNA sequences of *M. marina* KPMS1 (MH036351) strain were blasted and deposited in the GenBank of National Center for Biotechnology Information.

**Conclusion:** The results of the study were concluded that microbial compounds are the promising sources of nearly all of the antibiotics produced by marine *Micromonospora* sp. The detection of new biological compounds was considered to represent a novel species of the genus *Micromonospora* which have been used for clinical treatment against multidrug-resistant bacteria and were pharmaceutically important.

**Keywords:** Biochemical studies, 16S rRNA sequences, Antimicrobial, Antibiotics, Multidrug-resistant bacteria.

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**INTRODUCTION**

The Actinobacteria are one of the plentiful sources of novel biological metabolites. Among Actinobacteria, the genera of *Micromonospora* have been accommodated as a reservoir of antimicrobial agent and novel secondary metabolite producer next to the *Streptomyces* genera [1]. The genus *Micromonospora* has been documented as aerobic, spore-forming Gram-positive, filamentous bacteria which are characterized by aerial and substrate mycelia. *Micromonospora* sp. produced single non-motile spores on substrate mycelia. Many species of the genus *Micromonospora* have been isolated from diverse environments. More than 50% of bioactive compounds were derived from different regions in soil produced by soil-inhabiting genera *Micromonospora* [2]. The few are found in root nodules and sea sand [3]. Currently, *Micromonospora* sp. was isolated from marine sponge, marine water, and its sediments [4]. Not only soil region, the marine water and its sediments also harbor microbial rich environment which have abundant microbial communities ranging from  $10^6$  to  $10^9$  CFU/g [5]. The potential source of bioactive metabolites was produced by *Micromonospora* strains which are pharmaceutically important. Among these compounds, aminoglycoside group of antibiotics is predominant followed by macrolide antibiotics [6]. Among 23,000 bioactive compounds, more than 740 bioactive metabolites isolated from family Micromonosporaceae such as gentamicin, neomycin, sagamicin, mutamicin, verdamicin, rifamycins, sisomicin, erythromycin B, and fortimicins have been used for clinical treatment [7]. The marine *Micromonospora* not only have several new species but also have number of novel secondary metabolites that have different mechanisms of action in worldwide [8]. The present study was aimed to identify the antimicrobial compound isolated from marine *Micromonospora* sp. and its antibacterial activity against the multidrug-resistant bacteria.

**MATERIALS AND METHODS****Materials**

All chemicals were purchased from Merck (Germany), HiMedia (India), or Thermo Fisher Scientific Pvt., Ltd. (India) unless otherwise indicated. All buffers and reagents used in this study were prepared in distilled water.

**Sample collection and cultivation**

The marine sediment samples were collected and air dried at room temperature. 1 g marine sediment was suspended in 9 ml of distilled water and serially diluted upto  $10^{-9}$  dilution. 1 ml of each dilution series was inoculated into Actinomycete Isolation Agar (AIA) supplemented with cycloheximide (50 µg/ml) and incubated at 28°C for 2–3 weeks. The selected colonies were subcultured and maintained on AIA with 25% v/v glycerol at 4°C [9].

**Morphological identification***Gram reaction*

Most of the actinobacteria can be differentiated by their gram reaction due to differences in their cell wall structure. In this technique, the cell smear was made and flooded with complex chemical stains and focused on the microscope under  $\times 100$  magnification.

*Slide culture analysis*

The morphology of spore based on the aerial and substrate mycelium was observed by slide culture method. The agar blocks were prepared using AIA. The selected colonies were inoculated in every corner of the agar blocks. The setup was kept in moist chamber under incubation at

28°C for 1 week. After incubation, the aerial and substrate mycelia were examined under high power magnification using Sudan black stain. The spore morphology was examined under bright-field microscope using 0.1% crystal violet stain along with a drop of culture.

#### Biochemical reaction

The biochemical and physiological properties were identified by indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, catalase test, oxidase test, starch hydrolysis test, urea hydrolysis test, and utilization of triple sugar iron agar test [10].

#### L-asparaginase production

L-asparaginase production was carried out by submerged fermentation on minimal media broth incorporated with phenol red indicator [11]. The selected colonies were inoculated and incubated in a rotary shaker at 28°C for 24 h. At the end of the incubation, the crude enzyme was prepared by centrifugation at 10,000 rpm for 20 min. The cell-free supernatant was separated by centrifuging at 10,000 rpm for 20 min.

#### Antibacterial studies

For fermentation studies, the selected strains were inoculated on ISP 4 medium and incubated under 150 rpm in a rotary shaker at 28°C for 2 weeks. After incubation, the growth was analyzed by optical density at 600 nm, and the dry weight of the mycelium was measured and correlated with the growth of the isolate. The cell-free supernatant was collected by centrifugation at 10,000 rpm from fermented broth and filtrated by nitrocellulose filter paper. The cell-free culture filtrate was mixed with an equal amount of ethyl acetate and kept under 200 rpm overnight. The solvent phase was collected and tested against multidrug-resistant bacteria.

Preliminary antibacterial activity was performed on Mueller-Hinton agar by agar well diffusion method. The 100 µl of crude ethyl acetate extract of selected active strain was tested against multidrug-resistant bacteria and incubated at 37°C for 24 h. After incubation, all plates were examined for the zone of inhibition around the wells.

#### Biological activity

The purification of antimicrobial compounds was performed by thin-layer chromatography (TLC) plate [12]. The crude extract of selected strain was applied on marked spot using a capillary tube and the spots were dried. The solvent of n-butanol: ethyl acetate: water (9:9:1) was prepared, and the TLC plate was vertically placed into solvent chamber. The distance of solvent moved up to 2 cm below the top of TLC plate and the plate was removed from solvent chamber. The fractions were detected under ultraviolet chamber, and the *R<sub>f</sub>* values were recorded:

$$R_f = \text{Distance of solute} / \text{distance of solvent}$$

TLC fractions were separated and 100 µl of 5 mg/ml concentration of selected fraction was loaded on sterile disc. The multidrug-resistant bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were inoculated over the Mueller-Hinton agar plates. Standard drug cefotaxime (10 mg) was placed on the agar plates and incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured.

The minimal inhibitory concentration (MIC) was performed on Mueller-Hinton broth by tube dilution method. In each of the test tubes, 2 ml of Mueller-Hinton broth was added. Various concentrations of standard antibiotic and active compound were prepared by dilution of × 2 concentration of drug. The concentration of diluted antibiotics and sample was 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/ml. A tube without drug was negative control and uninoculated tube served as sterility control. After dilution, test organism was inoculated into all tubes and incubated for 24 h. After incubation, the lowest concentration of antibiotics and its growth inhibition was recorded by the addition of resazurin indicator [13].

#### Characterization of active compound Fourier-transform infrared (FT-IR) and nuclear magnetic resonance (NMR) spectrum

The active compound of *Micromonospora* was subjected to FT-IR spectral analysis. The FT-IR spectrum ranges between 4000 and 400 cm<sup>-1</sup> with the resolution of 0.9 cm<sup>-1</sup>. The FT-IR spectrum was recorded with 95% methanol. The resulting FT-IR spectral pattern was analyzed and confirmed the presence of functional groups in the absorption peaks which were compared with known signatures of identified standard.

The NMR (<sup>13</sup>C and <sup>1</sup>H NMR) spectroscopy was used to determine the chemical structural properties of active compound derived from *Micromonospora* strain. The NMR experiment was conducted on a 400 MHz JOEL NMR spectrophotometer at the National Institute of Technology, Tiruchirappalli, Tamil Nadu, India.

#### Molecular characterization and phylogenetic tree analysis

The chromosomal DNA isolation was performed by the exponential growth of *Micromonospora* on actinomyceye isolation broth. The cell pellet was collected and resuspended in 567 µl of TE buffer, 30 µl of 10% SDS, 3 µl of 20 mg/ml proteinase K, and incubated at 37°C. Addition of 100 µl of 5 M NaCl and 80 µl of CTAB-NaCl solution was mixed well on a vortex mixer until the cell suspension became translucent and incubated at 65°C for 10 min. An equal volume of chloroform and isoamyl alcohol was added and centrifuged at 5000 rpm for 5 min. The aqueous phase was aspirated and mixed with an equal volume of phenol, chloroform, and isoamyl alcohol which were centrifuged for 5 min. The aqueous phase was collected and transferred to 1.5 ml microfuge tube. Subsequently, 0.6 volume of isopropanol and 100 µl of 3 M sodium acetate were added. The precipitate was collected by centrifugation and washed with 70% ethanol, dried, and dissolved in 100 µl of TE buffer.

Polymerase chain reaction (PCR) amplification of 16S rRNA gene sequence was carried out by two primers for matching most of known eubacteria order. The amplification of 16S rRNA gene sequence was carried out using the forward primer F243 (5'-GGATGAGCCCGCGCCTA-3') and reverse primer R513GC (5'-CGGCCGCGCTGCTGGCAGTA-3') with an activation temperature of 95°C for 5 min, followed by denaturation of 94°C for 1 min, the primer annealing at 52°C for 0.5 min, elongation at 72°C for 2 min and the final elongation temperature at 72°C for 10 min. The 5 µl of the PCR product was loaded on 1.2% agarose gel in × 1 Tris-borate-EDTA buffer and stained with ethidium bromide 0.5 µl/ml. The PCR product was precipitated by polyethylene Glycol (PEG)-NaCl (20% PEG in 2.5 M NaCl) precipitation at 37°C for 30 min. The reaction mixture was centrifuged at 12,000 rpm for 30 min at room temperature. The supernatant was discarded and the pellet was washed twice with 70% ethanol. The forward and reverse DNA sequencing reaction of PCR amplicon was carried out using BDT v 3.1 cycle sequencing kit on ABI 3730 × 1 genetic analyzer.

The reference sequence required for comparison was downloaded from the GenBank using BLASTN sequence based retrieval system from GenBank (URL <http://www.ncbi.nlm.nih.gov>) using our sequence as the query. Based on the sequenced data, the phylogenetic tree was constructed using the bioinformatics tool MEGA 5.05 for aligning the sequences by neighbor-joining method. The 16S rRNA sequences for the *Micromonospora* have been deposited in GenBank using sequin (<http://www.ncbi.nlm.nih.gov/genbank>)

## RESULTS AND DISCUSSION

#### Collection site and morphological studies

The collection site of marine sediments was located at Gulf of Mannar, East Coastal Region, Kayalpatnam, Tuticorin district, Tamil Nadu, India. East Coastal Region is bestowed with rich natural flora which has been considered as a potential source of marine *Micromonospora* was studied for its bioactivity. The physiological and climatic condition of marine sediment region of this site has resulted in diverse type bio-organism

contributing to enrich the biological diversity. The collection marine sediments were done randomly. The sediments were taken 0–5 cm depth. The isolation of *Micromonospora* was performed using serial dilution and plating method by AIA supplemented with cycloheximide. The specific antibiotics were added into the isolation media which is used to suppress the growth of other actinobacteria [14]. The growths of marine *Micromonospora* colonies were recognized by their mycelium formation. All the *Micromonospora* colonies were subcultured on AIA slants. The marine sediments were screened and the *Micromonospora* colonies were isolated, and the numbers of colonies were calculated as  $6 \times 10^6$  CFU/g. All the isolates were identified at their generic level based on the colony morphology and microscopic morphology. The ecological niche of marine sediments may have the valuable source for the isolation of novel *Micromonospora* with the potential to produce new secondary metabolites [15].

The identification of strains was observed by both morphological and cultural characteristics, revealing that most of the isolates were showed the nature of color such as greenish gray, dull white, whitish ash, dark gray, brownish yellow, and whitish gray on AIA. The colonies were elevated 3–4 mm in diameter, initially had a smooth appearance, and later developed the rough colonies. The aerial mycelium absent in all isolates except KPMS10 developed a weft of aerial mycelium which is granular or powdery in nature. All isolates produced single and non-motile spore on substrate mycelium. The pigments were raised in four isolates except KPMS17 and KPMS19. Two isolates KPMS1 and KPMS10 were produced melanoid pigments [Table 1]. All the *Micromonospora* sp. showed septate hyphae with monospore on substrate mycelium by slide culture methods. The present study revealed that all *Micromonospora* isolates were found to be Gram-positive cell wall. The *Micromonospora* are currently updated with spore-forming Gram-positive nature of bacteria that form extensive branching of substrate mycelia [16].

#### Carbohydrate utilization

The Four isolates were found to be positive for triple sugar iron test with acid butt and alkaline slant. Only two strains of KPMS 9 and KPMS 19 produced acid butt and acid slant with gas production. The potent strain was tentatively identified by the utilization of different carbohydrate source that they belong to distinct taxonomic groups which have been well documented [17].

#### Biochemical screening

The biochemical characteristics of *Micromonospora* strains showed positive results on indole and urease. Four isolates showed methyl red except KPMS10 and KPMS17. Two isolates KPMS10 and KPMS17 were positive in Voges–Proskauer. All the strains were negative on citrate utilization test. Among six strains, three were catalase and oxidase

positive and two were starch positive [Table 2]. The *Micromonospora* genera were further identified using physiological and biochemical tests. This finding gives an initial recognition as novel taxa isolated from marine sediments [18]. The ability of starch, casein, and urease hydrolysis is immense importance for both practical and taxonomical approaches considered for the proper identification of *Micromonospora* genera which has been documented [19].

Among six isolates, KPMS1 and KPMS10 showed L-asparaginase activity on minimal broth medium incorporated with phenol red indicator, glucose, and yeast extract as carbon and nitrogen substrates. After incubation, the medium turned pink color due to the production of ammonia which indicated the positive result of L-asparaginase production. The marine actinobacteria are serving as a good source of L-asparaginase enzyme and used as a chemotherapeutic agent [20].

#### Antibacterial studies

The antibacterial screening was carried out for *Micromonospora* strains on ISP 4 medium using ethyl acetate extract at a concentration of 2:1 (v/v). The quantity of concentrated product was calculated by the initial weight of watch glass (g) subtracted by final weight of watch glass (g) equal to concentrated component weight (g). The concentrated components were redissolved in distilled water and analyzed for secondary screening process. In screening process, all *Micromonospora* strains were tested against multidrug-resistant bacteria *E. coli*, *P. aeruginosa*, and *E. faecalis* isolated from urinary tract infection (UTI) infection.

In preliminary screening, *Micromonospora marina* KPMS1 was highly sensitive to multidrug-resistant bacteria isolated from UTI. The highest inhibition showed in *Klebsiella pneumoniae* ( $18 \pm 0.04$  mm) with a relative zone of inhibition of 88%, *P. aeruginosa* ( $17 \pm 0.01$  mm) with relative zone of inhibition of 76%, and *Enterococcus faecium* ( $16 \pm 0.08$  mm) with relative zone of inhibition of 80%. The comparison was done by standard antibiotic cefotaxime, and the percentage of relative inhibitory zone was found to be ranged between 76% and 88% [Table 3].

The bioactive compound extracted from *M. marina* KPMS1 and concentrated with ethyl acetate was subjected to TLC analysis using three mobile phase, namely chloroform: benzene: methanol: ethyl acetate (4:2:2:1), chloroform: methanol: ethyl acetate (2:1:1), and chloroform: methanol: ethyl acetate (2:1:1). After airdrying, the plates were visualized with iodine vapors. Among three mobile phases, the plate developed with solvent system 3 gave the best separation. A total of three different spots were obtained from *M. marina* KPMS1 sample with  $R_f$  values of 0.76, 0.62, 0.51, and 0.42.

Table 1: Identification of *Micromonospora* isolates

Isolated strains	Mycelium	Color of mycelium	Pigmentation	Carbohydrate utilization	Melanoid pigments
KPMS 1	SM	Greenish gray	Yellowish green	Acid butt and alkaline slant	Positive
KPMS 9	SM	Dull white	Diffusible yellowish brown	Acid butt, acid slant with gas production	Negative
KPMS 10	AM/SM	Whitish ash	Diffusible red wine	Acid butt and alkaline slant	Positive
KPMS 13	SM	Dark gray	Pale yellow	Acid butt and alkaline slant	Negative
KPMS 17	SM	Brownish yellow	-	Acid butt and alkaline slant	negative
KPMS 19	SM	Whitish gray	-	Acid butt, acid slant with gas production	negative

Table 2: Biochemical properties of *Micromonospora* isolates

Isolated strain	Indole	MR	VP	Citrate	Catalase	Oxidase	Starch	Urease
KPMS 1	+	+	-	-	+	+	+	+
KPMS 9	+	+	-	-	-	-	-	+
KPMS 10	+	-	+	-	+	+	-	+
KPMS 13	+	+	-	-	-	+	+	+
KPMS17	+	-	+	-	-	-	-	+
KPMS 19	+	+	-	-	+	-	-	+

In secondary antibacterial screening of *M. marina* KPMS1, the fraction one showed highly sensitive to *E. coli*  $18 \pm 0.07$  mm with 87%, *P. aeruginosa*  $17 \pm 0.06$  mm with 77%, and *E. faecalis*  $17 \pm 0.05$  mm with 81% of the relative zone of inhibition. As compared to standard antibiotic cefotaxime, the percentage of the relative inhibitory zone was found to range between 77% and 87% [Table 4]. The results clearly demonstrate that a Gram-negative bacterium *E. coli* was highly susceptible to the tested crude extracts compared to Gram-positive *E. faecalis* bacteria. The *M. marina* KPMS1 showed significant antibacterial activity against multidrug-resistant Gram-negative and Gram-positive bacteria and it proved a promising antibacterial strains. The screening of the antimicrobial metabolites derived from *Micromonospora* isolates is often encountered and showed antibacterial activity against multidrug-resistant bacteria isolated from human beings [21]. The unusual antibiotic profile exhibited from *Micromonospora* strains was isolated from marine sediment and its biopotential activity against multidrug-resistant bacterial pathogens [13].

The active fraction showed a dose-dependent antimicrobial activity against the pathogen tested with zones of inhibition ranging from 17 and 19 mm in size at 2 mg/ml. The minimal concentration to inhibit the tested bacterial pathogen was determined by microdilution method, indicating that the minimum inhibitory concentration of active fraction was 6.2–12.5  $\mu\text{g/ml}$ . This could be either due to the low concentration of the active inhibitory molecules or the presence of molecules with high inhibition constant. The MIC varied among the tested compounds against *E. coli*. These results were similar with the other report [22].

#### Detection of bioactive compound

In chemical screening, the crude potential fractions of *M. marina* KPMS1 recovered after fermentation were purified by TLC and identified with

**Table 3: Preliminary screening of *M. marina* KPMS1 against multidrug-resistant bacteria with standard drug**

Samples	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>
Cefotaxime	16 mm	17 mm	15 mm
KPMS 1	$18 \pm 0.04$ mm	$17 \pm 0.01$ mm	$16 \pm 0.08$ mm
Negative control	4 mm	4 mm	4 mm
RIZD (%)	88	76	80

*M. marina*: *Micromonospora marina*, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *E. faecalis*: *Enterococcus faecalis*

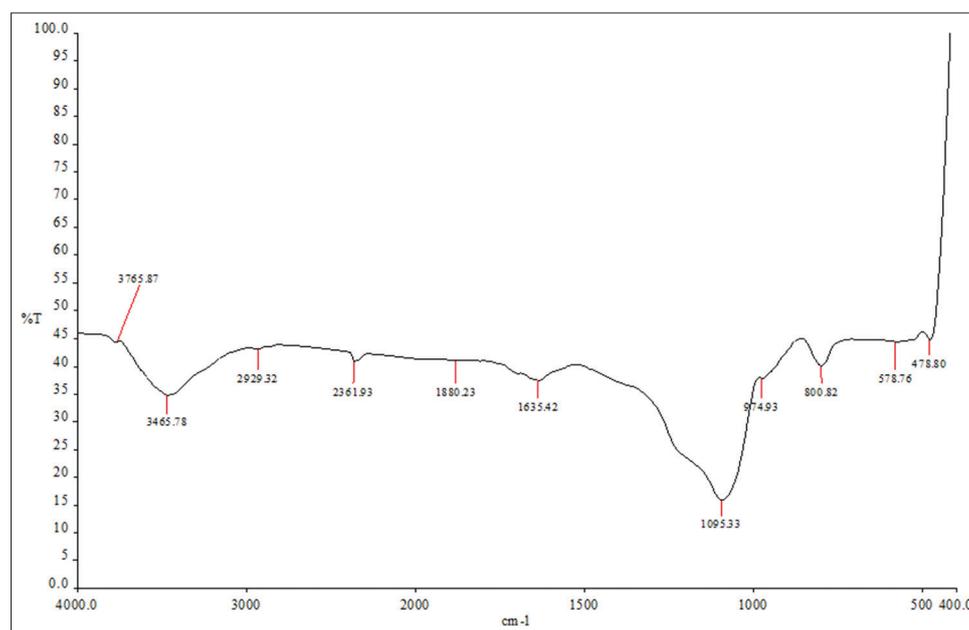
FT-IR and NMR analysis [23]. The FT-IR spectral analysis was carried out to determine the functional groups present in the *M. marina* KPMS1. The active fraction of spectra was scanned in the  $4000\text{--}400\text{ cm}^{-1}$  and plotted as intensity and wave number. The absorption bands were exhibited at  $3465.78\text{ cm}^{-1}$ – $1095.33\text{ cm}^{-1}$  (Fig. 1). A broad stretching in the region  $3465.78\text{ cm}^{-1}$  (O-H stretching),  $2929.32\text{ cm}^{-1}$  (C-H stretching),  $2361.93\text{ cm}^{-1}$  (C-N stretching),  $1635.42\text{ cm}^{-1}$  (C=O stretching), and  $1095.33\text{ cm}^{-1}$  (C-O stretching) was observed. The NMR analysis of *M. marina* KPMS1 chemical structure was predicted as 2-(2E, 4E)-3, 7-dimethylocta-2,4-dienyl)-7-hydroxy-2,3,4,5-tetrahydrobenzo[c] azepin-1-one. The molecular weight of compound was 313.43388 and the molecular formula was  $\text{C}_{20}\text{H}_{27}\text{NO}_2$ . The FT-IR and NMR analysis was more or less similar trend which was observed with other researcher [24]. The active compounds derived from marine sediments showed a good actinobacterial activity and can be used as an industrially important one [25].

#### Phylogenetic relationship

The monitoring of different *Micromonospora* strains in species levels was done by molecular techniques. The genomic DNA was extracted from *M. marina* KPMS1 and amplified for 16 S rRNA gene using the primers F243 and R513GC [26]. The size of amplified 16S rRNA gene product of *M. marina* KPMS1 was approximately 900 bp. The samples were sequenced and compared for homology with other bacterial strain sequences deposited in the public databases using the National Center for Biotechnology Information BLAST function. The GenBank accession number of *M. marina* KPMS1 was MH036351.

The phylogenetic evolutionary analysis was performed in MEGA 5.05 [27]. The sequence analysis usually includes alignment of sequences, construction of a phylogenetic tree, and testing the reliability of the constructed tree. The relationships of the aligned sequences are usually shown as a tree, in which the branching pattern of the tree displays the evolutionary relationships of the *M. marina* KPMS1. The most commonly applied tree construction method is distance method in which neighbour joining uses pair-wise distances calculated from aligned sequences before correcting to evolutionary distances in a substitution model [28]. The shortest distant sequences were clustered together in a tree and its length was optimized to the correspondent distance matrix.

The phylogenetic tree of *M. marina* KPMS1 was constructed using 16S rRNA gene sequence with that of other *Micromonospora* species.



**Fig. 1: Fourier-transform infrared spectral analysis of *Micromonospora marina* KPMS1**

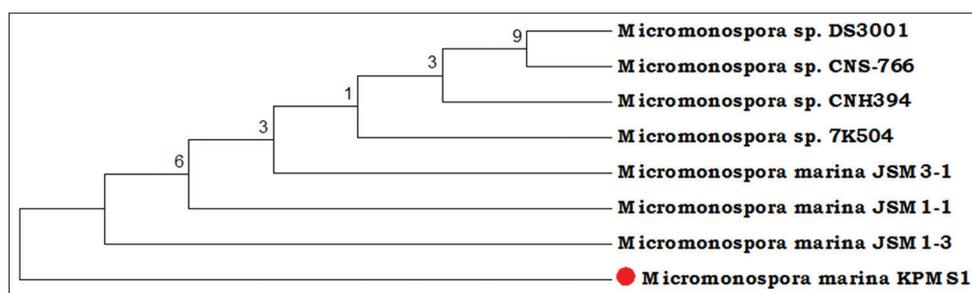


Fig. 2: Phylogenetic relationship of *Micromonospora marina* KPMS1

Table 4: Secondary screening of *M. marina* KPMS1 against multidrug-resistant bacteria

Samples	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>
KPMS1	18±0.07 mm	17±0.06 mm	17±0.05 mm
RIZD (%)	87	77	81

*M. marina*: *Micromonospora marina*, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *E. faecalis*: *Enterococcus faecalis*

The optimal tree with the sum of branch length 1.15000000 was analyzed [Fig. 2]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using p-distance method and are in the units of the number of base substitutions per site. The phylogenetic tree showed two clades (I and II). The Clade I was subdivided into six smaller groups which encompass *M. marina* JSM1-3, *M. marina* JSM1-1, *M. marina* JSM3-1, *Micromonospora* sp. 7K504, *Micromonospora* sp. CNH394, and *Micromonospora* sp. DS3001. The Clade II encompassed the strain *M. marina* KPMS1. This analysis involves eight nucleotide sequences. The codon positions included were 1<sup>st</sup> + 2<sup>nd</sup> + 3<sup>rd</sup> + noncoding. All positions containing gaps and missing data were eliminated. There were a total of 50 positions in the final dataset.

## CONCLUSION

A genus *Micromonospora* are widely distributed in the marine environments and documented as a potential source of bioactive compounds. Among actinomycetes groups, *Streptomyces* sp. is considered as the dominant group found in the marine environment and also used for antibiotic research. *Micromonospora* is a rare group of actinomycetes which has been regarded as reservoir for antimicrobial agents. It had been emphasized that marine sediments might be valuable for the isolation of a novel strain of *Micromonospora* which potentially yield a broad spectrum of secondary metabolites. In reports suggested from the East Coast of India, soil is a major and suitable source of actinomycetes isolation. Correspondingly, the Kayalpatnam marine ecosystem is not only rich in biodiversity but also recognized a world heritage site and may provide the rich source for the discovery of *Micromonospora* sp. This study concludes that marine *M. marina* KPMS1 is well-organized novel secondary metabolites synthesizer and holds potential for the treatment of clinical associated infection, acute lymphocytic leukemia, and cancer in other types. The screening of antimicrobial metabolite is a significant role toward the greater demand for new metabolites for the benefit of mankind and synthesized a lot of promising genera derived from marine sediment of *Micromonospora* sp. having medicinal importance in future.

## AUTHORS' CONTRIBUTIONS

Each author has contributed to the best of our knowledge for the research carried out in this article.

## CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

## ACKNOWLEDGMENTS

The authors thank the PG and Research Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli - 620 020, Tamil Nadu, India, toward this research work hereby acknowledged.

## REFERENCES

- Zhao H, Kassama Y, Young M, Kell DB, Goodacre R. Differentiation of *Micromonospora* isolates from a coastal sediment in wales on the basis of fourier transform infrared spectroscopy, 16S rRNA sequence analysis, and the amplified fragment length polymorphism technique. *Appl Environ Microbiol* 2004;70:6619-27.
- Genilloud O. Genus *Micromonospora* Ørskov 1923, 156AL. In: *Bergey's Manual of Systematic Bacteriology*. Vol. 5. New York: Springer; 2012. p. 1039-57.
- Tanasupawat S, Jongrungruangchok S, Kudo T. *Micromonospora marina* sp. Nov. isolated from sea sand. *Int J Syst Evol Microbiol* 2010;60:648-52.
- Supong K, Suriyachadkun C, Pittayachajonwut P, Suwanborirux K, Thawai C. *Micromonospora spongicola* sp. Nov. an actinomycete isolated from a marine sponge in the Gulf of Thailand. *J Antibiot (Tokyo)* 2013;66:505-9.
- Fenical W, Jensen PR. Developing a new resource for drug discovery: Marine actinomycete bacteria. *Nat Chem Biol* 2006;2:666-73.
- Chambers HF. General principles of antimicrobial therapy. In: Brunton LL, Lazo J, Parker K, editors. *Goodman and Gilman's Pharmacological Basis of Therapeutics*. 11<sup>th</sup> ed. New York: McGraw-Hill; 2006. p. 1095-111.
- Bérdy J. Bioactive microbial metabolites. *J Antibiot (Tokyo)* 2005;58:1-26.
- Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine natural products. *Nat Prod Rep* 2012;29:144-222.
- Pathom-aree W, Stach JE, Ward AC, Horikoshi K, Bull AT, Goodfellow M. Diversity of actinomycetes isolated from challenger deep sediment (10.898M) from the Mariana trench. *Extremophiles* 2006;10:181-9.
- Abbas IH. A biological and biochemical studies of actinomycetes isolated from Kuwait saline soil-Kuwait. *J Appl Sci Res* 2006;2:809-15.
- Savitri NA, Astana N, Azmi W. Microbial lasparagenase: A potent anti-tumor enzyme. *Indian J Biotechnol* 2003;2:184-94.
- Laidi RF, Kansoh AL, Elshafei AM, Cheikh B. Taxonomy, identification and biological activities of novel isolate of *Streptomyces tendae*. *Arab J Biotechnol* 2006;9:427-36.
- Sharma D, Kaur T, Chadha BS, Manhas RK. Antimicrobial activity of actinomycetes against multidrug resistant *Staphylococcus aureus*, *E. coli* and various other pathogens. *Trop J Pharm Res* 2011;10:801-8.
- Subramani R, Aalbersberg W. Culturable rare actinomycetes: Diversity, isolation and marine natural product discovery. *Appl Microbiol Biotechnol* 2013;97:9291-321.
- Tiwari K, Dilip JU, Eva M, Roderich S, Rajinder KG. Culturable bioactive actinomycetes from the great Indian Thar desert. *Ann Microbiol* 2015;65:1901-14.
- Bérdy J. Thoughts and facts about antibiotics: Where we are now and where we are heading. *J Antibiot (Tokyo)* 2012;65:385-95.
- Sánchez López JM, Martínez Insua M, Pérez Baz J, Fernández Puentes JL, Cañedo Hernández LM. New cytotoxic indolic metabolites from a marine *Streptomyces*. *J Nat Prod* 2003;66:863-4.
- Nagashu P, Gayatri Devi V, Kumar BA, Kumari S, Mohan GM, Malla RR. Antioxidant and antiproliferative potentials of marine actinomycetes. *Int J Pharm Pharm Sci* 2016;8:277-84.

19. Sallam LA, El-Refai AF, Osman ME, Hamdy AA, Ahmed EM, Mohamed MA. Some physiological factors affecting rapamycin production by *Streptomyces hygroscopicus* ATCC 29253. *J Am Sci* 2010;6:188-94.
20. Sarquis MI, Oliveira EM, Santos AS, Costa GL. Production of L-asparaginase by filamentous fungi. *Mem Inst Oswaldo Cruz* 2004;99:489-92.
21. Kokare CR, Mahadik KR, Kadam SS, Chopade BA. Isolation, characterization and antimicrobial activity of marine halophilic *Actinopolyspora* species AH1 from the West coast of India. *Curr Sci* 2004;86:593-7.
22. Sibanda T, Mabinya LV, Mazomba N, Akinpelu DA, Bernard K, Olaniran AO, et al. Antibiotic producing potentials of three freshwater actinomycetes isolated from the Eastern Cape province of South Africa. *Int J Mol Sci* 2010;11:2612-23.
23. Tiwari K, Gupta RK. Rare actinomycetes: A potential storehouse for novel antibiotics. *Crit Rev Biotechnol* 2012;32:108-32.
24. Janardhan A, Kumar AP, Viswanath B, Saigopal DV, Narasimha G. Production of bioactive compounds by actinomycetes and their antioxidant properties. *Biotechnol Res Int* 2014;2014:217030.
25. Sheik GB, Maqbul MS, Shankar SG, Ranjith MS. Isolation and characterization of actinomycetes from soil of ad-dawadmi, Saudi Arabia and screening their antibacterial activities. *Int J Pharm Pharm Sci* 2017;9:276-9.
26. Ravikumar S, Fredimoses FM, Gokulakrishnan R. Biodiversity of actinomycetes in Manakkudi mangrove ecosystem, South West coast of Tamil Nadu, India. *Ann Biol Res* 2011;2:76-82.
27. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, et al. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731-9.
28. Nei M, Kumar S. *Molecular Evolution and Phylogenetics*. New York: Oxford University Press; 2000.