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Screening and partial purification of antifungal metabolite from *Streptomyces rochei* MSA14: an isolate from marine mining soil of Southwest coast of India.

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A total of fourteen actinobacterial strains were isolated from the mining sediment of Manavalakurichi, Southeast coast of India. Primary screening results through agar well diffusion method revealed that 28.57% actinobacterial strains had in vitro antifungal activity. Most potent actinobacterial isolate MSA14 showed strongest inhibitory activity and was identified as Streptomyces rochei through morphological, physiological, biochemical and 16S rRNA gene sequence characteristics. Crude ethyl acetate extract of S. rochei exhibited wide spectrum antifungal activity which was ranged between 12 and 17 mm. Further evaluation of Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) showed the values ranged from 50 to 200 and 100 to 200 µg/ml, respectively. Partial purification of crude extract through TLC using various gradient solvent system recorded different spots of active principles with the respective Rf values between 0.22 and 0.90. TLC autobiography assay evidenced that, spot with the Rf value of 0.54 had promising antagonistic activity.

[**Keywords:** Mining sediment, Antifungal activity, *Streptomyces rochei*, MIC and MFC]

Introduction

Recent medical reports evidently inferred that fungal infections have reached a level of crisis in immunocompromised individuals. Of the different fungal species affecting humans, *Candida* and *Aspergillus* species are the most prevalent opportunistic pathogens reported to be resistant to numerous antifungal agents[1]. Many antifungal compounds have been identified, but safe and effective antifungal drugs have not yet been developed because of the high degree of similarity between fungi and mammalian cells[2]. Antibiotics such as amphotericin - B, miconazole, ketoconazole, fluconazole, and itraconazole are the predominant antifungal agents used towards fungal infections. However, these compounds were reported to cause vomiting in patients[3]. This has significantly necessitated the search of newer source of antifungal agents from marine organisms, in particular from marine microorganisms.

Marine microorganisms are potentially active and so far numerous bioactive compounds with unique biological properties against human, veterinary and agriculture field have been explored[4]. Actinomycetes are one of the major components of the microbial populations present in soil and they are most economically and biotechnologically valuable prokaryotes[5,6]. Actinomycetes isolated from marine sediments were reported to be excellent source of bioactive compounds, since thousands of natural compounds were described from different actinomycetes strains. According to Lazzarini et al[7] of the 8000 antimicrobial products described in the ABL (AntiBiotic Literature) database, 45.6% were reported to be produced by *Streptomyces* spp, 16% were produced by strains belonging to rare genera of actinomycetes and 38.4% antibiotics were produced by other groups of microorganisms like fungi and other bacteria. Some of the novel antibiotics isolated from marine actinobacteria include the anticancer metabolite salinosporamide - A from a *Salinispora* sp., the structurally unique marinomycins from *Marinophilus* sp. and abyssomicin - C, a potent inhibitor of the Para - aminobenzoic acid pathway from *Verrucosispora* sp. and Marino pyrroles from a *Streptomyces* strain[8-11]. More recently, Chronakova

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et al[12] screened marine actinobacterial strains isolated from post mining soil of North-West Bohemia, and evidenced that, the soil isolate *Streptomyces* sp. had predominant antimicrobial effect against bacterial and unicellular fungal strains. However, investigations on biopotential of actinomycetes from mining soils of Indian coastal regions are still lacking. India is fortunately blessed with three important mining sites, which are being operated by Indian Rare Earths Limited (IRE). Manavalakurichi is one among the three sites (IRE), located at Kanyakumari District, Tamilnadu. IRE of Manavalakurichi acquires much importance, because of the presence of rare heavy minerals such as ilemnite, monazite, titanium dioxide and zircon in this coastal sediment[13]. In general, microbial counts in mining sediments often remain lower than microbial counts noticed in the normal sediments. Microorganisms that thrive in mining sediments were found to be more potent and synthesize numerous unusual secondary metabolites with excellent biomedical potentials. Thus based on the above literature and considering the biomedical importance of microorganisms of the mining soils, in the present study, an attempt was made to study the fungicidal property of actinomycetes isolated from mining soil.

Materials and Methods

For the present study, sediment samples were collected from mining region (IRE) of Manavalakurichi coast (Lat 8°8' 35" and Long 77°8' 00"), Tamilnadu, India. Briefly, 50 g each of sediment samples were aseptically collected in sterile polythene bags from different localities of mining area and brought to the laboratory in ice cold box. Samples were air dried at room temperature for 48 h and then pretreated in hot water bath at 55ºC for 45 min. One gram each of pretreated sediment samples were weighed and serially diluted up to 10^{-6} using 50% sterilized seawater. Starch casein agar (SCA) medium was prepared in 50% filter sterilized seawater and autoclaved at 121ºC for 15 minutes. After sterilization, the medium was cooled to pourable temperature (32ºC) and supplemented with 20 µg/mL nalidixic acid and 100 µg/mL cycloheximide to avoid the bacterial, fungal and yeast contaminations[14]. Then 0.2 mL of each aliquot was spreaded individually over SCA plates and then incubated at 28 ± 2ºC for 7-10 days. After the specified period of incubation, the morphologically distinct 14 actinobacterial colonies (MSA1 to MSA14) were isolated and purified. Then the pure cultures were maintained as slant at 4ºC for further study.

For the present study, most prevalent disease causing fungal strains such as *Candida albicans* MTCC 183, C. *tropicalis* MTCC 184, C. *glabrata* MTCC 3984, *Aspergillus fumigatus* MTCC 4333, A. *niger* MTCC 961 and *Rhizomucor miehei* MTCC 546 were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Antagonistic efficiency of the isolated actinobacterial strains was determined through agar well diffusion method[15]. Before the startup of experiment, all the actinobacterial isolates were grown individually in 100 mL Erlenmeyer flasks containing 50 mL starch casein broth and incubated at 150 rpm for seven days at 28 ± 2ºC. After incubation, 2 mL of individual culture broth was taken and centrifuged at 10,000 rpm for 15 min at 4ºC and the supernatant obtained was then used for antifungal assay. The antifungal assay was performed in sterile Sabouraud's Dextrose Agar plates (SDA). After solidification, wells of 6 mm diameter were made in the agar plates using sterile cork borer and 72 h old individual culture of fungal strains were spreaded over agar plates using sterile swabs. Then each well was loaded with 100 µl of respective culture free supernatant (CFS) and incubated at 28 ± 2ºC for 48 – 96 h. After the incubation period, growth inhibitory activity in terms of zone of inhibition (mm) was measured from the edge of well. The assay was carried out in triplicate.

Based on the primary screening result, the broad spectrum antagonistic activity rendering actinobacterial strain MSA14 was subjected to identification on the basis of morphological, physiological and biochemical characteristics. Starch Casein Agar plate (SCA) was used as standard medium to determine the morphological characters of the promising actinobacterial strain through cover slip method[1617].

The extraction of genomic DNA of candidate strain was followed by the method of Kumar *et al*[18]. Growth from mature slant culture of MSA14 was inoculated aseptically into 250 mL Erlenmeyer flasks, each containing 100 mL of Starch casein broth (SCB) medium prepared by 50% sterilized seawater and incubated in a rotary shaker at 30°C for 4 days at 150 rpm. Cultures were centrifuged at 10,000 rpm for

10 minutes at 4ºC. Then 100 mg of mycelium pellet was transferred into sterile mortar and pestle and crushed with liquid nitrogen. Crushed mycelium was transferred into fresh tube containing 500 µl of TE buffer supplemented with lysozyme (20 mg/mL). Tube was incubated at 37°C for 30 min. After the incubation period, 20 µl of 10% SDS (w/v) and 20 µl of proteinase - K were added into the tube and incubated at 55°C for 30 min. Lysate was cooled down and extracted once with equal volume of phenol: chloroform solution (v/v, 1:1) at 10,000 rpm for 5 min. Aqueous phase was transferred carefully to a fresh tube and DNA was precipitated by adding 70 - 90% and keeping at -20°C for 30 minutes. After the specified incubation period, it was centrifuged at 10,000 rpm for 10 minutes. Pellet obtained was then washed twice with 90% ethanol and dissolved in TE buffer. Sample was once again extracted with equal volume of phenol: chloroform and precipitated as described above.

The 16S rRNA of the potent strain MSA14 was amplified from the extracted genomic DNA using universal eubacterial primers; Forward primer-5'AGAGTTTGATCCCTGGCTCAG3' and Reverse primer 5'GTACGGCTACCTTGTTACGAC3'. Polymerase chain reaction was performed in 50 µl reaction mixture containing 20 µl (~100 ng) of template DNA, 1U of each primer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP, 1U of Taq DNA polymerase and 1X PCR buffer (Genei, India). Amplification was performed in Eppendorf gradient thermocycler 96, according to the following profile: an initial denaturation step for 2 min at 94°C, thereafter 30 amplification cycles consisting of denaturation at 94°C for 1 min, followed by annealing at 55°C for 1 min and then a final extension step consisting of 2 min at 72°C. PCR product was detected by 1% of agarose gel electrophoresis and was visualized by ultraviolet (UV) fluorescence after ethidium bromide staining.

The partial sequences (1413 bp) of potent strain MSA14 obtained were matched with previously published sequences available in NCBI using BLAST[19]. In brief, the phylogenetic position of the candidate strain MSA14 was determined by retrieving sequences of closely related bacterial strains from NCBI—Gen Bank and aligned through multiple sequence alignment using CLUSTAL X software. Nucleotide substitution rates (K_{nuc} values) were also calculated[20]. Thereafter, a phylogenetic tree was constructed by Neighbor- Joining (NJ) and Kimura two pair method and topologies were evaluated by performing bootstrap analysis of 1000 sets by using MEGA 4.0 software[21]. Sequence obtained in this study was then deposited in the Gen Bank, NCBI Database.

The selected antagonistic actinomycete was enriched in starch casein broth at 37°C for 4 days. Then 5% of the prepared seed culture was transferred to 10 L of starch casein broth and incubated at 30°C in orbital shaker (150 rpm) for seven days. After incubation, the culture was centrifuged at 10,000 rpm for 20 min, and the supernatant was then filtered through 0.45 µ membrane cellulose filter paper for complete removal of mycelial cells. CFS obtained was then mixed with equal volume of ethyl acetate. Ethyl acetate phase was then separated and concentrated under rotary evaporator and stored in preweighed glass vial. Crude extract was then dissolved in DMSO and tested for antifungal activity at 100 µl volume containing 250 µg of the crude extract through agar well diffusion method. A positive control (100 µg of fluconazole) was also tested against the pathogenic fungal strains. Growth inhibitory activity in terms of zone of inhibition (mm) was measured. The assay was carried out in triplicate.

Minimum inhibitory concentration (MIC) of the crude extract was tested by micro broth dilution method using the 96 well microtitre plates[22]. For this, different concentrations of crude extract (10 to 250 µg/ml) were prepared using Methanol: Ethyl acetate (3:1) as carrier solvent and coated over 96 well microtitre plate (Flat bottom; Polypropylene plate) and allowed for evaporation under UV for 2 h. Thereafter, 100 µl inoculum of pathogenic fungal strain (1×10^7 cell/ml) was added to each well. Only fungal spore suspensions were used as negative control, while broth containing fluconazole was used as positive control. Microtitre plates were then incubated at 37°C for 48 – 96 h. The assay was carried out in six replicates. MIC values were taken as the lowest concentration of the crude extract that showed no turbidity after incubation. Turbidity of the wells was noticed based on the visible growth of test fungal strains. Minimum fungicidal concentration (MFC) was determined by streaking a loop full of inoculum from each well in SDA plates. Plates which showed no apparent growth of fungal strains was recorded as MFC value.

The partial purification of crude extract of MSA14 was performed by the method described by Bhavya

et al[23]. For this, the crude extract of MSA14 was dissolved in ethyl acetate and made up to a volume of 100 mg/mL. From this, 0.6 µL was taken and made up to a volume of 3 µl with ethyl acetate and spotted on TLC plates (TLC aluminium sheets, 20 × 20 cm, silica gel $60F_{254}$, Merck co, USA) using various gradient solvent system such as Benzene (100%), Hexane (100%), Ethyl acetate (100%) and Methanol (100%) and 1:1 ratio of combined solvent system such as Hexane: ethyl acetate, Benzene: ethyl acetate, Ethyl acetate: Methanol, and finally 1:1 ratio of Methanol: Distilled water was also used as mobile phase. Thereafter, the TLC plates were dried at room temperature and observed under UV/Vis absorbtion (Bio-Rad; AlphaImager™ 3300) for detection at different wave lengths of 254 and 365 nm. The Rf values of all the TLC plates were then calculated.

Modified method of Bhavya *et al*[23] was followed for the TLC autobiography overlay assay. Before startup of the experiment, the developed TLC plates were sterilized by UV lamp for 30 min before enchased in the base SDA in the petridish. It was then covered by melting soft SDA (46ºC) containing test fungal strains. After 10 h diffusion process at 8ºC, plates were incubated at 37ºC for 48 – 96 h and the zone of inhibition (mm) around the TLC spots was observed and recorded.

Results

Table 1 shows the antagonistic activity of isolated 14 actinomycetes against the test fungal strains. From the result it was observed that, supernatant of MSA1, MSA7, MSA11 and MSA14 had significant fungal growth inhibitory activity. However, among these four actinobacterial strains, MSA14 recorded maximum of 100% fungal growth inhibitory activity with the zone of inhibition ranged from 7 ± 0.05 to 15 ± 0.16 mm, with maximum growth inhibitory activity against C. *albicans* and minimum against two fungal strains such as A. *fumigatus* and A *niger*. Besides, MSA7 exhibited moderate fungal growth inhibitory activity of 66.66% with the zone of inhibition ranged between 5 ± 0.12 and 9 ± 0.14 mm. It exhibited maximum antagonistic activity against C. *tropicalis*. The other two actinobacterial strains like MSA1 and MSA11 recorded poor growth inhibitory activity (33.33%) and the zone of inhibition observed was ranged between 8 and 9 mm, respectively.

Based on the results of primary screening, the predominant result rendering actinobacterial strain MSA14 was subjected to morphological, physiological and biochemical characteristics for identification. Accordingly, it was identified as Gram-positive bacterium, possessing grey colour aerial and pale yellow colour substrate mycelium. The culture when examined under the light microscope (100X) showed spiral shaped spore morphology. Strain did not show the presence of melanoid and diffusion of pigments (Fig. 1). Strain showed excellent growth at temperature 45ºC, NaCl 2% and pH 8. Biochemical characters such as nitrate, MR, citrate, catalase and oxidase were found to show positive reaction; whereas, it was found to be negative for urease, H_2S, VP and indole production tests. Often, sugar pattern / utilization test was more effective for the identification of actinomycetes groups. The candidate strain MSA14 showed positive results for the utilization of carbon sources such as glucose, fructose, maltose, mannitol, galactose and inositol. Candidate strain MSA14 was also subjected to various substrate hydrolysis. Result displayed that it could effectively hydrolyze gelatin, starch, and pectin. Based on the above biochemical characteristics the strain MSA14 was identified as *Streptomyces* sp. (Table 2). Further, the blast search of the 16S rRNA sequence showed 99% similarity to *Streptomyces rochei* SM3 (JN128892). Then the 16s rRNA sequence of the candidate strain S. *rochei* MSA14 was deposited

Table 1—Antifungal activity of culture free supernatant of marine actinomycetes through agar well diffusion method (Zone of inhibition - mm)

Pathogenic fungal strains	MSA 1	MSA 2	MSA 3	MSA 4	MSA 5	MSA 6	MSA 7	MSA 8	MSA 9	MSA 10	MSA 11	MSA 12	MSA 13	MSA 14
C. albicans	-	-	-	-	-	-	8 ± 0.17	-	-	-	8 ± 0.21	-	-	15 ± 0.16
C. tropicalis	9 ± 0.14	-	-	-	-	-	9 ± 0.14	-	-	-	9 ± 0.14	-	-	13 ± 0.21
C. glabrata	9 ± 0.21	-	-	-	-	-	8 ± 0.17	-	-	-	-	-	-	12 ± 0.09
A. fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	7 ± 0.05
A. niger	-	-	-	-	-	-	5 ± 0.12	-	-	-	-	-	-	7 ± 0.05
R. miehei	-	-	-	-	-	-	-	-	-	-	-	-	-	9 ± 0.21
Inhibition (%)	33.33%	-	-	-	-	-	66.66%	-	-	-	33.33%	-	-	100%

Each value is the mean ± 8D of three replicates

in Gen Bank under the accession number (JQ045775) (Fig. 2).

Fig.3 shows the antifungal activity of crude ethyl acetate extract of S. rochei MSA14. From the result, it was observed that the crude extract had maximum growth inhibitory activity of 17 mm against C. *albicans,* followed by 12 and 10 mm against C. *tropicalis* and C. *glabrata*, respectively. Minimum growth inhibitory activity of 6 mm was noticed against R. *miehei*. But, the other two fungal strains such as A. *fumigatus* and A. *niger* were found to be resistant to the crude extract of MSA14. On the other hand, the positive control fluconazole (100 µg/well) showed the inhibitory level ranged between 5 and 13 mm against all the tested fungal strains.

The crude extract of S. *rochei* MSA14 rendered better fungistatic (MIC) and fungicidal (MFC) activity against the test fungal strains. In the present investigation, lowest MIC value of 50µg/ml was recorded against C. *glabrata;* whereas the MIC value against C. *albicans*, C. *tropicalis* and R. *miehei* was ranged between 100 – 200 µg/mL, thus the result of the present study clearly manifested the promising fungistatic activity of the crude extract. However, the extract was ineffective against A. *fumigatus* and A. *niger*. On the other hand, MFC values of the crude extract exhibited relatively higher concentration against the test fungal strains and it varied from 150 – 250 µg/mL (Fig. 4). Standard antibiotic (Fluconazole) evidenced 100% fungistatic (MIC) and fungicidal activity (MFC) at least concentration and it ranged between 10 to 100 and 50 – 150 µg/mL, respectively.

Partial purification of crude extract of S. *rochei* MSA14 was performed through thin layer chromatogram using different gradient solvent system. Result displayed significant difference in separation of active principles and recorded Rf values ranged from 0.22 to 0.90. Result of autobiogram showed that compound with the Rf value of 0.54

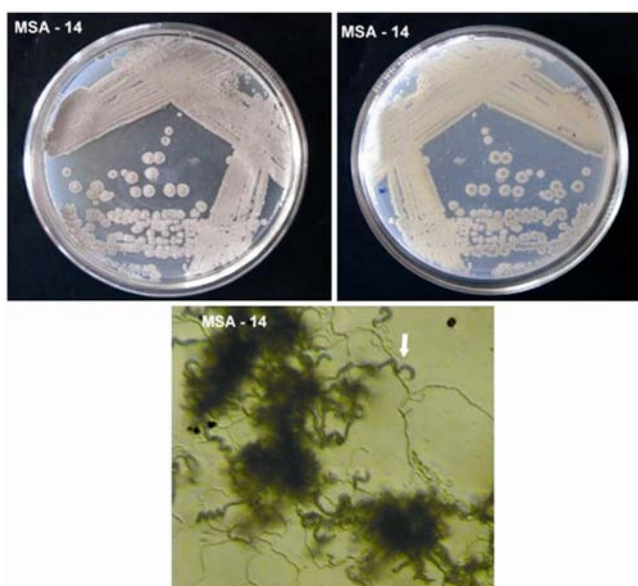

Fig. 1—Culture characteristics and spore morphology of the candidate strain S rochei MSA14

Table 2—Morphological and physiological characteristics of MSA14

S.No	Characters	MSA14
1	Cell Shape	Mycelium
2	Colony Morphology	Round; Concave; Margin- irregular
3	Aerial mycelium Colour	Grey
4	Substrate mycelium Colour	Pale Yellow
5	Spore Colour	Grey
6	Spore Shape	Spiral
7	Diffusion Colour	-
8	Grams Reaction	+
9	Production of melanin pigment	-
10	Range of temperature	20 to 45ºC
11	Optimum temperature	35ºC
12	Range of pH for growth	4 to 9
13	Optimum pH	8
14	NaCl tolerance (%)	0.5 to 6 %
15	Optimum NaCl	2 %
16	Urease	-
17	H_2S Production	-
18	Nitrate Reduction	+
19	MR Reaction	+
20	VP Reaction	-
21	Indole Production	-
22	Citrate Utilization	+
23	Catalase	+
24	Oxidase	+
25	Glucose	+
26	Fructose	+
27	Sucrose	-
28	Lactose	-
29	Maltose	+
30	Mannitol	+
31	Galactose	+
32	Inositol	+
33	Sorbitol	-
34	Xylose	-
35	Protein	-
36	Gelatine	+
37	Starch	+
38	Cellulose	-
39	Pectin	+

+: Positive; - : Negative

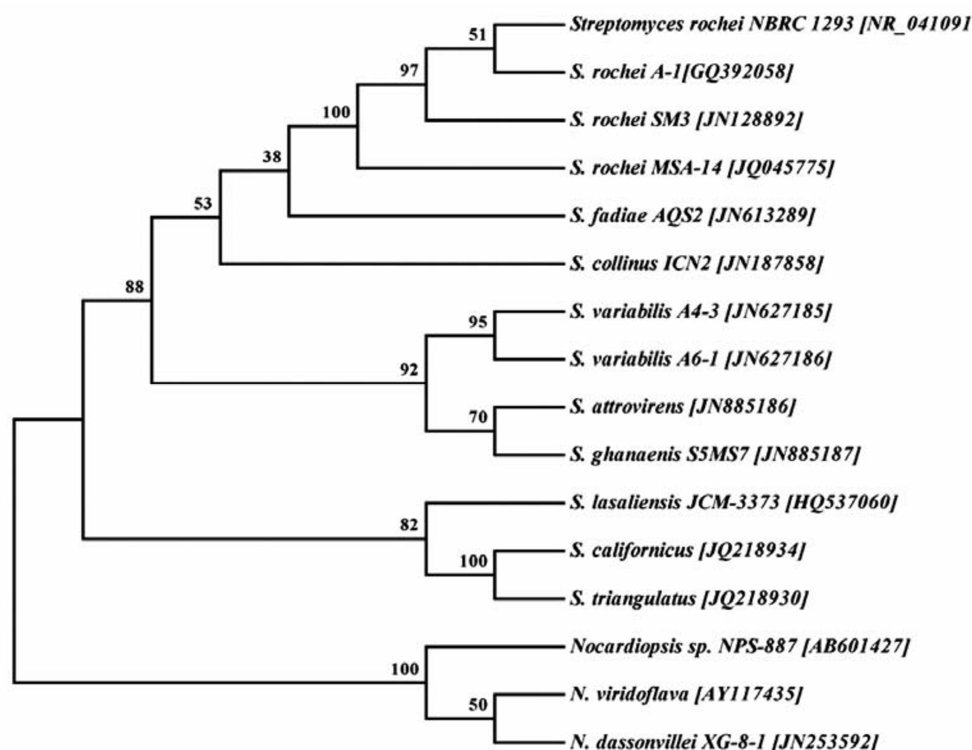

Fig. 2—Phylogentic relationship of *Streptomyces rochei* MSA-14 (JQ045775) with refrence actinobacterial 16S rRNA gene

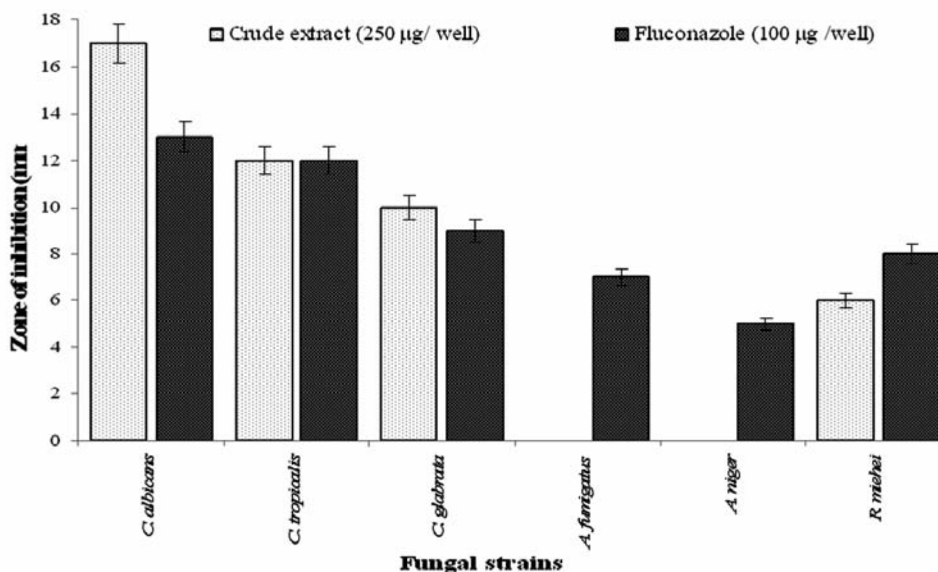

Fig. 3—Antifungal activity of crude extract of *S. rochei* MSA 14 against test fungal starins

(separated using Ethyl acetate: Methanol) was found to be more active against the test fungal strain. It showed better antagonistic activity against fungal strains such as C. *albicans*, C. *tropicalis*, C. *glabrata* and R. *miehei* and recorded the zone of inhibition ranged between 9 and 12 mm. However, A. *fumigaus* and A. *niger* were found to be resistant to the active compound (Rf 0.54) (Table 3).

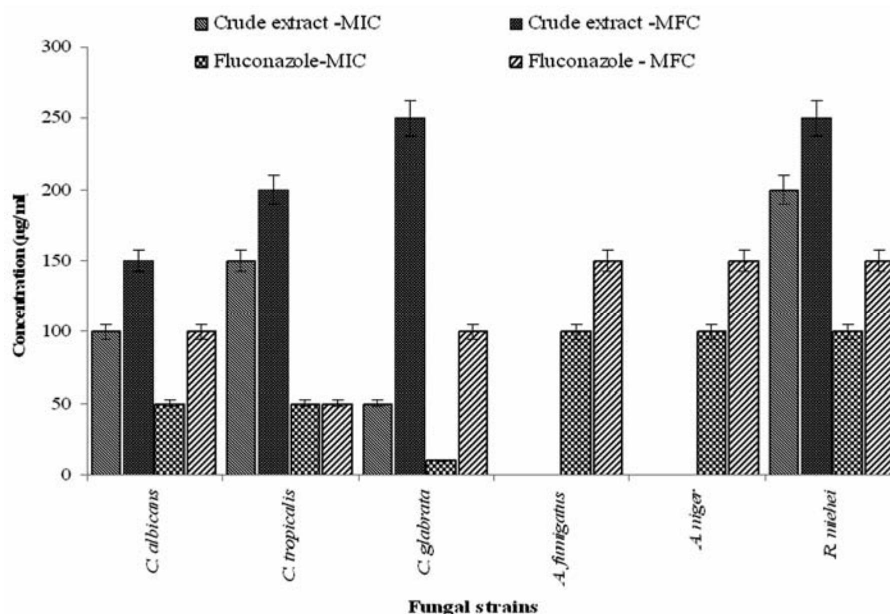

Fig 4—MIC and MFC of crude ethyl acetate extract of S. rochei MSA14 against test fungal starins

Table 3—TLC autobiogram of *S. rochei* MSA14 against test fungal strains

S.No	Gradient Solvents	Rf values	Zone of inhibition – mm					
			C. albicans	*C. tropicalis*	*C. glabrata*	*A. fumigatus*	*A. niger*	*R. miehei*
1	Hexane	-	-	-	-	-	-	-
2	Hexane: Benzene	0.22, 0.26, 0.88	-	-	-	-	-	-
3	Benzene	0.76	-	-	-	-	-	-
4	Hexane: Ethyl acetate	0.34, 0.90	-	-	-	-	-	-
5	Benzene: Ethyl acetate	0.29, 0.46, 0.82	-	-	-	-	-	-
6	Ethyl acetate	0.57, 0.67, 0.78,	-	-	-	-	-	-
7	Ethyl acetate: Methanol	0.54, 0.74	+ (12mm)	+ (10mm)	+ (10mm)	-	-	+ (9mm)
8	Methanol: H_2O	0.75, 0.89	-	-	-	-	-	-

+: growth inhibition; - : No growth inhibition

Discussion

The emergence of pathogenic microbes with increased resistance to established antibiotics provides a major incentive for the discovery of new antimicrobial agents[24]. However, investigation on isolation and identification of new bioactive compounds from marine microorganisms will be more effective in controlling disease related to bacterial and fungal pathogens. Ability to produce a large number of chemically different secondary metabolites is associated mostly with the actinomycetes. In particular, the genus *Streptomyces* sp is widely reported for the production of various antibiotics which are used therapeutically[25]. Yedir *et al*[26] showed that 21.88% of actinobacterial colonies isolated from terrestrial environment had significant growth inhibitory activity against pathogenic fungal strains. Abo-Shadi *et al*[27] reported that the *Streptomyces* sp. strain $A_{24}S_4$ isolated from plant rhizosphere region at Al-Madinah Al-Munawwarah, Saudi Arabia, exhibited 62.5% growth inhibitory activity against the clinically important fungal pathogens.

In the present study, actinomycetes isolated from mining sediments were subjected to primary screening through agar well diffusion method. Based on the results, it was observed that, CFS of S. *rochei* MSA14 exhibited growth inhibitory activity against all the tested fungal strains such as C. *albicans*, C. *tropicalis*, C. *galbrata*, A. *fumigatus*, A. *niger* and R. *miehei* with the zone of inhibition ranged between 5 and 15 mm. In accordance with the above findings, Kavitha *et al*[28] evidenced that, CFS of *Streptomyces* sp. A1 tested for antifungal activity had 100% bioactivity against pathogenic fungal strains such as A. *flavus*, A. *niger*, C. *albicans* and F. *oxysporum*

with the zone of inhibition ranged from 3 to 15 mm. Thus the result of the present study corroborates with the previous findings and emphasize that the active principle localized within the extract has effectively inhibited the growth of pathogenic fungal strains.

Most of the actinomycetes were identified by classical method i.e. based on the colour of mycelium, sporophore arrangement, pigment diffusion and sugar utilization patterns. Augustine *et al*[29] identified S. *rochei* AK9 through classical method, and inferred that the colour of aerial and substrate mycelium to be grey and yellow, the spore morphology to be spiral in nature and arranged in aerial spore mass with optimum temperature and *p*H (37ºC and *p*H 7). Likewise, Kavitha and Vijayalakshmi[30] showed the taxonomical position of S. *rochei* MTCC 8376 based on its physiology and colony morphology and emphasized that, the colour of aerial and substrate mycelium to be grey to white and yellow to brown and the morphology of spore to be spiral in nature with aerial spore mass. Similarly in the present study, the classical identification of candidate actinobacterial S. *rochei* MSA14 showed the presence of grey and pale yellow coloured aerial and substrate mycelium. Likewise, the morphology of spore was spiral with aerial spore mass and the optimum temperature, *p*H and NaCl concentration required for the growth of *S. rochei* MSA14 evidenced that it requires 45ºC, *p*H 8 and 2% NaCl, respectively.

The *Streptomyces* spp. is today responsible for production of about 75% of commercially and medically useful antibiotics[31]. In the present study, the crude extract of S. rochei MSA14 was tested for antifungal activity and it showed marked influence in growth inhibitory activity of 6 to 17 mm against fungal strains such as C. *albicans*, C. *tropicalis*, C. *glabrata* and R. *miehei*; whereas the other two filamentous fungi such as A. *fumigatus* and A. *tubingensis* were insensitive to the crude extract. Nevertheless, Fluconazole (Positive control) exhibited antifungal activity against all the tested fungal strains in the following order: A. *niger* < A. *fumigatus* < R. *miehei* < C. *glabrata* < C. *tropicalis* < C. *albicans* with the zone of inhibition ranged from 5 to 13 mm. In consonance with the result of the present study, antifungal activity of crude extract of terrestrial actinomycetes strain S. *rochei* MTCC 8376 has shown broad spectrum antifungal activity against C. *albicans*, followed by A. *niger*, P. *citrinum*, A. *flavus*, F. *oxysporium* and A. *alternate* with the zone of inhibition ranged between 13 and 20 mm[30]. Furthermore, Reddy *et al*[32] investigated the antimicrobial activity of S. *rochei* (MTCC10109) and pointed out its wide spectrum antimicrobial activity against both bacterial and fungal pathogens.

The autobiography assay is one of the more convenient methodology for testing crude extract of microorganisms, plants and purified bioactive active compounds against both pathogenic bacterial and fungal strains. In the present study, wide spectrum result yielding crude extract of S. *rochei* MSA14 was subjected to TLC for partial purification using different gradient solvent system as mobile phase. Results showed the significant variation in separation of active principle within the extract with respect to solvent system used and recorded Rf values ranged from 0.22 to 0.90. The developed TLC plates with respective spots were tested individually for their inhibitory activity against the tested fungal strains. The results of autobiogram showed that, TLC with the Rf value of 0.54 (separated using Ethyl acetate: Methanol) was found to be exhibited wide spectrum of antifungal activity against fungal strains such as *C. albicans, C. tropicalis, C. glabrata and R. miehei* with the zone of inhibition ranged from 9-12 mm. Accordingly, Selvakumar and Sumantha[33] separated the crude extract of Streptomyces spp. AQBCD24 and AQBCD54 through thin layer chromatography by saturated solvent system (butanol: acetic acid: water (4:1:2) and recorded the compounds with the Rf values of 0.40 and 0.78. Both these compounds had better antifungal activity. Similarly, Trejo-Estrada *et al*[34] isolated and purified nigericin an antifungal compound from the crude extract of *S.violaceusniger* YCED-9 through TLC using butanol: acetic acid: water (4:1:2) as gradient solvent system and recorded a prominent spot with the Rf value of 0.54. They reported that based on the autobiography overlay assay, that the compound had broad spectrum antifungal activity against the tested fungal strains such as *F. oxysporum*, *Phytophthora* spp. *Pythium ultimum* and *C. albicans*.

Conclusion

The overall results of the present study clearly emphasized that, the actinobacterium S. *rochei* MAS14 isolated from marine mining sediment is a promising source of antifungal metabolite. Furthermore, the TLC autobiography assay pointed out that the active principle resides within the

partially purified extract could probably be a good source of bioactive compound. However, further study is underway to purify and structure predict the biologically active compound for the development of new class of antifungal metabolite.

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