



Original Research Article

Isolation, Characterization of Bioinspired Secondary Metabolites Producing Actinomycetes from Marine Soil Samples

K.Saraswathi¹, A.M.Sabitha Rani², S.Sindhu³ and P. Arumugam^{1*}

¹Armats Biotek Training and Research Institute, Maduvankarai, Guindy, Chennai 600032, India

²Department of Biotechnology, Prince Shri Venkateshwara Arts and Science College, Venkateshwara Nagar, Gowrivakkam, Chennai, India

³Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Kancheepuram, Chennai – 603 308, India

*Corresponding author

ABSTRACT

Keywords

Marine actinomycetes, Antimicrobial activity, Pathogenic bacteria, Thin layer chromatography, Antioxidant, DPPH

A total of sixteen isolates of Actinomycetes were collected from marine sediments of Chennai. Each isolate was tested against four pathogenic bacteria and also against pathogenic fungi. Among the sixteen isolates, one of the isolates showed potent activity against specific bacteria. The selected isolate appear to produce high anti-bacterial compounds on starch casein agar medium and nutrient agar medium respectively, by using the cross streak method. The potent actinomycetes were characterized by morphological methods consist of macroscopic and microscopic methods. The mycelium colour and the appearance were observed as well as various biochemical tests such as starch hydrolysis, carbohydrate utilization, catalase test, etc. was performed. Further purification of the crude compound resulted in three distinct bands in different solvent system. Also, the anti-bacterial activity was effective for the crude compound.

Introduction

The marine environment of Indian peninsula is rich in microbial diversity. However, the wealth of marine micro-flora has not been fully investigated till date (Ramesh, 2009). Natural products remain to be the most propitious source of antibiotics (Bull *et al.*, 2007). There are approximately 32,500 natural products reported from microbial sources including about 1000 derived from marine microbes (Singh and Pelaez, 2008).

Marine microbes appeared to have adapted excellently to the marine environment, thus have gained special attention because of their diverse metabolic capabilities. Marine

ecosystems contain several unique features that set them apart from other aquatic ecosystems, the main factor being the presence of dissolved compounds in seawater, particularly sodium chloride. Salinity is expressed as the amount (in grams) of total dissolved salts present in 1kg of water. Normal seawater with a salinity of 35g/kg (or litre) of water is expressed as 35%. For marine micro-organisms, their cellular adaptation to moderate and high salt content is a fundamental biological process needed for survival and growth. It is postulated that marine microorganisms have

different characteristics from those of terrestrial counter parts and therefore, might produce different types of bioactive compounds (Jenson *et al.*, 2000).

Actinomycetes, the filamentous bacteria, are primarily saprophytic microorganisms of the soil, where they contribute significantly to the turnover of complex biopolymers, such as lignocellulose, hemicellulose, pectin, etc. The recently proposed class actinobacteria (Stackebrandt *et al.*, 1997) is comprised of high G+C content gram-positive bacteria and includes the actinomycetes (order Actinomycetales), whose members have an unparalleled ability to produce diverse secondary metabolites (Mincer *et al.*, 2002).

The oceans found to be an attractive field and great efforts have been accomplished worldwide aiming the isolation of new novel products from marine microorganisms. It is a boon in marine bioprospecting for the exploration and exploitation of the rich biological and chemical diversity found in marine organisms that inhabits the oceans.

Actinomycetes are prokaryotes that may look like fungi. Actinomycetes grow as filamentous mycelia and form spores. There are two characteristics that distinguish actinomycetes from fungi (Bull *et al.*, 2005).

1. Absence of cell nucleus, thus resembling prokaryotes.
2. They form hyphae 0.5–1.0µm in diameter, which is smaller than the fungal hyphae.

For discovering novel bioactive compounds of biotechnological interest is important. Several factors such as:

- Choice of screening source,
- Pretreatment,
- Selective medium,

- Culture condition and
- Recognition of actinomycetes colonies on a primary isolation plate, are used for identification of unique actinomycetes (Baskaran *et al.*, 2011).

The microbial fibrinolytic enzymes, especially those from actinomycetes, have the potential to be developed as drugs to prevent cardiovascular diseases (Sasirekha *et al.*, 2012).

Early evidence supporting the existence of marine actinomycetes came from the description of *Rhodococcus marinonascene* the first marine actinomycete species to be characterized (Helmke and Weyland, 1984).

Actinomycetes with some exceptions are aerobic – requiring oxygen for growth. As a result they do not grow well in wet soils. They are not tolerant to desiccation, but the spores they produce can tolerate (Goodfellow and Williams, 1989). Actinomycetes grow very little at 5°C. They are isolated more commonly from hotter soils than colder soils. Optimum growth is between 28°C and 37°C. Actinomycetes are tolerant to alkaline conditions. In alkaline soils, 95% of the microbial isolates may be actinomycetes (Lee and Hwang, 2002). With this background, the present work has been focused to screen the actinomycetes, to explore their antimicrobial activity against various human pathogenic bacteria and to study antioxidant activity of actinomycetes.

Materials and Methods

Study area and collection of sediment samples

Marine soil sample was collected from Marina beach (Wet soil) and Thiruvanmaiur beach (Dry soil) (Chennai, India). The soil sample was stored in sterile

plastic bags and water samples that are stored in sterile bottles. The samples were kept for drying in room temperature.

Pre-treatment of soil samples for enhancement of actinomycetes

0.1g of Calcium Carbonate (CaCO₃) mixed with 10g of soil sample. These samples were kept for 3 days.

Isolation and inoculation of Actinomycetes

One gram of pre-treated soil sample was suspended in 10ml of distilled water. The serial dilution technique was performed and the range of dilution is 10⁻¹ to 10⁻⁹. The dilution 10⁻¹, 10⁻², 10⁻³, 10⁻⁵, 10⁻⁷ were selected for isolating actinomycetes. The serial dilution was performed in wet and dry soil. The medium used for isolation of actinomycetes was starch casein agar (SCA).

The medium was prepared according to the composition using sea water and sterilized. The medium was cooled and the media were supplemented with fluconazole to avoid fungal contamination. The prepared medium was then poured onto the petriplates. After the solidification of the medium, plates were labeled and the samples were inoculated with different dilution (10⁻¹, 10⁻², 10⁻³, 10⁻⁵, 10⁻⁷) 100µl of suspension was transferred and plated over the surface of SCA medium by (spread plate method). The plates were incubated at 28 ± 2°C for 5 days.

Maintenance of isolated actinomycetes

The isolated actinomycetes were maintained on starch casein agar slants by frequent sub culturing; and they were named as ABTRI3000, ABTRI3001, ABTRI3002, ABTRI3003, ABTRI3004, ABTRI3005, ABTRI3006, ABTRI3007, etc...

Primary screening against human pathogens

Starch casein Agar and Nutrient agar was prepared in equal volume, sterilized and poured into sterile petriplates by mixing both the media and allowed to solidify. The actinomycete strains were inoculated (5cm measurement) on each plate by single streak method. The plates were incubated for 4-5 days. 24 hours culture *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* were streaked (3cm measurement) by perpendicular streaking method, incubated overnight and observed for inhibition. Similarly, antifungal activity was screened against *C. albicans* using SCA and PDA (Potato Dextrose Agar) plates where SCA and PDA were used in equal ratio. Based on primary screening, the effective strain was selected for further characterization.

Characterization of Actinomycetes

The selected foremost actinomycetes were studied by cultural, morphological and biochemical methods. The classical method for the culture characterization was based on aerial mass colour substrate mass colour and pigmentation. The microscopic characterization was done by cover slip culture method.

Morphological identification - Cover slip culture method

Morphological method is done by using coverslip method in which spore suspension culture of the actinomycetes (ABTRI 3037) was inoculated at the intersection of the SCA medium and cover slip was buried in the solid SCA medium at an angle of 45°C (Williams and Cross, 1971). These plates were inoculated for 5 days. Then the cover slip was kept under glass slide in an inverted position and viewed under microscope.

Biochemical characterization

Various biochemical tests were performed for identification of the potent isolate (ABTRI 3037). The cultures were grown on SCA plates for 5 days and used for various tests.

Gram staining

The culture (ABTRI 3037) was smeared in a microscopic slide and then subjected to primary staining which involves staining with crystal violet for 1 min, followed by washing with tap water. Then gram's iodine mordant was added on the slide and left for 30 seconds, which is followed by addition of 95% ethanol or acetone. The safranin was added on the slide and kept for 1 min. The slide was washed with tap water and the slide was blotted on tissue paper in order to make it dry. The slide was observed under microscope

Other biochemical tests for ABTRI 3037 such as indole production, methyl-red, Voges-Proskauer, oxidase test, Starch hydrolysis, etc. was been carried out according to Bergey's Manual of Systematic Bacteriology.

Antibiotic production of actinomycetes strain

The medium used for antibiotic production was ISP- 2 (International *Streptomyces* project) also called Yeast Malt Agar. Yeast Malt Broth was prepared according to the composition using sterile Sea water and sterilized by autoclave. The ABTRI 3037 strain was been inoculated into the Yeast Malt Broth. The inoculated broth was been kept in an orbital shaker for incubation for 10days to obtain for maximum growth, followed by filtration and centrifugation at 10,000 rpm for 10 min. The supernatant was been collected and equal volume of ethyl

acetate was added. After 24 hours of incubation, the upper layer was collected and kept in condensation at 40°C in round bottom flask. The extract was been carefully poured on to sterile petriplates, allowed for drying for 2 days. The plates were scrapped using 1ml of ethyl acetate and the crude compound was been transferred to sterile tubes. The crude extract thus obtained was used for various applicable activities.

Partial purification of crude compound by thin layer chromatography

TLC is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography was been performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. The adsorbent layer was the stationary phase.

TLC is a solid-liquid system of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a quick, inexpensive micro scale technique that could be used to determine the number of components in a mixture and to verify a substance identity. It consist two various phases such as stationary and mobile phase. Stationary phase was the silica gel and the mobile phase was (Ethyl Acetate: Toluene) the solvents.

Calculation of Rf value:

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Antioxidant activity

Estimation of Radical Scavenging Activity (RSA) using DPPH assay (Nenadis and Tsimidou, 2002).

The RSA activity of the extract was

determined using DPPH assay according to Nenadis and Tsimidou (2002), with small modification. The decrease of the absorption at 517nm of the DPPH solution after addition of the antioxidant was measured in a cuvette containing 1ml of 0.1mM ethanolic DPPH solution was mixed with varying concentration (20-100µg/ml) of the crude compound (from stock: 10mg/ml of 5%DMSO). Ascorbic acid served as standard. The ability to scavenge DPPH radical was calculated by the following equation.

% of DPPH radical scavenging activity,

$$(\%RSA) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control is the absorbance of DPPH radical + ethanol: Abs sample is the absorbance of DPPH radical + crude compound.

Qualitative bioassay of the compound – Secondary Screening for antibacterial activity

For the screening of bio-activity nutrient agar was prepared and autoclaved. The prepared medium was then poured onto sterile petridishes and allowed it for solidifying. After solidification, the plates were labelled and accordingly the lawns of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were formed using sterile cotton swabs. After lawn formation four well of diameter 12mm was cut using cork-borer. To these cut wells the prepared crude compound (ABTRI 3037) was loaded at different concentrations such as 250, 500, 750 and 1000µg/ml (from stock: 10mg/ml of 5% DMSO). Tetracycline served as control. The plates were incubated for 24h and the zone of inhibition was measured.

Results and Discussion

Pre-treatment of soil samples for enhancement of actinomycetes

The soil samples were initially pretreated with 0.1g of Calcium carbonate (CaCO₃) mixed with 10g of soil sample and was allowed to air dry for three days. By pretreatment method the unwanted microbes was removed.

Isolation and inoculation of actinomycetes

All the plates were observed with dried powdery shape colonies after an incubation of five days. The distinct colonies were taken using a sterile loop and are sub-cultured onto SCA slants. A total of 16 colonies were isolated from two different Soil sample and sub-cultured. The isolates were labelled as ABTRI3000, ABTRI 3001, ABTRI 3002, ABTRI 3003, ABTRI 3004, ABTRI 3005, ABTRI 3006, ABTRI 3007, ABTRI 3008, ABTRI 3009, ABTRI 3015, ABTRI 3025, ABTRI 3031, ABTRI 3037, ABTRI 3043 and ABTRI 3027.

Maintenance of isolated actinomycetes

The incubated sub-culture tubes were observed with a well grown adhered to the surface of the test tube. These cultures were further used for identification process (Fig. 1).

Primary screening against human pathogens

The 16 actinomycetes strains (ABTRI3000, ABTRI 3001, ABTRI 3002, ABTRI 3003, ABTRI 3004, ABTRI 3005, ABTRI 3006, ABTRI 3007, ABTRI 3008, ABTRI 3009, ABTRI 3015, ABTRI 3025, ABTRI 3031, ABTRI 3037, ABTRI 3043 and ABTRI 3027) were tested for their antibacterial

activity by perpendicular streaking. From the 16 strains, the effective strain, ABTRI 3037 (from dry soil) was been selected and the selected strain was re-checked for antibacterial activity for confirmation. The zone of inhibition was recorded (Fig. 2 and Table 1).

ABTRI 3037 was effective and it was selected for further analysis. However, all the strains did not inhibit the tested fungal pathogen *C. albicans*.

Characterization of actinomycetes

Morphological identification - coverslip culture method

The microscopic image of the strain ABTRI 3037 revealed that the isolate was filamentous in nature with spiral shaped spores (Fig. 3).

Characterizing different cultures revealed with distinguishable features in colour, colony formation and range (Table 2 and 3). The results of biochemical characterization revealed that the strain ABTRI 3037 was Gram positive, indole positive, methyl red negative, Voges proskauer negative, oxidase negative, catalase positive, carbohydrate utilization: sucrose negative, maltose negative, galactose positive, fructose positive, Starch hydrolysis positive, Casein hydrolysis negative and gelatin liquefaction negative.

Antibiotic production of actinomycetes strain

The Ethyl Acetate (EA) layer and the supernatant layer was been separated from the separating funnel and the solvent layer was transferred to round bottom flask for condensation at 40°C. The condensed extract was been transferred to sterile

petriplates for drying, followed by collection of the crude compound for further analysis.

Partial purification of crude compound by thin layer chromatography (TLC)

The crude compound was been subjected to TLC in order to identify the bioactive compounds. The crude extract was spotted on pre-coated silica sheet (5cm×1.5cm) using capillary tube. The most appropriate TLC system for analysis was shown to be (Ethyl acetate: Toluene V/V) in the ratio 0.3:0.7, in which the separation of compound were found with R_f values of 0.842, 0.631 and 0.315 (Fig. 4).

The TLC profile of the crude extract of ABTRI 3037 showed 3 distinct bands with R_f values 0.842, 0.631 and 0.315 under UV and iodine illumination (Table 4).

The results of DPPH assay suggest that the Ethyl acetate extract of ABTRI 3037 possessed significant antioxidant activity (Fig. 5).

The DPPH scavenging potential of the bioactive compound varied from 45-64% at a concentration range of 20-100µg/ml. Also, the IC₅₀ value was recorded as 58.22µg/ml. It is evident from the results that the bioactive compounds produced by ABTRI 3037 have potent antioxidative property.

Screening of bioactivity of the produced secondary metabolites

The extracted bioactive compound was subjected to well diffusion assay to study its antibacterial property against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *B. subtilis* (Table 5). The compound showed maximum inhibition against *P. aeruginosa* with ZOI of 17mm at a concentration of 1000µg/ml.

Primary screening against human pathogens

In the current study 16 marine actinomycete strains were isolated from soil samples collected from Marina and Thiruvannamiyur beaches. A similar study on isolation of antibacterial actinomycetes was carried out by Devi *et al.* (2006). It has been reported that 10 strains were isolated from marine water collected from Dhanushkodi, among which, 3 strains were found to be *Actinopolyspora*, *Nocardia* and *Streptomyces* sp. The *Streptomyces* species showed best level of antibacterial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi* and *A. niger*.

Whereas, the other 2 species *Actinopolyspora* and *Nocardia*, showed antifungal activity only. Totally 164 strains were isolated from 39 sediment samples collected from the Bay of Bengal coast of Puducherry and Marakkanam and the isolate VITSVK9 showed antibacterial activity against *Bacillus subtilis* (18 mm) and antifungal activity against *Aspergillus niger* (17 mm) (Thenmozhi and Kannabiran, 2012). The results of the current study revealed that the isolated strains possessed significant antibacterial activity and no significant inhibition of fungal strains.

Actinomycetes, “the reservoir of secondary metabolites and enzymes”, dominant and significant microbes inhabiting soil environment comprises about 50% of the uncultivable soil microbes. The immense source of novel metabolites and their therapeutic applications, specifically as drug lead molecules serves as a natural blue print for developing new drugs. Fungal drug resistance is a growing problem worldwide and search for new and effective antifungals to overcome drug resistance is of current importance. Actinomycetes isolate

producing polyene type of metabolite has been reported to be effective against pathogenic bacteria and fungi. A polyketide antibiotic extracted from *Streptomyces* sp. AP-123 have been shown to be very effective against *C. albicans* and filamentous fungi *A. niger*. New triazole antifungal drugs and combination drugs are underway to overcome invasive fungal infections and emergence of resistance.

Penicillin, Tetracycline, cephalosporins are important soil derived antibiotics. Vancomycin (isolated in 1956) from actinomycete species found in Indian and Indonesian soils, is very powerful and currently serves as the last line of defense to treat bacterial infections. Actinomycetes (C11 and C12) isolated from marine environment has been shown to be effective against group of bacterial pathogens. Marine actinobacteria isolated from salt pan environment, SRB25 has been reported to be effective against multidrug resistant *Staphylococcus aureus* (MDRSA). However, the results obtained from the current study revealed that the isolated strains did not possess significant antifungal activity against *C. albicans* (Subashini and Kannabiran, 2013).

Evaluation of Antioxidant activity

In recent years much attention has been devoted to natural antioxidant and their association with health benefits. There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al.*, 2001).

Table.1 Primary screening - zone of inhibition of antibacterial activity

S.No	Strain	Zone of inhibition (mm)			
		<i>E.coli</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>B.subtilis</i>
1	ABTRI 3037	12mm	16mm	19mm	19mm

Table.2 Culture characterization of actinomycetes strains

S.NO	STRAIN NUMBER	AERIAL MYCELIUM	SUBSTRATE MYCELIUM
1	ABTRI3015	Peach powdery	Light peach
2	ABTRI3025	White dotted colonies	Light brown
3	ABTRI3031	Greyish powdery	Medium brown
4	ABTRI3001	Medium yellowish, dotted powdery	Light sandal
5	ABTRI3043	Dotted yellow colonies	Light sandal
6	ABTRI3027	Light white, flat powdery	Light sandal
7	ABTRI3000	Whitish leathery powder	Light sandal
8	ABTRI3037	Puffy white, greyish powdery	Light sandal
9	ABTRI3002	Greyish puffy powdery	Dark grey
10	ABTRI3003	Dotted white colonies	Light brown
11	ABTRI3004	Medium grayish	Light grey
12	ABTRI3005	Peach white powder	Light peach
13	ABTRI3006	Whitish leathery powder	Light brown
14	ABTRI3007	Medium white dotted colonies	Light sandal
15	ABTRI3008	Peach powdery	Light peach
16	ABTRI3009	Greyish powdery	Light grey

Table.3 Biochemical characterization of ABTRI 3037

S.No	TEST	RESULT
1	Gram staining	+
2	Indole	+
3	Methylred	-
4	Voges-Proskauer	-
5	Oxidase	-
6	Catalase	+
7	Carbohydrate utilization	
7.1	Sucrose	-
7.2	Maltose	-
7.3	Galactose	+
7.4	Fructose	+
8	Gelatin liquefaction	-

Table.4 TLC profile of ABTRI 3037

S.No	RATIO Ethyl acetate : Toluene	Rf Value	
		UV	IODINE
1.	0.3: 0.7	0.842	0.842
		0.631	0.631
		0.315	0.315

Table.5 Secondary screening - antibacterial activity of the extracted bioactive compound

Organism	Zone of inhibition (mm)			
	250µg/ml	500µg/ml	750µg/ml	1000µg/ml
<i>E. coli</i>	-	10mm	10mm	11mm
<i>K. pneumoniae</i>	10mm	11mm	12mm	13mm
<i>P. aeruginosa</i>	14mm	15mm	16mm	17mm
<i>B. subtilis</i>	-	-	10mm	10mm
Tetracycline	23mm			

Fig.1 Maintenance of actinomycetes on SCA slants

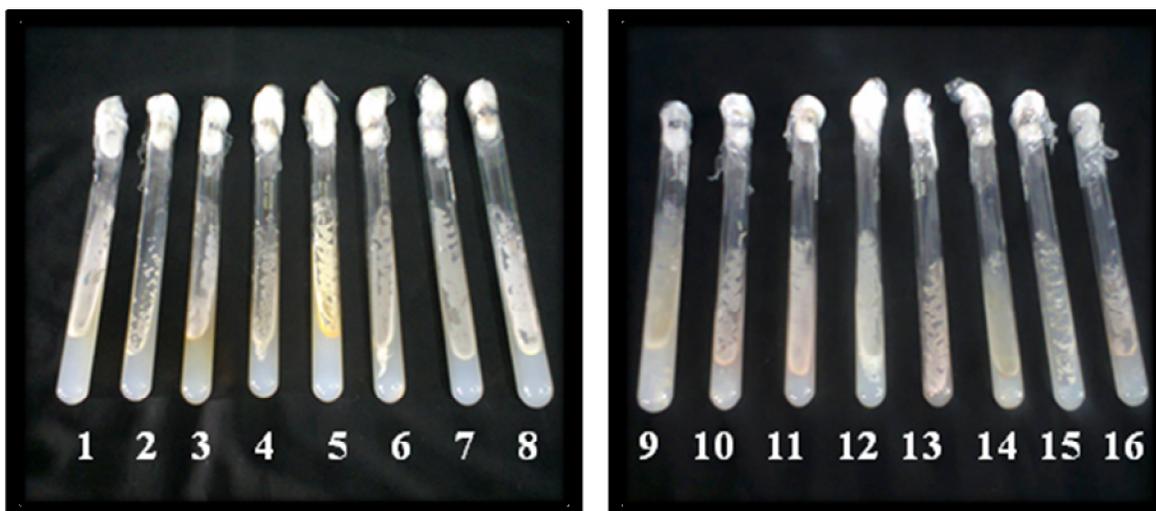


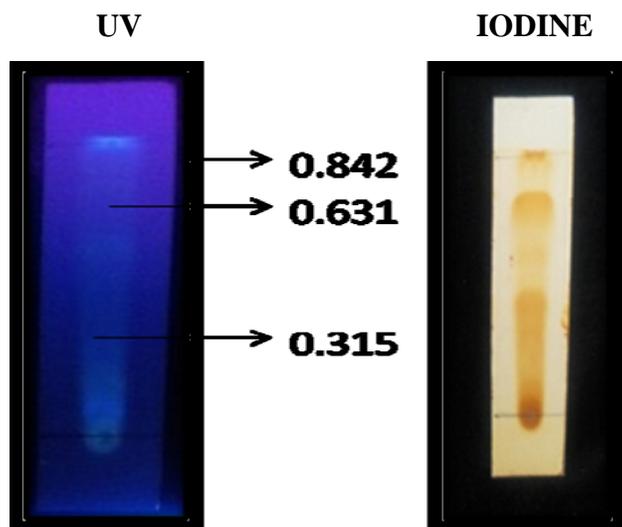
Fig.2 Antibacterial activity of selected actinomycetes against human bacterial pathogens [(a) *E. coli*, (b) *K. pneumoniae*, (c) *P. aeruginosa* and (d) *B. subtilis*]



Fig.3 Morphological identification by coverslip culture method

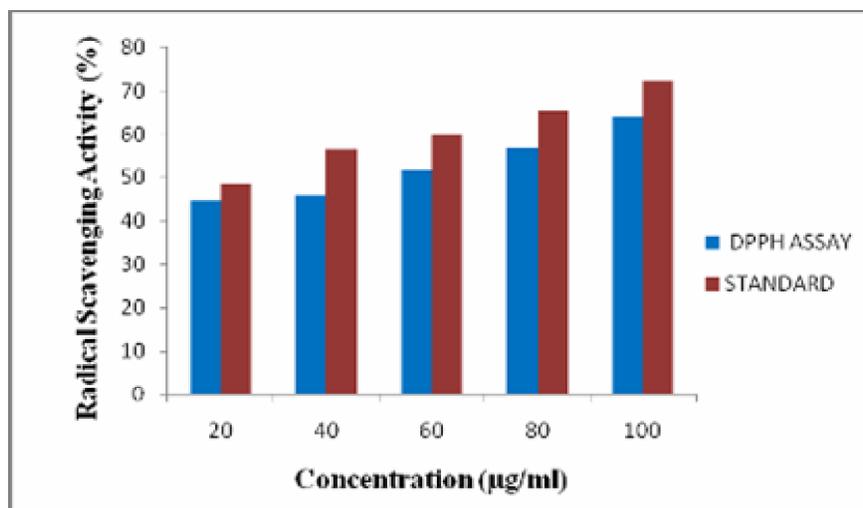


Fig.4 TLC profile of ABTRI 3037



Evaluation of antioxidant activity

Fig.5 Antioxidant activity of crude compound of ABTRI 3037



In this study, the scavenging activity of ethyl acetate extracts was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. A recent work on isolation of antioxidant and antimicrobial actinomycetes strains from marine sample collected from Puducherry coastal region (Priya and Perumal Arumugam, 2012).

The secondary metabolite showed 83% inhibition at 0.2mg/ml and the inhibition was compared with the standard antioxidant ascorbic acid which showed 84% inhibition at 0.2mg/ml concentration. This could be well compared with the current result which showed 64% inhibition of DPPH radicals at maximum concentration of 100µg/ml. The crude compound possessed IC_{50} of 58.22µg/ml at concentration of 60µg/ml.

The research study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Partial purification of Secondary metabolites

The bioactive compounds isolated from the strain ABTRI 3037, when subjected to TLC, showed the presence of 3 distinct compounds with R_f values 0.842, 0.631 and 0.315. A similar study on the extracts obtained from *Streptomyces* isolated from sediment soil showed nine bioactive regions with R_f values from 0.21 to 0.96 (Ilic *et al.*, 2007). Similar result was obtained from the cultural extracts of marine sponges associated *Streptomyces* was reported (Dharmaraj and Sumantha, 2009). IR spectra of crude extract showed some different vibrational peaks of these functional groups in the extract depicts that the diverse activity they exhibited against test organisms during the susceptibility screening. The distribution

of the antibiotic inhibition phenotype of *Streptomyces* with great antibacterial and antifungal activity which gave a similar spectra profile has also been reported (Ilic *et al.*, 2007). Nevertheless, further investigation is needed in order to purify and determine the structure of the active components in the extracts.

Acknowledgement

We are highly thankful to Armats Biotek Private Limited for providing all facilities and support to meet our project requirements. Last but not least, we take great pleasure in expressing thanks to our parents and friends who helped us a lot in finishing the project that made us for the indeed success.

References

Baskaran, R., Vijayakumar, R., Mohan, P.M. 2011. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Malaysian J. Microbiol.*, 7: 26–32.

Bull, A.T., Stach, J.E.M., Ward, A.C., Goodfellow, M. 2005. Marine actinobacteria: perspectives, challenges, future directions. *Antonie Van Leeuwenhoek*, 87: 65–79.

Devi, N.K.A., Jeyarani, M., Balakrishnan, K. 2006. Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. *Pak. J. Biol. Sci.*, 9(3): 470–472.

Dharmaraj, S., Sumantha, A. 2009. Bioactive potential of *Streptomyces* isolated from marine sponges. *World J. Microbiol. Biotechnol.*, 25: 1971–1979.

Goodfellow, M., O'Donnell, A.G. 1989. Roots of bacterial systematic. In: The

Handbook of new bacterial systematic. Academic Press, London. Pp. 3–5.

Helmke, E., Weyland, H. 1984. *Rhodococcus marinonascens* sp. Nov., an actinomycete from the sea. *Int. J. Syst. Bacteriol.*, 34: 127–138.

Ilic, S.B., Konstantinovic, S.S., Todorovic, Z.B., Lazic, M.L., Veljkovic, V.B., Jokovic, N., Radovanovic, B.C. 2007. Characterization and antimicrobial activity of the bioactive metabolites in *Streptomyces* isolates. *Microbiology*, 76: 421–428.

Jensen, P.R., Mincer, T.J., Williams, P.G., Fenical, W. 2005. Marine actinomycete diversity and natural product discovery. *Antonie Van Leeuwenhoek*, 87: 43–48.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N. 2001. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Analysis*, 13: 8–17.

Lee, J.Y., Hwang, B.K. 2002. Diversity of antifungal actinomycetes in various vegetative soils of Korea. *Rev. Can. Microbiol.*, 48: 407–417.

Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W. 2002. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl. Environ. Microbiol.*, 68(10): 5005–5011.

Mohankumar Thenmozhi, Krishnan Kannabiran, 2012. Antimicrobial and antioxidant properties of marine actinomycetes *Streptomyces* sp VITSTK7. *Oxid. Antioxid. Med. Sci.*, 1(1): 51–57.

Nikolaos Nenadis, Maria Tsimidou, 2002. Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-

- 2-picrylhydrazyl (DPPH) tests. *J. Oil Fat Ind.*, 79(12): 1191–1195. doi: 10.1007/s11746-002-0626-z
- Priya, A.J., Perumal Arumugam, 2012. Detection of antioxidant and antimicrobial activities in marine actinomycetes isolated from Puducherry Coastal Region. *J. Modern Biotechnol.*, 1(2): 63–69.
- Ramesh, S. 2009. Marine actinomycetes diversity in Bay of Bengal, India: isolation and characterization of bioactive compounds from *Streptomyces fungicidicus* MML 1614. India. Dissertation, University of Madras, Chennai, India.
- Sasirekha, C. *et al.* 2012. Fibrinolytic enzyme from actinomycetes. *J. Pharm. Res.*,
- Singh, S.B., Pelaez, F. 2008. Biodiversity, chemical and drug discovery. *Progr. Drug Res.*, 65: 143–74.
- Stackebrandt, E., Rainey, F.A., Ward-Rainey, N.I. 1997. Proposal for a new hierarchic classification system. *Actinobacteria* classis nov. *Int. J. Syst. Bacteriol.*, 47: 479–491.
- Subashini, J., Kannabiran, K. 2013. Antimicrobial activity of *Streptomyces* sp. VITBT7 and its synthesized silver nanoparticles against medically important fungal and bacterial pathogens. *Der. Pharmacia Lett.*, 5(3): 192–200.
- Williams, S.T., Cross, T. 1971. Actinomycetes isolation from soil. In: Booth, C. (Ed.). *Methods in microbiology*. Academic Press, London, New York. Pp. 295–334.