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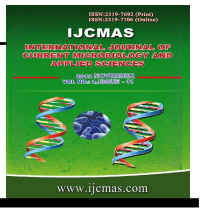

Original Research Article

Isolation and characterization of Endophytic Actinomycetes from mangrove plant for antimicrobial activity

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A B S T R A C T

Endophytes are Microbes that colonize living, internal tissues of plants without carrying any immediate over negative effects. In this present investigation the endophytic actinomycetes were isolated from root, stem and leaf of two species of mangrove such as, *Rhizopora apiculata* and *Avicennia marina* for their antimicrobial activity. Totally 6 endophytic actinomycetes were obtained from all the samples. Population enumeration, phenotypic characters like substrate mycelium, aerial mycelium, aerial mass colour, reverse side pigment, melanoid pigment, spore chain morphology, salt tolerance on growth, growth on different pH, effect of different temperature on growth, Biochemical characteristics, assimilation of different carbon sources, nitrogen sources and enzymatic activity were studied. For antimicrobial activity 7 bacterial pathogens and 4 fungal pathogens were used. Up on the 6 isolate 1 endophytic actinomycetes isolate (MAR1) shows good activity against all the isolates. Based on the above characteristics the potential isolate MAR1 is identified as *Streptomyces* sp. and species may be *Streptomyces coelicolor.*

K e y w o r d s

Endophytes; Actinomycetes; Mangrove; Antimicrobial activity; *Streptomyces coelicolor.*

Introduction

"Endophytes are Microbes that colonize living, internal tissues of plants without carrying any immediate over negative effects" an inclusive and widely accepted definition of endophytes by Bacon and white (2000). Endophytes could be better protected from biotic and abiotic stresses than rhizosphere bacteria (Gayathri and Muralikrishnan, 2013). The mangrove forests are one among the world's most productive ecosystems with great ecological and economic significance (Kathiresan, 2000). However, mangroves exist under condition of high salinity, extreme tides, strong winds, high temperature and muddy, anaerobic soils, These plants, and the associated microbes, fungi, plants and animals, constitute the mangrove forest community or mangal (Kathiresan and Bingham, 2001). Actinomycetes are gram positive bacteria, with a high guanine (G) plus cytosine (C)

ratio in their DNA (>55mol %), which are phylogenetically related from the evidence of 16S ribosomal cataloguing and DNA: rRNA pairing studies (Goodfellow and Williams, 1983). The name "Actinomycetes" was derived from Greek "atkis" (a ray) and "mykes" (fungus), and has features of both Bacteria and fungi (Das *et al*., 2008). In the strict taxonomic sense, actinomycetes are clubbed with bacteria in the same class of Schizomycetes but confined to the order Actinomycetales (Kumar *et al*., 2005).

As the frequency of novel bioactive compounds obtained from terrestrial Actinobacteria decreases with time, Actinobacteria from diverse environments have been increasingly screened for their ability to produce new secondary metabolites. In the recent years, terrestrial and marine microorganisms have known for antimicrobial, antiviral, antitumour, anticoagulant, antidiabetic and cardio active properties. Antibiotic effect of actinomycetes has been used in many fields including agriculture, veterinary and pharmaceutical industry.

Based on the above information the present study is conducted to isolate bacterial endophytic actinomycetes from different parts of mangrove plants to identify better antimicrobial compound.

Materials and Methods

Sample collection and transport

Samples were collected from Parangipettai and Pichavaram. All the Samples were collected in a sterile plastic covers, transferred to laboratory and processed immediately.

Sample pretreatment and endophytic actinomycetes isolation

For pretreatment and isolation modified method of Lixiang Coa *et al.*, (2004) were adopted. The root, leaf and stem samples were washed in running tap water to remove soil particles and sterilized by sequential immersion in 70% ethanol for 5 min and a solution of sodium hypochlorite (0.9% available chlorine) for 20 min. Samples were washes in sterile water three times to remove surface sterilization agents. Each samples was divided into small fragments and plated on starch casein agar medium (Kuster and Williams, 1964). Nystatin and Cycloheximide (50 µg/ml of each) were added to media to suppress the fungal growth (William and Davies, 1965) and incubated at room temperature for 7 – 10 days. After incubation plates were observed for growth of endophytic actinomycetes.

Phenotypic characterizations

The classification of actinomycetes was originally based largely upon the morphological observations. So, morphology is still an important characteristic for the description of taxa and it is not adequate in itself to differentiate between many genera. In fact, it was the only characteristic which was used in many early descriptions, particularly of *Streptomyces* species in the first few editions of Bergey's Manual. These observations are best made by the variety of standard cultivation media. Several of the media suggested for the International Streptomyces Project and by Pridham *et al.*, (1957) have proven to be useful in our hands for the characterization of strains accessioned into the ARS

Actinomycetales Culture Collection (Labeda, 1985). It includes some basic tests *viz.,* Aerial mass colour, Reverse side pigment, Melanoid pigments, Spore chain morphology and Spore morphology (Shirling and Gottileb, 1966).

Aerial mass colour

For the grouping and identification of *Actinomycetes* sp. the chromogenicity of the aerial mycelium was considered to be an important character. The colours of the mature sporulating aerial mycelium are white, gray, red, green, blue and violet. When the aerial mass colour falls between two colours series, both the colors are recorded. In the cases where aerial mass colour of a strain showed intermediate tints, then in that place both the colour series should be noted.

Reverse side pigments

The strains are divided into two groups according to their ability to produce characteristic pigments on the reverse side of the colony, called as distinctive (+) and not distinctive or none (-). A colour with low chroma such as pale yellow, olive or yellowish brown occurs, it is included in the latter group (-).

Melanoid Pigments

The grouping is made on the production of melanoid pigments (i.e. greenish brown, brownish black or distinct brown pigment modified by other colours) on the medium. The strains are grouped as melanoid pigment produced (+) and not produced (-). For the melanoid pigment observation the inoculated plates were kept under incubator for 4 to 5 days. The strains which shows cultures forming a greenish brown to brown to black diffusible pigment or a distinct brown pigment modified by other color are recorded as positive (+) total absence of diffusible pigment, are recorded as negative (-) for melanoid pigment production.

Spore chain morphology

A characteristic of the spore bearing hyphae and spore chains is determined by the direct microscopic examination of the culture area. Adequate magnification used to establish the presence or absence of spore chains and to observe the nature of spore chains is 40x. By the standard protocol of cover slip culture technique, the plates were prepared and after the incubation of 7 to 10 days it was observed. During this method of spore morphological study, ISP 2 medium Plates were prepared. After solidification, by a sharp scalpel from the central portion of the plate, medium should be scooped out making a rectangular area. Then, three sterile cover slips were placed on the hollow rectangular space. Slowly Actinomycetes spores have to be inoculated at the edge of the cover slips touching the medium. The plates were incubated at 28±2°C for 5 days and examined periodically taking out the cover slips.

Assimilation of carbon sources

The ability of different actinomycetes strains in utilizing various carbon compounds as source of energy was studied by following the method recommended in International *Streptomyces* Project. Stock solution of 10 sugars i.e., Xylose, Inositol, Sucrose, Raffinose, Fructose, Rhamnose and Mannitol having concentration of 10x was prepared in autoclaved water and sterilized by filtering through 0.22µm pore size

Table.1 Stress tolerance test for endophytic actinomycetes (Saurav and Kannabiran, 2009)

S. No	Stress tolerance test	Concentrations or differentiation used	Medium	Incubation	Method of detection
1.	Different NaCl concentration	2.5, 5.0, 7.5, 10 and 12.5 %	Starch casein agar	30°C for 7 – 15 days	Growth indicates positive
2.	Different pH	4, 5, 6, 7, 8, and 9 pH	Starch casein agar	30°C for 7 – 15 days	Growth indicates positive
3.	Different temperature	25°C, 30°C, 35°C, 40°C and 45°C	Starch casein agar	For 7 – 15 days	Growth indicates positive

Table.2 Biochemical characteristics of endophytic actinomycetes isolates (Shriling and Gottlieb., 1966)

S. No	Biochemical Test	Medium used	Incubation	Indicator used	Method of detection
1.	Indole Test	Peptone broth	30°C for 3 – 4 days	Kovac's reagent	Cherry red colour ring indicates positive
2	Methyl red	MR broth	30°C for 2 – 4 days	Methyl Red	Red colour indicates positive
3.	Voges – Proskaur	VP broth	30°C for 2 – 4 days	Solution – A, Solution - B	Red colour indicates positive
4.	Citrate utilization	Simmon's citrate agar slant	30°C for 2 – 4 days	-	Blue colour indicates positive
5.	Hydrogen-sulphide production	Tryptone-Yeast extract agar slants	30°C for 7 – 15 days	-	Black colour indicates positive
6.	Urease test	Urea agar	30°C for 2 – 4 days	-	Pink colour indicates positive
7.	Catalase	Treat with 3% H_2O_2	30°C for 2 – 4 days	-	Bubble formation indicates positive
8.	Oxidase	Starch casein broth	30°C for 2 – 4 days	Oxidase disc	Blue colour indicates positive

Table.3 Enzymatic activity of endophytic actinomycetes isolates (Stolpe and Godkeri, 1981)

S. No	Enzymatic test	Medium used	Incubation	Indicator used	Method of detection
1.	Lipolytic activity	Tween 20 agar	30°C for 7 – 10 days	-	Zone around the colonies
2.	Hydrolysis of starch	Starch agar	30°C for 7 – 10 days	Lugal's Iodine	Blue colour indicates positive
3.	Hydrolysis of casein	Skim milk agar	30°C for 7 – 10 days	-	Zone around the colonies
4.	Hydrolysis of Gelatin	12% Gelatin agar	30°C for 7 – 10 days	-	Liquification of medium
5.	Cellulose Utilization	Cellulose agar	30°C for 7 – 10 days	-	Growth on the medium
6.	Chitin Degradation	Colloidal chitin agar	30°C for 7 – 10 days	-	Zone around the colonies

membrane filters and stored at 4^0C. Media was prepared by adding 1% carbon source in sterile ISP2 medium. Inoculated and incubated at 28^0C for 7 to 10 days. Growth was observed by comparing them with positive and negative control (Pridham and Gottlieb, 1948).

Assimilation of nitrogen sources

The ability of different actinomycetes strains in utilizing various nitrogen compounds as source of energy was studied by following the method recommended in International Streptomyces Project. Stock solution of nitrogen sources namely L-Aspergine, L-Argine, L-Coralline, L-Histidine, Glysine, L-Lysine and L-Proline having concentration of 10x was prepared in autoclaved water and sterilized by filtering through 0.22µm pore size membrane filters and stored at 4^0C. Medium was prepared by adding 1% nitrogen source in ISP2 media. Inoculated and incubated at 28^0C for 7 to 10 days. Growth was observed by comparing them with positive and negative control (Pridham and Gottlieb, 1948).

Screening of Anti-bacterial and antifungal activity of endophytic *Actinomycetes* isolates (Sathish kumar *et al.*, 2012)

Primary screening of actinobacterial isolates were performed by cross streak method on modified nutrient agar plates (MNA). The actinobacterial isolates were inoculated in straight line on MNA plates and incubated for 7 days. Bacterial and fungal Pathogenic strains were cross streak on the same plate in perpendicular manner. The plates were incubated at 37°C for 24 hours. The plates were examined for the zone of inhibition of pathogens.

Fermentation procedure (Umasankar *et al.*, 2010)

The potent actinobacterial isolate were inoculated into production broth (SS Media) containing soluble starch-25g, glucose-10g, yeast extract-2g, CaCO3- 3g, trace elements- 1ml, Distilled water - 1000ml. Flasks were lodged on the flask shaker at a speed of 120 rpm at room temperature for 7 days.

Table.1 Isolation and enumeration of endophytic actinomycetes from mangrove plant

S. No	Locations	Isolates	Total actinomycetes population (x 10^4 cfu g^{-1})
1	Pichavaram	MAR1	16.94
2		MAS1	14.23
3		MAL1	12.45
4	Parangipettai	MAR2	16.23
5		MAS2	14.65
6		MAL2	12.23

Table.2 Phenotypic Characterization of endophytic *Actinomycetes* isolates

S. No	Isolates	Substrate mycelium	Aerial mycelium	Aerial mass colour	Melanoid pigments	Reverse side pigments	Spore chain morphology
1	MAR1	+	+	Gy	_	+	S
2	MAS1	+	+	Gy(W)	+		R
3	MAL1	+	+	W	_	_	BIV-S
4	MAR2	+	+	Gy	+	_	S
5	MAS2	+	+	W	+	_	S
6	MAL2	+	+	Gy(W)	+	+	RA

S - Spirals; RA - Retinaculum-apertum; BIV-S - Biverticillus-spira with +: Positive -: Negative W: White, Gy: Grey, D: Dark ash and Y: Yellow

Table.3 Sodium chloride tolerance on the growth of endophytic *Actinomycetes* isolates

S. No	Isolates	2.5%	5%	7.5%	10%	12.5%
1	MAR1	+++	+++	+++	+++	+++
2	MAS1	+++	++	++	+	-
3	MAL1	+++	++	+	-	-
4	MAR2	+++	+++	++	+	-
5	MAS2	+++	++	+	+	-
6	MAL2	+++	++	++	+	-

+++: Good growth, ++: Moderate growth, +: Fair growth -: No growth

Table.4 Effect of different pH on the growth of endophytic *Actinomycetes* isolates

S. No	Isolates	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
1	MAR1	++	++	++	++	++	++
2	MAS1	+	++	++	++	++	++
3	MAL1	-	+	++	++	++	+
4	MAR2	++	++	++	++	++	++
5	MAS2	+	++	++	++	++	+
6	MAL2	-	+	+	++	++	+

++: Good growth, +: Moderate growth -: No growth

Table.5 Effect of different Temperatures on the growth of *Actinomycetes* Isolates

S. No	Isolates	Temperature (^{0}C)				
		25	30	35	40	45
1	MAR1	+	++	++	+	+
2	MAS1	+	++	+	-	-
3	MAL1	+	++	++	-	-
4	MAR2	+	++	+	+	-
5	MAS2	+	++	+	-	-
6	MAL2	+	++	++	-	-

++: Good growth, +: Moderate growth -: No growth

Table.6 Biochemical characterization of endophytic *Actinomycetes* Isolates

S. No	Isolates	Biochemical characteristics							
		Indole	MR	VP	Citrate	H_2S	Urease	Catalase	Oxidase
1	MAR1	+	+	-	+	+	+	+	+
2	MAS1	-	+	+	-	+	+	+	+
3	MAL1	+	-	+	+	+	+	+	+
4	MAR2	+	+	-	+	-	+	+	+
5	MAS2	-	+	+	-	+	+	_	+
6	MAL2	+	+	-	+	+	+	_	+

+: Positive, -: Negative

Table.7 Assimilation of carbon sources by the endophytic *Actinomycetes* isolates

S. No	Isolates	Xylose	Inositol	Sucrose	Raffinose	Fructose	Rhamnose	Mannitol
1	MAR1	+	+	±	±	+	+	+
2	MAS1	+	+	+	±	+	-	+
3	MAL1	-	+	-	-	+	+	+
4	MAR2	+	-	+	+	±	-	-
5	MAS2	+	+	+	±	+	+	+
6	MAL2	-	+	+	_	+	+	-

+: Positive, ±: Not defined, -: Negative.

Table.8 Assimilation of Nitrogen sources by endophytic *Actinomycetes* Isolates

Isolates	Nitrogen sources						
	L-Aspergine	L-Arginine	L-Citrulline	L-Histidine	Glysine	L-Lysine	L-Proline
MAR1	+	-	-	-	-	+	+
MAS1	±	±	+	±	+	_	+
MAL1	+	+	_	±	_	_	+
MAR2	±	+	_	+	±	+	_
MAS2	±	+	±	±	±	_	_
MAL2	+	+	±	_	_	±	+

+: Positive, ±: Not defined, -: Negative

Table.9 Enzymatic activity of endophytic actinomycetes isolates

S. No	Isolates	Enzymatic activity					
		Lipase	Amylase	Caseinase	Gelatinase	Cellulase	Chitinase
1	MAR1	+	+	+	-	+	+
2	MAS1		+	+	-	+	+
3	MAL1	-	-	-	-	+	+
4	MAR2	-	+	-	-	+	+
5	MAS2	-	+	+	-	+	+
6	MAL2	-	+	+	-	+	+

+: Positive, -: Negative

Table.10 Results obtained from the keys given for 458 species of actinomycetes included in ISP (International Streptomyces Project) for probable identification

S. No	ISOLATES	SPECIES NAME
1	MAR1	*Streptomyces coelicolor*
2	MAS1	*Streptomyces hygroscopicus*
3	MAL1	*Streptomyces mirabilis*
4	MAR2	*Streptomyces xantholyticus*
5	MAS2	*Actinomycetes longiporus*
6	MAL2	*Actinomycetes malachitorectus*

Table.11 Screening of Anti-bacterial activity of endophytic *Actinomycetes* isolates

S. No	Isolates	Zone of inhibition (mm)					
		P. aeruginosa	***E.coli***	***K.pneumoniae***	***P.vulgaris***	***S.aureus***	***S.typhi***
1	*Streptomyces coelicolor* (MAR1)	+++	+++	+++	+++	+++	+++
2	*Streptomyces hygroscopicus*(MAS1)	++	++	++	-	++	++
3	*Streptomyces mirabilis*(MAL1)	++	-	+	++	-	+
4	*Streptomyces xantholyticus*(MAR2)	-	++	++	++	+	++
5	*Actinomycetes longiporus*(MAS2)	++	+	-	-	++	-
6	*Actinomycetes malachitorectus*(MAL2)	-	++	-	++	++	-

+++: Good Activity, ++: Fair activity, +: Moderate activity –: No activity

Table.12 Screening of Anti-fungal activity of endophytic *Actinomycetes* isolates

S. No	Isolates	Zone of inhibition (mm)			
		A.niger	***A.flavus***	***Penicillium sp***	***A.fumigatus***
1	*Streptomyces coelicolor* (MAR1)	+++	+++	+++	++
2	*Streptomyces hygroscopicus* (MAS1)	+	++	-	-
3	*Streptomyces mirabilis* (MAL1)	++	-	+	-
4	*Streptomyces xantholyticus* (MAR2)	++	++	++	-
5	*Actinomycetes longiporus* (MAS2)	++	+	++	-
6	*Actinomycetes malachitorectus* (MAL2)	+	++	-	-

+++: Good Activity, ++: Fair activity, +: Moderate activity –: No activity

Table.13 Anti-bacterial activity of selected endophytic *Actinomycetes* isolates

S. No	Isolates	Zone of inhibition (mm)					
		P.aeruginosa	***E. coli***	***K.pneumoniae***	***P.vulgaris***	***S.aureus***	***S. typhi***
1	*Streptomyces coelicolor* (MAR1)	35	39	32	37	32	33
2	*Streptomyces hygroscopicus* (MAS1)	18	10	16	NZ	16	12
3	*Streptomyces xantholyticus* (MAR2)	NZ	13	10	14	9	11

NZ – No zone of inhibition

Table.14 Anti-fungal activity of selected endophytic *Actinomycetes* isolates

S. No	Isolates	Zone of inhibition (mm)			
		A.niger	***A.flavus***	***Penicillium sp***	***A.fumigatus***
1	*Streptomyces coelicolor* (MAR1)	25	22	20	17
2	*Streptomyces xantholyticus* (MAR2)	11	13	10	NZ
3	*Actinomycetes longiporus* (MAS2)	12	9	13	NZ

After fermentation, the medium was harvested and centrifuged to remove cell debris. Filtrate was collected and stored for further use.

Antibacterial and antifungal activity of selected endophytic actinomycetes by Agar well diffusion method (Modified method of Gaurav kumar *et al.*, 2010)

For antibacterial and antiulgal activity of selected endophytic actinomycetes was detected by agar well diffusion method on Mueller Hinton agar. All bacterial and fungal pathogens was inoculated in nutrient broth and potato dextrose broth respectively and incubated for 24 hours at 37°C. The turbidity of the broth was adjusted at 0.5 (optical density) using spectrophotometer. The bacterial and fungal cultures were inoculated on MHA plates using sterilized cotton swabs. In each of these plates, wells were cut out using a sterilized gel borer. The selected endophytic actinomycetes culture supernatant was used against test pathogen, 100 µl of supernatant were loaded into each well. Plates were incubated at 37°C for 24 hours. After incubation, all plates were examined for the presence of zone of inhibition around the Wells and measure the zone size.

Result and Discussion

Results of Population enumeration, phenotypic characters (substrate mycelium, aerial mycelium, aerial mass colour, reverse side pigment, melanoid pigment, spore chain morphology), Stress tolerance on growth such as., Salt tolerance on growth, growth on different pH, effect of different temperature on

growth, Biochemical characteristics, Assimilation of different carbon sources, Nitrogen sources and enzymatic activity of endophytic actinomycetes isolates were given in the Tables 1 to Table 9 respectively.

The probable identification of endophytic actinomycetes isolates was obtained from the keys given for 458 species of actinomycetes included in ISP (International Streptomyces Project) were given in table 10.

Screening of antibacterial and antifungal activity of endophytic actinomycetes were given in the table 11 and table 12 respectively. Based on the screening 3 isolates for antibacterial and 3 isolates for antifungal activity were selected.

Antibacterial and antifungal activity of selected endophytic actinomycetes were given in table 13 and table 14. One isolate MAR1 (*S. Coelicolor*) shows good activity against all the bacterial and fungal pathogens.

Based on the above results obtained by endophytic actinomycetes isolated from mangrove plant parts have the great capacity of biological activities viz., Stress tolerance on growth, Assimilation of different carbon and nitrogen sources, enzymatic activity and potential antimicrobial activity. The present study concludes that this work may be the first report on potential endophytic actinomycetes (*Streptomyces coelicolor*) from mangrove in state of Tamilnadu. This study evidenced that mangrove are the potential for bioactive endophytic actinomycetes. Detailed investigations on Mangrove endophytic actinomycetes were needed to prove its potential further and it will leads to the discovery of numerous high value metabolites.

References

Bacon, C.W., and White, J.F. 2000. Microbial endophytes. Marcel Dekker Inc., New york, N.Y. 341-388.

Das, S., P.S. Lyla and Khan, S.A. 2008. Distribution and generic composition of culturable marine actinomycetes from the sediments of Indian continental slope of Bay of Bengal. Chinese .J. Oceanol. Limnol. 26 (2): 166-177.

Gaurav Kumar, L. Karthik and Bhaskara Rao, K.V. 2010. In vitro anticandida activity of Calotropis gigantea against clinical isolates of candida. J. Pharm. Res. 3: 539-542.

Gayathri,.P., and Muralikrishnan,V. 2013. Bioprospecting of endophytic bacteria from mangrove bananas And sugarcane plants for their pgpr activity. Europial. J.Biol.Rev. 21: 456-469.

Goodfellow, M., and Williams, S.T. 1983. Ecology of actinomycetes. Ann. Rev. Microbiol. 37:189-216.

Kathiresan, K**.,** 2000. A review of studies on pichavaram mangrove, southeast India. Hydrobiologia. 430: 185-205.

Kathiresan, N.K., and Bingham, B.L. 2001. Biology of Mangroves and Mangrove Ecosystems. Adv. Marine Biol. 40: 81-251.

Kumar, S.V., M.K. Sahu and Kathiresan, K. 2005. Isolation and characterization of streptomycetes producing antibiotics from a mangrove environment, Asian J. Microbial. Biotech. 3: 457-464.

Kuster, E., and Williams, S.T. 1964. Selective media for the isolation of *Streptomycetes*. Nature. 202: 928-929.

Labeda, D.P., 1985. Actinomycete taxonomy: generic characterization,

Developments in Industrial Microbiology, J. Indus. Microbiol. Suppl. No.2. Editor: G. Pierce, 28: 115-121.

Lixiang Cao., Zhiqi Qiu, Xin Dai, Hongming Tan, Yongcheng Lin and Shining Zhou, 2004. Isolation of endophytic actinomycetes from roots and leaves of banana (*Musa acuminata*) plants and their activities against *Fusarium oxysporum* f. sp. *Cubense*. World J. Microbiol. Biotechnol.. 20: 501–504.

Pridham, T.G., P. Anderson, E. Foley, L.A. Lindenfelser, E.W. Hesseltine and Benedict, R.G. 1957. A selection of media for maintenance and taxonomic study of *Streptomyces*. Antibiot. 947-953.

Pridham, T.G., and Gottlieb. D. 1948. The utilization of carbon compounds by some Actinomycetes as an aid for species determination. J. Bacteriol. 56: 107-114.

Sathish Kumar S.R, and Kokati Venkata Bhaskara Rao. 2012. In-vitro antimicrobial activity of marine actinobacteria against multidrug resistance *Staphylococcus aureus*. *Asian* Pacific. J. Trop. Biomed. S1802-S1807.

Saurav, K., and Kannabiran, K. 2009. Chromium Heavy metal resistance activity of marine *Streptomyces* VITSVK5 spp. (GQ848482). Pharmacologyonline. 3: 603-613.

Shirling, E.G., and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313-340.

Stolpe., and Godkeri. 1981. Non-pathogenic members of genus *Pseudomonas*. In: The prokaryotes, Ed. Marthiner et al., Springer Verlag, New York, pp. 719-741.

Umasankar, M.E., Gaurav Kumar, L. Karthik and Bhaskara Rao, K.V. 2010. Exploration of antagonistic actinobacteria from Amirthi forest. Int J Curr Pharm Res. 2: 16-19.

Williams, S. T., and Davies F. L. 1965. Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. J. Gen. Microbiol. 38: 251-261.