Advances in Biological Research 4 (2): 108-115, 2010
ISSN 1992-0067

Production of Biosurfactant and Heavy Metal Resistance Activity of *Streptomyces Sp.* VITDDK3-a Novel Halo Tolerant Actinomycetes Isolated from Saltpan Soil

[1]T. Deepika Lakshmipathy, [2]A.S. Arun Prasad and [1]Krishnan Kannabiran

[1]Division of Biomolecules and Genetics,
[2]Division of Environmental Biotechnology,
School of Biosciences and Technology, VIT University, Vellore- 632014, India

Abstract: Marine actinomycetes, *Streptomyces* spp. VITDDK3 was screened and evaluated for biosurfactant production and heavy metal resistance activity. Biosurfactant production was confirmed by conventional screening methods, including hemolytic, drop collapsing and lipase production activity. The isolate was also screened for dye decolourization activity. Haemolytic, drop collapsing and lipase production studies revealed that the isolate produced surface active agents. Agar diffusion assay for heavy metal resistance showed that the isolate was resistant to cadmium and lead. Biodegradation activity of our isolate can be envisaged from its ability to degrade nearly 98% of the azo dye and Reactive red 5B. Based on Hideo Nonomura's classification system, the isolate was considered to be a novel *Streptomyces* spp. Considering the biosurfactant production and heavy metal resistant activity of the strain, it could be used as potential strain for large scale production of the lead compound.

Key words: Nonomura's classification · Biosurfactant · Biodegradation · Azo dye degradation

INTRODUCTION

For the last few decades actinobacteria have been categorized as extremely fascinating organisms with unexhausted reserve of bioactive compounds that are being exploited for various commercial applications which includes environmental, biomedical and industrial applications. Among the diverse marine microbial communities, actinobacteria have occupied a prominent and significant position as potential producers of structurally complex and unique metabolites. Our search for a potential *Streptomyces* species led to the discovery of VITDDK3, a novel species with diverse properties. Over the years the marine environment has produced nearly 15,000 compounds which have been exploited for therapeutic purposes [1, 2]. Drugs are not the only target for which the marine system has been continuously explored but also other applications which include environmental, industrial and biotechnological implications [3]. Marine microorganisms in comparison with the terrestrial organisms have developed self protective mechanism in the course of their evolution and competition for survival. This self defense results in the production of unique and complex chemical entities which are being unraveled for multiple purposes [4-6]. Among the microbial communities, *Actinobacteria* are illustrious for their ability to produce numerous secondary metabolites and till date it remains matchless [7-9]. *Actinobacteria* are a collection of filamentous, Gram-positive prokaryotes with high G+C content in DNA of 69-78% [10-12]. *Actinobacteria,* especially *Streptomyces* spp. are often screened for production of secondary metabolite. The genus *Streptomyces* was reported initially in the year 1943 by Waksman and Henrici [13]. *Streptomyces* are most-striking aerobic, chemoorganotrophic organisms with oxidative metabolism capable of producing a powdery, granular or velvety colonies [14].

Chemical surfactants are surface active agents that reduce the surface tension. Most of the surfactants available are petroleum derivatives which are highly toxic and non-degradable [15]. In an effort to overcome these disadvantages microorganisms were investigated for the production of biosurfactant molecules with low toxicity and biodegradability [16, 17]. Microbial surface active agents or biosurfactant molecules are surface active

Corresponding Author: Dr. K. Kannabiran, Biomolecules and Genetics Division, School of Biosciences and Technology, VIT University, Vellore-632014, India

agents synthesized by microbial cells [17]. Of late these agents have received much attention and interest for industrial applications. The amphipathic nature of these agents helps in reducing the surface tension of media [18]. These agents not only helpful in the uptake and utilization of hydrocarbons by the organisms but also facilitate the biodegradation of toxic hydrocarbons [19]. The other advantages of microbial surfactants are eco-friendly nature, high foaming ability, efficiency at extreme temperatures, pH and salt concentrations [16].

Industrialization of coastal regions has drastically increased over the last three decades [20]. Heavy metals and dyes are being introduced into the nearby water bodies thereby contaminating the water bodies [21, 22]. Heavy metals such as lead, cadmium, copper, zinc, mercury, arsenic and chromium are released into the environment and water. Effluents from tanneries and fertilizer industries are released into near by streams and rivers [21]. Azo dyes are the predominant class of dyes that is extensively used in textile, food, paper, leather and cosmetics industries [23]. These sulphonated azo dyes are not only toxic in excessive quantities but also be carcinogenic [24]. Release of these dyes and metals are of major concern since they cause a serious health hazards to humans and animals [25]. The present study was aimed to screen and to evaluate the biosurfactant and heavy metal resistant activity of *Streptomyces* spp. VITDDK3.

MATERIALS AND METHODS

Sample Collection: The coastal region of Tamil Nadu, Ennore saltpan (Lat. 13°C.14' N, Long. 80°C.22' E) was chosen for sample collection. Soil samples were collected in sterile polythene covers, transported to the laboratory aseptically and refrigerated until further use.

Isolation of Actinobacteria: Soil samples were serially diluted (10^{-6}) and 1ml from each dilution was plated on Starch Casein agar by pour plate technique [26]. The plates were incubated at 30°C for 7-10 days. The colonies which showed morphological difference were selected, purified by streak plate technique and maintained for further studies.

Screening Methods for Biosurfactant Activity: Biosurfactant activity was determined for the pure culture of actinobacteria by three different methods namely *(1) Haemolysis (2) Drop collapsing test and (3) Lipase production.*

Hemolytic Activity: Haemolysis was carried out using blood agar plate and it was prepared by adding human blood (5%) to blood agar base. The purified cultures were inoculated and the blood agar plates were incubated at 28°C for 7 days. The plates were then examined for zone of clearance around the colonies [27].

Drop Collapsing Test: Biosurfactant production was screened using the qualitative drop-collapse test described by [28]. Mineral oil (2µl) was added to 96-well microtitre plates. The plate was equilibrated for 1 h at 37°C and 5 µl of the culture supernatant was added to the surface of the oil in the well. The shape of drop on the oil surface was observed after 1 min. The culture supernatant makes the drop collapsed was indicated as positive result and if the drops remains intact indicates negative result. Distilled water was used as negative control.

Lipase Production: Lipase production by the actinobacteria pure culture was determined using Tributyrin agar plates. To Actinomycetes isolation agar (AIA), tributyrin (1%) was added and the pH of the medium was adjusted to 7.3–7.4 using 0.1N NaOH. The cultures were inoculated on to the tributyrin agar plates and incubated at 28°C for 7 days. The plates were later examined for clear zone around the colonies [17].

Screening for Heavy Metal Resistance

Heavy Metals: Screening for heavy metal resistance was carried out using standard heavy metal solutions (Mercury, Lead, Cadmium, Zinc and Copper) as well as heavy metal salt solutions (Cadmium sulphate, Nickel sulphate, Copper sulphate, Zinc sulphate, Mercuric chloride, Lead acetate, Potassium dichromate, Cobalt nitrate and Sodium arsenate). The concentration of the standard heavy metal solutions was 1000ppm. The concentration of heavy metal salt solutions was ranged from 10mM, 50mM, 100mM, 500mM, 1000mM, 5M and 10M. The salt solutions were prepared with phosphate buffer saline, PBS (pH 6.8). The standard and salt solutions were sterilized separately for 15mins at 110°C.

Tube Method: The culture medium (ISP1 broth) was dispensed in test tubes and sterilized for 15 min at 121°C. To each of the labeled tubes, 500µl of the appropriate metal standard or salt solutions was added and incubated at 28°C for 7 days [29]. The tubes were qualitatively measured for turbidity. The tubes with growth were considered as resistant and without growth as sensitive to the particular metal and salt solutions.

Agar Diffusion Method: Lawn culture of the isolates, grown for 7 days in ISP 1 (International Streptomycetes Project) broth was prepared on Starch Casein agar (SCA). Using a sterile well borer wells were made on the surface of SCA seeded with the *Streptomyces* isolates. To each well 500µl of the standard metal solution or the salt solutions were added and incubated at 28°C for 7 days. The area of inhibition (mm) was measured as the distance from the edge of growing colonies to the edge of the well [30].

Screening for Dye Degradation: A loopfull of pure culture was inoculated in 100ml of ISP1 broth sterilized for 15mins at 121°C. The flasks were incubated at 28°C for 7 days and after adequate growth of the organism, filter sterilized Reactive Red 5B (RR5B) azo dye (50mg/Lt) was added and incubated for 24hrs in static condition. The culture supernatant was obtained by spinning down the culture at 10,000 rpm for 20 minutes. The degradation activity of the organism was determined by measuring the absorbance of the culture supernatant (2ml) at OD_{542}nm using UV spectrophotometer [31]. The percentage of decolourization by the organism was calculated using the formula:

$$\% \text{ of decolourization} = \left\{ \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \right\} \times 100$$

Identification of the Isolate VITDDK3: The potential isolate VITDDK3 was inoculated on Oat meal agar (ISP3) and the aerial spore mass colour and reverse side and soluble pigment production other than melanin was studied. Melanin pigment production was studied on Peptone yeast extract iron agar (ISP6). Spore chain morphology and orientation was studied using scanning electron microscope (S3400, 20 KV, 5.00 µm) for which the isolate was grown on SCA for 7days. Assimilation of various sugars such as arabinose, xylose, inositol, mannitol, fructose, rhamnose, sucrose and raffinose as sole carbon source was determined by inoculating the isolate in modified Bennets' broth supplemented with the respective carbon source. After inoculation the tubes were incubated for 7 days at 30°C [32].

RESULTS

In the current study *Streptomyces* spp. VITDDK3 was evaluated for biosurfactant production and also screened for heavy metal resistance and biodegradation of dye. On serial dilution and plating on SCA about 100 colonies of *Streptomyces* strains were obtained. Morphologically distinct colonies were selected for further analysis. The selected strains were screened for production of surface active molecules. Inoculation of the isolate on blood agar plate produced a clear zone around the colonies, indicates the biosurfactant activity by the surface active molecules produced by *Streptomyces* spp. VITDDK3 (Fig. 1a). In the drop collapsing test a flat drop was observed around the colonies of the isolate, which indicates a biosurfactant activity. Inoculation of the isolate on Tributyrin agar plate produced a clear zone which indicates production of the enzyme, lipase by *Streptomyces* spp. VITDDK3. These results confirmed that *Streptomyces* spp. VITDDK3 is a potential producer of surface active molecules (Fig. 1b).

(A) (B)

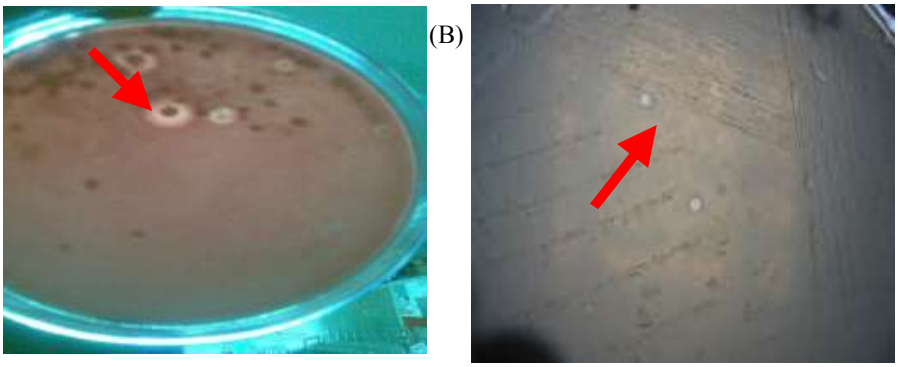

Fig. 1: Biosurfactant activity exhibited by *Streptomyces* spp.VITDDK3 A)B) Assay of biosurfactant activity of *Streptomyces* spp.VITDDK3. (A) A clear zone around the colonies grown on blood agar plate indicates haemolysis of the blood cells. (B) A clear zone around the colonies on tributyrin agar indicates the production of the enzyme lipase by the organism *Streptomyces* spp.VITDDK3.

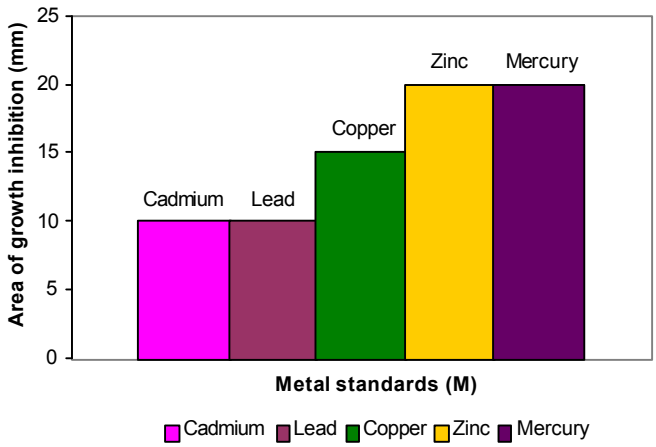

Fig. 2: Heavy metal resistance pattern exhibited by *Streptomyces* spp.VITDDK3 against metal standards by agar diffusion method Heavy metals resistance exhibited by *Streptomyces* spp.VITDDK3 against metal standards tested by agar diffusion method. An inhibition zone of 10mm is arbitrarily set as the tolerance limit. Hence the organism *Streptomyces* spp.VITDDK3 is resistant to the metals cadmium and lead

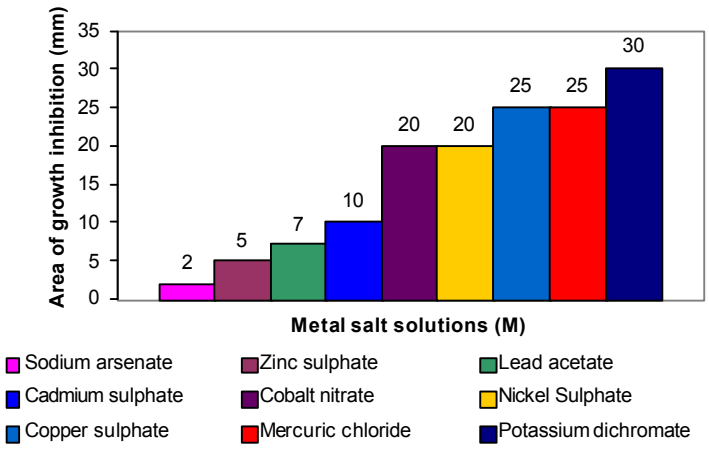

Fig. 3: Heavy metal resistance pattern exhibited by *Streptomyces* spp.VITDDK3 against metal salt solutions by agar diffusion method Heavy metals resistance exhibited by *Streptomyces* spp.VITDDK3 against metal salt solutions tested by agar diffusion method. An inhibition zone of 10mm is the arbitrary tolerance limit. Hence the organism *Streptomyces* spp.VITDDK3 is resistant to the metal salt solutions sodium arsenate, zinc sulphate, lead acetate and cadmium sulphate.

The isolate was also tested for its ability to remove heavy metals and their respective salt solutions. In the tube method the strain produced visible turbidity for sodium arsenate, zinc sulphate, lead acetate, cadmium sulphate, cadmium and lead (Fig. 2) whereas no visible turbidity was seen with other metals. It also showed resistance to the metal salt solutions (Fig. 3). Based on the results of the tube method, agar diffusion method was performed for semi quantitative determination of heavy metal resistance. An inhibition zone of 10mm on the agar surface was considered as resistant to the particular metal standard and metal salt solution. The strain produced an inhibition zone of 2-7mm for sodium arsenate, zinc sulphate and lead acetate. While a zone of 10mm was produced for cadmium, lead and cadmium sulphate and a clearance zone of 15-20mm was recorded for copper, zinc mercury, cobalt nitrate and nickel sulphate. An inhibition zone of 25-30mm was observed for copper sulphate, mercuric chloride and potassium dichromate. Hence the isolate was found to be resistant to sodium arsenate, zinc sulphate, lead acetate, cadmium sulphate, cadmium and lead.

Table 1: Hideo and Nonomura's key for classification of *Streptomyces* spp.VITDDK3

S.No	Characteristics	*Streptomyces* spp. VITDDK3
1.	Aerial spore mass colour	White
2.	Pigmentation	
	a. Reverse side pigment	-
	b. Soluble pigment	-
	c.Melanin pigment	Dark brown pigment
3.	Spore surface	Smooth
4.	Spore chain orientation	Retinaculiaperti
5.	Sugars	
	a. Arabinose	+
	b. Xylose	+
	c. Inositol	+
	d. Mannitol	+
	e. Fructose	+
	f. Rhamnose	+
	g. Sucrose	+
	h. Raffinose	+

The strain was also evaluated for its ability to degrade the sulphonated azo dye RR5B. The absorbance was measured for control (medium+ dye) as well as the test. The organism was inoculated, incubated and grown adequately in the presence of dye and the absorbance was measured (medium+ organism+ dye). The isolate produced nearly 98% decolourization of the dye in static condition whereas when incubated on rotary shaker at 150rpm for the same time period no decolourization was observed.

The isolate produced large, irregular, raised, white, powdery colonies with pale yellow substrate mycelium on ISP3 agar surface. Dark brown melanin pigment was produced on ISP6 agar medium and no reverse side and soluble pigments by the isolate. It produced unfragmented, branched, looped hyphae with 2 curves (Rectiflexibles) and bearing non-motile spores with smooth surface. The isolate was capable of assimilating all 8 sugars tested. Based on Hideo Nonomura's classification key which takes into account the aerial mass colour, reverse side pigmentation and soluble pigment production on ISP3; melanin pigment production on ISP6; spore chain orientation and surface and assimilation of sugars the isolate was assigned to the genus *Streptomyces* (Table 1). Though it was assigned to the genus *Streptomyces*, the strain did not match with any of the species and therefore it may be a novel species.

DISCUSSION

Marine ecosystem and the micro fauna occupying this niche are an unexhausted reserve of hundreds of thousands of novel chemical entities. Recent advances in marine biotechnology brought many new chemical compounds from actinobacteria for various potential applications. Discovery of *Streptomycin* by Waksman and Henrici [13] from *Streptomyces griseus* has opened new avenues for exploring actinobacteria for novel antibiotics. Marine actinomycetes are good candidates for biosurfactant production, bioremediation and biodegradation [17]. Under stress condition the organisms are bound to elaborate several different chemical entities for their survival [5] and those secondary metabolites are used for several potential applications.

Out of 9 maritime states in India only four states namely Maharashtra, Tamil Nadu, Kerala and Andhra Pradesh have been studied extensively for actinobacterial diversity whereas other states, Gujarat, Goa, Karnataka, Orissa, West Bengal and Andaman and Nicobar islands are not extensively studied [33]. *Streptomyces* species was the most frequently documented genus among others in marine sediment soils [34]. A biosurfactant producing *Streptomyces* spp. was isolated from soil samples collected at the Ennore saltpan, Tamil Nadu, India and the Ennore coast of Bay of Bengal is situated 24 Kms north of Chennai, India [10]. Serial dilution of the soil sample yielded nearly 100 isolates and isolates with morphological differences were chosen for studying the biodegradation, bioremediation and biosurfactant activity. Out of 100, three isolates (VITDDK1, VITDDK3 and VITDDK3) exhibited moderate to good activity. *Streptomyces* spp. VITDDK3 exhibited significant biosurfactant activity when compared to other two strains. In our earlier investigation, the isolate *Streptomyces* VITDDK3 proved to be a halo tolerant organism with the ability to grow in the presence of 26% sodium chloride concentration [10].

The biosurfactant producing ability of the strain *Streptomyces* spp. VITDDK3 was tested by different screening methods. Both Gram positive and Gram negative bacteria capable of producing surface active agents and they are amphipathic extracellular lipopeptides [35]. Production of lipopeptide biosurfactant by a sponge associated marine actinomycetes *Nocardiopsis alba* MSA 10 was reported recently (17). A novel, lipopeptide biosurfactant-producing bacterium, *Rhodococcus* sp. TW53 was reported from Pacific Ocean deep-sea sediments [36]. Biosurfactant activity of free fatty acids and glycolipids extracted from *Rhodococcus erythropolis* (3C-9 strain) isolated from sea side soil was reported by Peng *et al.* [37].

The strain VITDDK3 was more resistant to sodium arsenate and least resistant to potassium dichromate among all the metal standards and salt solutions used in this study. Similar studies on actinobacteria and other bacteria have been reported by many workers [22]. Abbas and Edward [38] have reported that *Streptomyces coelicolor* tolerated cooper (0.047mM). Several bacteria associated with a marine sponge *Fasciospongia cavernosa*, *Streptomyces* sp. (MSI01), *Salinobacter* sp. (MSI06), *Roseobacter* sp. (MSI09), *Pseudomonas* sp. (MSI016), *Vibrio* sp. (MSI23), *Micromonospora* sp. (MSI28), *Saccharomonospora* sp. (MSI36) and *Alteromonas* sp. (MSI42) showed resistance against heavy metals, copper, lead, cobalt, nickel, mercury and cadmium [21].

The strain showed a maximum of 98% degradation of dye and no significant absorbance was recorded with the pellet solvent extract. Also the pellet remained colorless which is yet another indication of degradation mechanism. Release of dyes in general into the nearby water bodies pollutes the land and water-micro and macro flora and fauna but also directly and indirectly affects human beings and the dependent animals [23]. Degradation of sulphonated azo dyes by actinobacteria has been reported by Maria *et al*. [39]. Zhou and Zimmermann [40] have reported the degradation of azo dyes by actinobacteria and also proposed the degradation mechanism for the removal of the dye.

The stain was designated as *Streptomyces* spp. VITDDK3 as per Hideo Nonomura's classification. Since it did not match with any of the species described in the key for classification and identification of 458 species of the *Streptomycetes* included in ISP, may be a novel species. Several reports are available on the use of Hideo Nonomura key for the identification and classification of the actinobacteria isolated from mangrove environment and marine sediments respectively [41, 42]. To the best of our knowledge not much research has been carried out on marine actinobacteria of Indian peninsula with regard to its biosurfactant activity, heavy metal resistance, bioavailability, dye and oil degradation. Extraction and identification of lead compound having biosurfactant, heavy metal resistance and dye degradation activity will have wider applications in industrial process and bioremediation.

ACKNOWLEDGMENT

Authors thank the management of VIT University for providing the facilities to carry out this study. The authors are thankful to the International Foundation for Science (IFS, F /4185-1), Sweden and the Organization for the Prohibition of Chemical Weapons (OPCW), The Hague for providing financial support.

REFERENCES

1. Battershill, C., M. Jaspars and P. Long, 2005. Marine Biodiscovery: New Drugs from the Ocean Depths. Biologist, 52: 107-114.
2. Simmons, T.L., E. Andrianasolo, K. McPhail, P. Flatt and W.H. Gerwick, 2005. Marine Natural Products as Anti-Cancer Drugs. Molecular Cancer Therapeutics, 4: 333-342.
3. Bull, A.T. and J.E.M. Stach, 2007. Marine actinobacteria: new opportunities for natural product search and discovery. Trends in Microbiol., 15: 491-499.
4. Deepika, T.L. and K. Kannabiran, 2009. A morphological, biochemical and biological studies of halophilic *Streptomyces sp* isolated from saltpan environment. American Journal of Infectious Diseases, 5: 207-213.
5. Chiaki, I., K. Naoko, K. Masazumi, K. Takeshi and H.S. Naoko, 2007. Isolation and characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater. Actinomycetologica, 21: 27-31.
6. Sigrid, H., F. Espen, D.J. Kjell, I. Elena, E.E. Trond and B.Z. Sergey, 2008. Characterization of *Streptomyces* spp. Isolated from the Sea Surface Microlayer in the Trondheim Fjord, Norway. Marine Drugs, 6: 620-635.
7. Nathan, A.M., M.K. Jessica, B.M. Valerie and H.S. David, 2004. Isolation and Characterization of Novel Marine-Derived Actinomycete Taxa Rich in Bioactive Metabolites. Applied and Environmental Microbiol., pp: 7520-7529.
8. Jensen, P.R., E. Gontang, C. Mafnas, T.J. Mincer and W. Fenical, 2005. Culturable marine actinomycete diversity from tropical Pacific oceans sediment. Environmental Microbiol., 7: 1039-1048.
9. Suthindhiran, K. and K. Kannabiran, 2009. Cytotoxic and Antimicrobial Potential of Actinomycete Species *Saccharopolyspora salina* VITSDK4 isolated from the Bay of Bengal Coast of India. American Journal of Infectious Diseases, 5: 90-98.
10. Deepika, T.L. and K. Kannabiran, 2009. A report on antidermatophytic activity of actinomycetes isolated from Ennore coast of Chennai, Tamil Nadu, India. International Journal of Integrative Biology, 6: 132-136.

11. Deepika, T.L., K. Kannabiran and D. Dhanasekaran, 2009. Diversity of antidermatophytic S*treptomyces* in the coastal region of Chennai, Tamil Nadu, India. Journal of Pharmacy Research, 2: 22-26.
12. Stackebrandt, E., F.A. Rainey and N.L. Ward, 1997. Proposal for a new hierarchic classification system, *Actinobacteria classis* nov. International Journal of Systematic Bacteriology, 47: 479-491.
13. Waksman, S.A. and A.T. Henrici, 1943. The nomenclature and classification of the actinomycetes. Journal Bacteriology, 46: 337- 41.
14. Stanley, T.W., M. Elizabeth, J. Sharpe and G. Holt, 2001. Bergeys's manual of systematic bacteriology, 2nd edition. Williams and Wilkins. ISBN: 978-0-387-98771-2.
15. Seghal, G.K., T.A. Hema, R. Gandhimathi, S. Joseph, T.T. Anto, R.T. Rajeetha and K. Natarajaseenivasana, 2009. Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3. Colloids and Surfaces B: Biointerfaces, 73: 250-256.
16. Thavasi, R., V.R.M.N. Subramanyam, S. Jayalakshmi, T. Balasubramanian and M.B. Ibrahim, 2008. Biosurfactant Production by *Azotobacter chroococcum* isolated from the Marine Environment. Marine Biotechnology, DOI 10.1007/s10126-008-9162-1.
17. Gandhimathi, R., K. Seghal, T.A. Hema, S. Joseph, R.T. Rajeetha and P.S. Shanmughapriya, 2009. Production and characterization of lipopeptide biosurfactant by a sponge- associated marine actinomycetes *Nocardiopsis alba* MSA10. Bioprocess Biosystems Engineering, DOI 10.1007/s00449-009-0309-x.
18. Desai, J.D. and I.M. Banat, 1997. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews, 61: 47- 64.
19. Rahaman, K.S.M., I.M. Banat, J. Thahira, T. Thayumanavan and P. Lakshmanaperumalsamy, 2002a. Bioremediation of gasoline contaminated soil by bacterial consortium with poultry litter, coir pith and rhamnolipid biosurfactant. Bioresource Technology, 81: 25-32.
20. Rao, J.V., P. Kavitha, R.N. Chakra and T.G. Rao, 2006. *Petrosia testudinaria* as a biomarker for metal contamination at Gulf of Mannar, southeast coast of India. Chemosphere, 65: 634-8.
21. Joseph, S., P.S. Shanmugha, K.G. Seghal, T. Thangavelu and B.N. Sapna, 2009. Sponge-associated marine bacteria as indicators of heavy metal pollution. Microbiological Research, 164: 352-363.
22. Maria, J.A., R. Guillermo Castro, J.C. Federico, C.R. Nora, T.H. Russell and O. Guillermo, 1998. Screening of heavy metal-tolerant actinomycetes isolated from the Salí River. Journal of General and Applied Microbiol., 44: 129-132.
23. Chang, J., C. Chon, Y. Lin, P. Lin, J. Ho and T.L. Ho, 2001. Kinetic characteristics of bacterial azo dye decolorization by *Pseudomonas luteola*. Water Research, 35: 2841-2850.
24. Myslak, Z.W. and H.M. Bolt, 1998. Occupational exposure to azo dyes and risk of bladder cancer. Zbl. Arbeitsmed, 38: 310-321.
25. Volesky, B. and Z.R. Holan, 1995. Biosorption of heavy metals. Biotechnology Progress, 11: 235-250.
26. Collins, C.H., P.M. Lyne and J.M. Granje, 1995. In: Microbiological methods, Butterworth and Heinemann Publishers, London, pp: 129-31.
27. Carillo, P., C. Mardarz and S. Pitta-Alvarez, 1996. Isolation and selection of biosurfactant producing bacteria. World Journal of Microbiology and Biotechnol., 12: 82- 84.
28. Youssef, N.H., K.E. Dunacn, D.P. Nagle, K.N. Savage, R.M. Knapp and M.J. McInerney, 2004. Comparison of methods to detect biosurfactant production by diverse microorganism. Journal of Microbiological Methods, 56: 339-347.
29. Konopka, A. and T. Zakharova, 1999. Quantification of bacterial lead resistance via activity assays. Journal of Microbiological Methods, 37: 17-22.
30. Hassen, A., N. Saidi, M. Cherif and A. Boudabous, 1998. Resistance of environmental bacteria to heavy metals. Bioresource Technology, 64: 7-15.
31. Kalme, S.D., G.K. Parshetti, S.U. Jadhav and S.P. Govindwar, 2007. Biodegradation of benzidine based dye Direct Blue-6 by Pseudomonas desmolyticum NCIM 2112. Bioresource Technology, 98: 1405-1410.
32. Nonomura, H., 1974. Key for classification and identification of 458 species of the Streptomycetes included in ISP. Journal of Fermentation Technology, 52: 78-92.
33. Sivakumar, K., M.K. Sahu, T. Thangaradjou and L. Kannan, 2007. Research on marine Actinobacteria in India. Indian Journal of Microbiol., 47: 186-96.

34. Suthindhiran, K. and K. Kannabiran, 2009. Hemolytic activity of *Streptomyces VITSDK1* spp. isolated from marine sediments in Southern India. Journal de Mycologie Médicale, 19: 77-86.
35. Molitt, M.C. and B.A. Neilan, 2000. The expansion of mechanistic and organismic diversity associated with non ribosomal peptides. FEMS Microbiology Letters, 191: 159-167.
36. Peng, F., Y. Wang, F. Sun, Z. Liu, Q. Lai and Z. Shao, 2008. A novel lipopeptide produced by a Pacific Ocean deep-sea bacterium, *Rhodococcus* sp. TW53. Journal of Applied Microbiol., 105: 698-705.
37. Peng, F., Z. Liu, L. Wang and Z. Shao, 2007. An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. Journal of Applied Microbiol., 102: 1603-1611.
38. Abbas, A. and C. Edward, 1989. Effects of metals on a range of *Streptomyces* species. Applied Environmental Microbiol., 55: 2030-2035.
39. Maria, P.G., B. Neal, G. Stefan and L.C. Don, 1996. Transformation of azo dye isomers by *Streptomyces Chromofuscus* A11. Applied and Environmental Microbiol., pp: 1814-1817.
40. Zhou, W. and W. Zimmermann, 1993. Decolorization of industrial effluents containing reactive dyes by actinomycetes. FEMS Microbiol. Letters, 107: 157-162.
41. Sivakumar, K., K.S. Maloy and K. Kathiresan, 2005. Isolation and Characterization of Streptomycetes producing antibiotic from a mangrove environment. Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 7: 457-464.
42. Balagurunathan, R. and A. Subramanian, 2001. Antagonistic Streptomycetes from marine sediments. Advances in Biosciences, 20: 71-76.