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Molecular Characterization of Antagonistic *Streptomyces* Isolated from a Mangrove Swamp

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ABSTRACT

The main objective of the present study was to isolate and identify the secondary metabolite producing Actinomycetes and to analyze the phylogenetic relationship. The soil samples were collected from the rhizosphre soil of Manakkudy mangroves, West Coast of India and screened for its antimicrobial potential. The novel bioactive compound producing strains were cloned in pGEM-T vector and sequenced. The phylogenetic relationship among the 20 *Streptomyces* sp. were studied. Phylogeny analysis showed that all the 20 isolates were having strong similarity (98%) with *Streptomyces*. Eight different species of *Streptomyces* such as *Streptomyces olivoverticillatus, Streptomyces caelestis, Streptomyces roseoviridis, Streptomyces venezuelae, Streptomyces showdoensis, Streptomyces bikiniensis, Streptomyces griseoruber, Streptomyces roseus* and 8 new strains (*Streptomyces* JS-1, *Streptomyces* JS-8, *Streptomyces* JS-10, *Streptomyces* JS-12, *Streptomyces* JS-14, *Streptomyces* JS-17, *Streptomyces* JS-19 and *Streptomyces* JS-20) were identified among the 20 isolates. The *Streptomyces* isolates JS-1, JS-8, JS-10, JS-12, JS-14, JS-17, JS-19 and JS-20 were considered as the new lineages of *Streptomyces*. Biochemical characterization was carried out and showed highly active by having different extracellular enzyme production to metabolize the nutrients in the environment. The GC contents were calculated for all the 20 *Streptomyces* isolates and it ranges between 58 and 59.6% in all the 20 sequences. All the isolates (JS-1 to JS-20) were having strong antagonistic activity against various bacterial and fungal pathogens, but the activity was differing in all the 20 *Streptomyces*. Phylogenetic analysis revealed that the isolates are the divergent of *Streptomyces* which are associated in the rhizosphere soil of Mangrove. A study of this kind will provide more details about the bioactive potential of *Streptomyces* from the estuarine ecosystem.

Key words: Actinomycetes, bioactive compounds, estuarine ecosystem, phylogeny, GC content

INTRODUCTION

Mangroves, unique woody plant communities of intertidal coasts in tropical and subtropical coastal regions, are highly productive ecosystems (Costanza *et al*., 1997; Wang *et al*., 2003) though surprisingly little is known about the microbial communities living therein (Hong and Yan, 2008; Hyde and Lee, 1995; Yan *et al*., 2006) although there is evidence that mangrove sediments contain high populations of micromonosporae (Eccleston *et al*., 2008) and novel actinomycetes, as illustrated

by the isolation of *Asanoa iriomotensis* (Han *et al*., 2007) and *Nonomuraea maheshkhaliensis* (Takizawa *et al*., 1993). It is also encouraging that bioactive compounds have been obtained from mangrove plants (Takizawa *et al*., 1993; Huo *et al*., 2008; Wu *et al*., 2004), fungi (Gao *et al*., 2007; Lazzarini *et al*., 2000; Lin *et al*., 2001, 2002, 2008) and bacteria (Tang *et al*., 2007) including actinomycetes (Bull *et al*., 2005; Xie *et al*., 2006). *Streptomyces* have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. Around two-thirds of naturally occurring antibiotics have been isolated from actinomycetes (Okami and Hotta, 1988) and they are responsible for the production of about half of the discovered bioactive secondary metabolites (Berdy, 2005), notably antibiotics (Strohl, 1997), antitumor agents and enzymes (Oldfield *et al*., 1998). Excellent track record of actinomycetes in this regard, a significant amount of effort has been focused on the successful isolation of novel actinomycetes from terrestrial sources for drug screening programs in the past fifty years. Rate of discovery of new compounds from terrestrial actinomycetes has decreased, whereas the rate of re-isolation of known compounds has increased (Fenical *et al*., 1999). Thus, it is crucial that new groups of actinomycetes from unexplored or underexploited habitats be pursued as sources of novel bioactive secondary metabolites. The diversity of life in the terrestrial environment is extraordinary; the greatest biodiversity is in the oceans (Donia and Hamann, 2003). As marine environmental conditions are extremely different from terrestrial ones, it is surmise that marine actinomycetes have different characteristics from those of terrestrial counterparts and, therefore, might produce different types of bioactive compounds. The genetic and metabolic diversity of marine actinomycetes, which remains largely unknown. Indeed, the marine environment is a virtually untapped source of novel actinomycete diversity (Bull *et al*., 2005) and, therefore, of new metabolites (Fiedler *et al*., 2005; Jensen *et al*., 2005). However, the distribution of actinomycetes in the sea and marine ecosystem is largely unexplored and the presence of indigenous marine actinomycetes in the oceans remains elusive. Furthermore, skepticism regarding the existence of indigenous populations of marine actinomycetes arises from the fact that the terrestrial bacteria produce resistant spores that are known to be transported from land into sea, where they can remain available but dormant for many years (Bull *et al*., 2000). Genomic studies indicate that the genetic potential for producing secondary metabolites is not uniformly distributed within the bacterial world. The *Streptomyces* in the mangrove ecosystem were remains untapped. In this study the diversity of antibiotic producing *Streptomyces* were identified from the Manakkudy Mangrove ecosystem of Palayaru river estuary, Arabian Sea, India.

MATERIALS AND METHODS

Sample collection and isolation of actinomycetes: The samples were collected during January, 2007 from the Rhizosphere soil of *Rhizophora mucronata*, Manakkudy estuary of Arabian Sea, Tamil Nadu, India (8° 6' 12" N77° 28' 57" E) at 5 feet depth. Soil samples were serially diluted in sterile water and spread plated over the medium containing soluble starch 20 g, KNO_3 1 g, NaCl 0.5 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, $FeSO_4$ 20 μM, agar 15 g, seawater from mangrove habitat 1 L and 15 μg nalidixic acid were added to inhibit the growth of other bacteria and incubated at 28°C for 3 days. Biochemical characterization was carried out based on Cappuccino and Sherman (2002) and shown in Table 1.

Antimicrobial assay: The isolated actinomycetes were further grown on the medium with glucose 20 g, tryptone 5 g, yeast extract 5 g, KNO_3 1 g, NaCl 0.5 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, distilled

Table 1: Biochemical characterization of *Streptomyces* isolates JS-1 to JS-20

	Biochemical characterization																			
	JS-1	JS-2	JS-3	JS-4	JS-5	JS-6	JS-7	JS-8	JS-9	JS-10	JS-11	JS-12	JS-13	JS-14	JS-15	JS-16	JS-17	JS-18	JS-19	JS-20
Metabolite exudation	+	-	-	+	+	+	-	-	+	+	-	-	+	+	-	-	-	+	+	+
Gelatin hydrolysis test	-	+	+	-	+	-	-	-	+	+	+	-	-	+	+	+	-	++	++	-
Starch utilization test	+	+	+	-	+	+	++	+	+	+	-	++	+	+	+	-	++	-	-	+
Lipase test	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	+	-		++	+
Casein hydrolysis test	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	+		+	-	-
Simmons citrate utilization test	+	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	+	-
Triple sugar iron agar test	-	+	+	-	+	-	+	+	++	+	+	+	+	++	+	+	+	+		+
MR-VP test (methyl red test)	+	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	-	+		+
Voges proskaeur test	+	+	++	+	+	+	++	+	+	+	-	++	+	+	+	-	++	-	-	-
Hydrogen sulfide test	-	-	+	+	+	-	-	+	+	-	+	-	+	+	-	+	-	-	+	-
Melanin pigment production	+	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	+	-	-	-
Dextrose	+	-	-	-	+	+	+	-	-	-	+	+	-	-	+	-	-	-	++	++
Lactose	+	+	++	+	+	+	+	+	++	+	+	+	+	++	+	+	+	+	++	-
Sucrose	-	-	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	+	+	-

Table 2: Antimicrobial activity of *Streptomyces* isolates

	Zone of inhibition (mm)																			
Bacterial isolates pathogenic microorganism	JS-1	JS-2	JS-3	JS-4	JS-5	JS-6	JS-7	JS-8	JS-9	JS-10	JS-11	JS-12	JS-13	JS-14	JS-15	JS-16	JS-17	JS-18	JS-19	JS-20
S. aureus	22	19	28	19	-	-	26	12	16		26	14	23	36	-	-	32	34	-	22
Candida albicans	25	-	32		-	-	22	28	-	28	-		12	33	23	-	35	23	-	19
K. pneumoniae MTCC109	18	-	-	-	11	27	-	30	-		21	38	18	13	-	-	33	-	-	21
Enterobacter sp.	11	21	-	-	-	12	14	29	16		22	21	17	12	-	35	31	-	-	21
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. typhi MTCC 733	19	25	-	-	-	17	-	29	-		19	34	14	11	-	-	36	21	29	32
Bacillus subtilis	17	30	-	19	21	12	23	33	18		23	29	13	33	-	-	32	-	-	33
Aspergillus flavour	26	-	29	23	-	-	-	31	21	29	-	-	11	31	27	-	31	-	-	16
Aspergillus niger	24	-	27	24	-	-	-	29	27	30	-	-	18	31	24	-	32	-	-	11
P. auruginosa	29	29	-	26	-	11	16	19	-	-	13	29	21	12	-	25	35	17	26	9

water 1 L and assayed for their antagonistic effect against selected microorganisms. Antagonistic activity of the isolated strains JS-1 to JS-20 was performed by double-layer agar method (Gauthier *et al*., 1975). *Staphylococcus aureus, Enterobacter* sp., *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhi* (MTCC 733), *Bacillus subtilis* and *Klebsiella pneumoniae* (MTCC 109), *Proteus vulgaris* and *Candida albicans* were the microbes used for assay. The zone of inhibition is shown in Table 2.

Amplification and cloning of 16S rRNA gene: The genomic DNA was isolated by Phenol-Chloroform method. The amplification of 16 S rRNA gene was done by using universal forward (5' CAGGCCTAACACATGCAAGTC 3') and reverse (5' GGGCGGWGTGTACAAGGC 3') (Sigma, India). The following composition was made for the amplificatio n-PCR reaction buffer- 5 µL, $MgCl_2$ 2 µL, dNTPs 1.2 µL, Template DNA 2 µL, Reverse primer 4 µL, Forward primer

4 μL, Taq polymerase 5 μL, Water 26.8 μL were added for the reaction. The conditions were initial denaturation at 95°C for 3 min, denaturation at 95°C for 1 min, annealing at 56°C for 1 min, elongation at 72°C for 1.5 min and the final elongation at 72°C for 10 min for 30 cycles. The PCR amplified products were cloned into pGEM-T vector and then sequenced.

Sequencing and Phylogenetic analysis: DNA samples were sequenced by the sequencing instrument Macrogen 3730XL7-16112-010 and the sequences were processed by ABI 1.6.0. The sequence were merged in EMBOSS Merger. The merged sequences were blasted with the NCBI database and analyzed for homology and phylogenetic analysis using CLUSTAL W and Genebee online software (PHYLIP).

RESULTS AND DISCUSSION

Antimicrobial activity: The agar overlay method showed antimicrobial activity against different bacterial and fungal pathogens. The results were tabulated in Table 2. Same species of the *Streptomyces* which were collected from the same site showed different antimicrobial activity and they were physiologically different. The organisms were sequenced after their variations in the Biochemical test. The results of Biochemical tests were tabulated in Table 1.

Phylogenetic analysis and *Streptomyces* sp. diversity: The diversity of the colony morphology was observed and is shown in Fig. 1. Twenty different *Streptomyces* sp. were isolated from the Manakkudy Mangrove sediment. Among the 20 *Streptomyces* sp. JS-20, showed similarity to 7. *Streptomyces venezuluea* JS-11. *Streptomyces* JS-12 and *Streptomyces roseus* JS-18 and *Streptomyces roseaviridis* JS-9. This strain showed more antimicrobial properties, but they are biochemically different and antagonistic activity is different among the 99% similarity to the organisms. *Streptomyces Olivoverticillatus* strain JS-2 showed 98% of the sililarity to *Streptomyces caelestis* strain JS-6 and 97% homology to *Streptomyces roseorubens*, *Streptomyces ghanaensis* strain OSS 47 *Streptomyces griseoruber* strain JS-16 and *Streptomyces caelestis* strain JS-4. *Streptomyces* sp. JS-17 showed 96% similarity to *Streptomyces albogriseolus*. *Streptomyces* sp. JS-1 showed distant relation to all the other *Streptomyces* sp. isolated from the sediments. *Streptomyces* sp. JS-13 showed 97% similarity to *Streptomyces viridobrunneus*. *Streptomyces olivoverticillatus* strain JS-3 showed 97 and 98% homology to the strains. *Streptomyces caelestis* strain JS-6

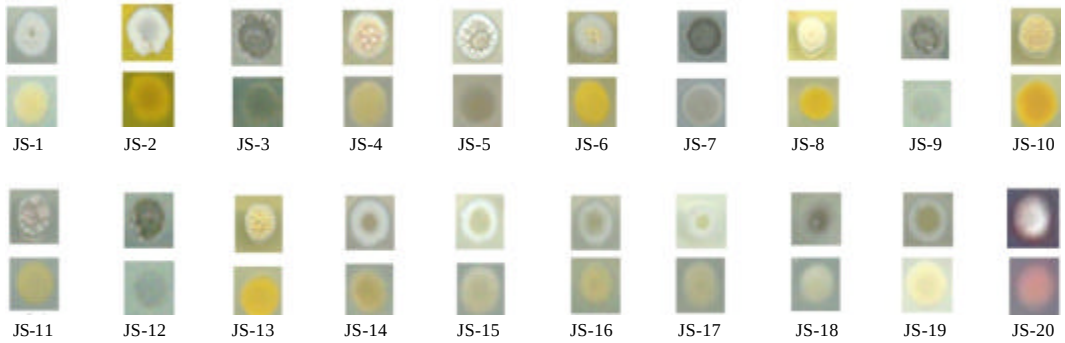

Fig. 1: Colony morphology of 20 *Streptomyces* JS-1 to JS-20 isolated from Mangrove swamp

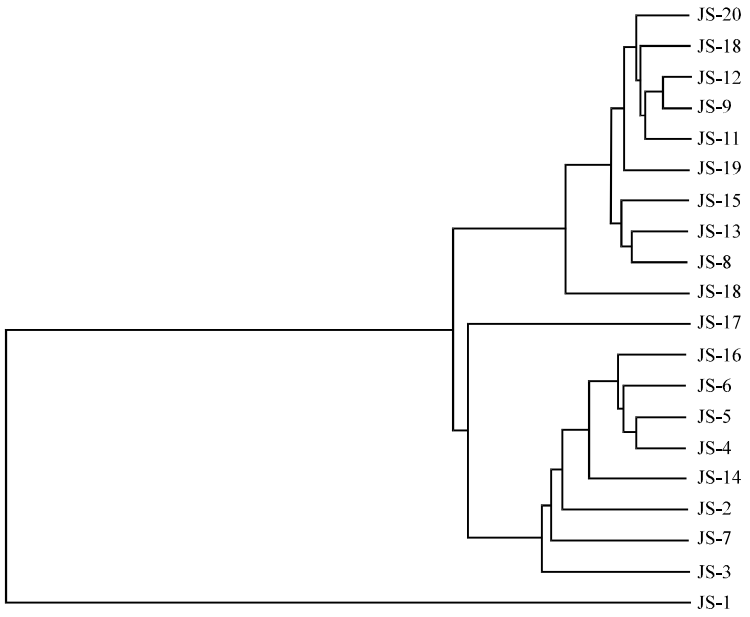

Fig. 2: Neighbourhood joining of Streptomyces strains JS-1 to JS-20 isolated from the rhizosphere soil of *Rhizophora mucronata*

Streptomyces griseoruber strain JS-16 *Streptomyces caelestis* strain JS-4 and *Streptomyces caelestis* strain JS-5. JS-7 JS-5, JS-6, JS-16 JS-4 These four *Streptomyces* showed different. Biochemical properties but they are evolutionary oriented from *Streptomyces venezuluea* JS-10 in the phylogenetic tree analysis. *Streptomyces olivoverticillatus* strain JS-2 showed 98% of the similarity to *Streptomyces caelestis* strain JS-6 and 97% homology to *Streptomyces roseorubens*, *Streptomyces ghanaensis* strain OSS 47 *Streptomyces griseoruber* strain JS-16 and *Streptomyces caelestis* strain JS-4. The neighborhood analysis of the 20 *Streptomyces* sp. were shown in Fig. 2. Antibacterial and antifungal activities were different in all the 20 *Streptomyces* sp. isolated from the soil rhizosphere of *Rhizophora mucronata* sediments.

Nucleotide sequence accession numbers: The 16S rRNA gene sequences of representative isolates were deposited in the NCBI databank under the accession EU124770 to EU124788 and EF536325 shown in Table 3.

Analysis of length and GC content of *Streptomyces*: We analysed the number of nucleotide base pairs in the 16S rRNA gene sequencing of all the 20 *Streptomyces* and the GC content was calculated. The GC content was varied in all the eight species of *Streptomyces* ranging from 58-9.8%. The GC content variation range among the 20 *Streptomyces* and the basepairs for all the 20 sequences were tabulated in Table 4.

Actinomycetes are a group of prokaryotic microorganisms capable of producing many types of secondary metabolites, which are Gram-positive bacteria that grow extensively in soils with rich organic matter (Henis, 1986; Demain, 1999). Actinomycetes are isolated from rhizosphere soil of Mangroves and all of them showed antibacterial and antifungal properties against human pathogens. Actinomycetes are previously reported in the rhizosphere sample by Sembiring *et al.* (2000). The bioactive compound of same *Streptomyces* are showing different antifungal and

Table 3: Taxan with accession number of the 16 S rRNA gene sequences of *Streptomyces* JS-1 to JS-20

No	Strain	Name	Accession no
1	JS-1	*Streptomyces* sp.	EF536325
2	JS-2	*Streptomyces olivoverticillatus*	EU124770
3	JS-3	*Streptomyces olivoverticillatus*	EU124771
4	JS-4	*Streptomyces caelestis*	EU124772
5	JS-5	*Streptomyces caelestis*	EU124773
6	JS-6	*Streptomyces caelestis*	EU124774
7	JS-7	*Streptomyces caelestis*	EU124775
8	JS-8	*Streptomyces* sp.	EU124776
9	JS-9	*Streptomyces roseoviridis*	EU124777
10	JS-10	*Streptomyces* sp.	EU124778
11	JS-11	*Streptomyces venezuelae*	EU124779
12	JS-12	*Streptomyces* sp.	EU124780
13	JS-13	*Streptomyces showdoensis*	EU124781
14	JS-14	*Streptomyces* sp.	EU124782
15	JS-15	*Streptomyces bikiniensis*	EU124783
16	JS-16	*Streptomyces griseoruber*	EU124784
17	JS-17	*Streptomyces* sp.	EU124785
18	JS-18	*Streptomyces roseus*	EU124786
19	JS-19	*Streptomyces* sp.	EU124787
20	JS-20	*Streptomyces* sp.	EU124788

Table 4: The length and GC content of the 20 *Streptomyces* JS-1 to JS-20 isolated from mangrove swamp

Taxon	Lengths (bp)	GC content (%)
Streptomyces sp. JS-1	1289	58.5
Streptomyces olivoverticillatus JS-2	1427	59.4
Streptomyces olivoverticillatus JS-3	1447	58.9
Streptomyces caelestis JS-4	1421	58.8
Streptomyces caelestis JS-5	1424	59.2
Streptomyces caelestis JS-6	1425	59.6
Streptomyces caelestis JS-7	1428	59.2
Streptomyces sp. JS-8	1425	58.9
Streptomyces roseoviridis JS-9	1428	58.6
Streptomyces sp. JS-10	1444	58.0
Streptomyces venezuelae JS-11	1428	58.9
Streptomyces sp. JS-12	1430	58.5
Streptomyces showdoensis JS-13	1429	59.1
Streptomyces sp. JS-14	1424	58.1
Streptomyces bikiniensis JS-15	1433	59.0
Streptomyces griseoruber JS-16	1432	59.4
Streptomyces sp. JS-17	1443	59.0
Streptomyces roseus JS-18	1425	58.3
Streptomyces sp. JS-19	1435	58.7
Streptomyces sp. JS-20	1424	58.7

antibacterial activities which shows the chemodiversity of the bioactive compounds present in all the 20 *Streptomyces* JS-1 to JS-20 isolates. Studies on the antimicrobial property of the actinomycetes are showing variations in the antimicrobial activities (Zheng *et al*., 2000). In the present work similar results are shown and highly supporting the present research. Actinomyctes were reported for the production of bioactive compounds based on their distribution in various

habits (Huck *et al.*, 1991). Mangrove ecosystem is poorly studied environment of bioactive compounds (Hong *et al.*, 2009). Hence, the development and application of new strategies for the detection, isolation and subsequent description of novel actinomycetes, from natural mangrove habitat was an essential need for the bioactive compound discovery (Zengler *et al.*, 2002). Actinomycetes are one of the most prolific producers of antibiotics (Strohl, 1997; Berdy, 2005). Among 52 Actinomyctes isolated in the present study, twenty isolate showed antimicrobial potential against the human pathogens. Similar results were previously reported by Huck *et al.* (1991). Antimicrobial property to human pathogens and secondary metabolite production from *Streptomyces* sp. was also observed (Thangapandian *et al.*, 2007; Rabah *et al.*, 2007; Krishna Kumari *et al.*, 2006).

Most bioactive products of microbial origin are derived from few taxonomic groups and terrestrial habitats (Berdy, 2005; Lam, 2007). The phylogenetic relationship of the *Streptomyces* sp. isolates are proving that, they are the lineages of the same genus and originated from the terrestrial environment which are acclimatized to the halophilic and the chemophilic environment of the rhizosphere soil of the mangrove *Rhizophora mucronata*. The *Streptomyces* isolates JS-1, JS-8, JS-10, JS-12, JS-14, JS-17, JS-19 and JS-20 are considered as the new lineages of *Streptomyces* because of its variations in biochemical, antimicrobial properties and phylogenetic analysis. The range of GC content of all the 16S rRNA sequences of the *Streptomyces* shows the divergence among the *Streptomyces* sp. The chemical diversity of the *Streptomyces* of same species is due to the rhizosphere environment which produces soil exudates and the halophilic nature of mangrove soil. Hence, the different lineages of *Streptomyces* sp. were adapted to the complex environment in the rhizosphere soil.

Antibiotic production is more in the Actinomycetes (Berdy, 2005; Strohl, 1997) and produces over half of the bioactive compounds in the Antibiotic Literature Database (Lazzarini *et al.*, 2000). Exploitation of terrestrial actinobacteria over many years estimated 95% rediscovery rate of known compounds (Fenical *et al.*, 1999), but *Streptomyces* from the estuarine ecosystem were halophilic nature and were adapted to the rhizosphere soil will produce novel antibiotics. It is becoming evident that mangrove rhizosphere habitats are an abundant, novel source of actinobacteria for discovering natural products originated from terrestrial environment. In the present work, the *Streptomyces* sp. were believed to be of terrestrial origin, transported to rivers by rain or irrigation water and finally to the marine environment where they are exposed to water with salt concentrations and temperatures that differ from those of the terrestrial environment. As a result, some metabolic changes may occur in the organisms (Okazaki and Okami, 1975) and secondary metabolite would be produced with good antibacterial and antifungal potential. Actinomycetes in marine and estuarine sediments have not been investigated extensively although their ubiquitous presence in marine sediments has been well documented (Takizawa *et al.*, 1993; Moran *et al.*, 1995). The purification and structural characterization studies of the bioactive secondary metabolites being carried out in the laboratory.

REFERENCES

Berdy, J., 2005. Bioactive microbial metabolites. J. Antibiot., 58: 1-26.

Bull, A.T., A.C. Ward and M. Goodfellow, 2000. Search and discovery strategies for biotechnology: The paradigm shift. Microbiol. Mol. Biol. Rev., 64: 573-606.

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

Cappuccino, J.G. and N. Sherman, 2002. Microbiology a Laboratory Manual. 6th Edn., Pearson Education, New York, pp: 133-198.

Costanza, R., R. d'Arge, R. Degroot, M. Grass and B. Hannon *et al.*, 1997. The value of the world's ecosystem services and natural capital. Nature, 387: 253-260.

Demain, A.L., 1999. Pharmaceutically active secondary metabolites of microorganisms. Applied Microbiol. Biotechnol., 52: 455-463.

Donia, M. and M.T. Humann, 2003. Marine natural products and their potential applications as anti infective agents. Lancet Infect. Dis., 3: 338-348.

Eccleston, G.P., P.R. Brooks and D.I. Kurtboke, 2008. The occurrence of bioactive micromonosporae in aquatic habitats of the sunshine coast in Australia. Mar. Drugs, 6: 243-261.

Fenical, W., D.M.B. Burg, C.V. de Goyet, J.D. Grimes and M. Katz *et al.*, 1999. Marine Derived Pharmaceuticals and Related Bioactive Compounds. National Academies Press, Washington, DC., pp: 71-86.

Fiedler, H.P., C. Bruntner, A.T. Bull, A.C. Ward and M. Goodfellow *et al.*, 2005. Marine actinomycetes as a source of novel secondary metabolites. Antonie van Leeuwenhoek, 87: 37-42.

Gao, H., K. Hong, X. Zhang, H.W. Liu, N.L. Wang, L. Zhang and X.S. Yao, 2007. New steryl esters of fatty acids from the mangrove fungus *Aspergillus awamori*. Helv. Chim. Acta, 90: 1165-1178.

Gauthier, M.J., J.M. Shewan and D.M. Gibson, 1975. Taxonomic position and seasonal variations in marine neritic environment of some Gram-negative antibiotic-producing bacteria. J. Gen. Microbiol., 87: 211-218.

Han, L., X.S. Huang, I. Sattler, H.Z. Fu, S. Grabley and W.H. Lin, 2007. Two new constituents from mangrove *Bruguiera gymnorrhiza*. J. Asian Nat. Prod. Res., 9: 327-331.

Henis, Y., 1986. Soil Microorganisms, Soil Organic Matter and Soil Fertility. In: The Role of Organic Matter in Modern Agriculture, Chen, Y. and Y. Avnime-Lech, (Eds.). Nijhoff Publishers, Boston, pp: 159-168.

Hong, K. and B. Yan, 2008. Uncultured Microorganisms in Hainan Mangrove Soil: Diversity and Functional Genes. In: Microbes and the Environment: Perspective and Challenges, Liu S.J. and H.L. Drake, (Eds.). Science Press, Beijing, China, pp: 52-58.

Hong, K., A.H. Gao, Q.Y. Xie, H. Gao and L. Zhuang *et al.*, 2009. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Mar. Drugs, 7: 24-44.

Huck, T.A., N. Porter and M.E. Bushell, 1991. Positive selection of antibiotic-producing soil isolates. J. Gen. Microbio., 137: 2321-2329.

Huo, C., H. Liang, G. Tu, Y. Zhao and W. Lin, 2008. A new 5, 11-epoxymegastigmane glucoside from *Acanthus ilicifolius*. Nat. Prod. Res., 22: 896-900.

Hyde, K.D. and S.Y. Lee, 1995. Ecology of mangrove fungi and their role in nutrient cycling: What gaps occur in our knowledge?. Hydrobiologia, 295: 107-118.

Jensen, P.R., E. Gontang, C. Mafnas, T.J. Mincer and W. Fenical, 2005. Culturable marine actinomycete diversity from tropical pacific ocean sediments. Environ. Microbiol., 7: 1039-1048.

Krishna Kumari, K., P. Ponmurugan and N. Kannan, 2006. Isolation and characterization of *Streptomyces* sp. from soil sample for secondary metabolite production. Biotechnology, 5: 478-480.

Lam, K.S., 2007. New aspects of natural products in drug discovery. Trends Microbiol., 15: 279-289.

Lazzarini, A., L. Cavaletti, G. Toppo and F. Marinelli, 2000. Rare genera of Actinomycetes as potential producers of new antibiotics. Antonie van Leeuwenhoek, 78: 399-405.

Lin, Y., X. Wu, S. Feng, G. Jiang and J. Luo *et al.*, 2001. Five unique compounds: Xyloketals from the mangrove fungus *Xylaria* sp. from the South China Sea coast. J. Org. Chem., 66: 6252-6256.

Lin, Y., X. Wu, Z. Deng, J. Wang, S. Zhou, L.L.P. Vrijmoed and E.B.G. Jones, 2002. The metabolites of the mangrove fungus *Verruculina enalia* No. 2606 from a salt lake in the Bahamas. Phytochemistry, 59: 469-471.

Lin, Z., T. Zhu, Y. Fang and Q. Gu, 2008. 1H and 13C NMR assignments of two new indolic enamide diastereomers from a mangrove endophytic fungus *Aspergillus* sp. Magn. Reson. Chem., 46: 1212-1216.

Moran, M.A., L.T. Rutherford and R.E. Hodson, 1995. Evidence of indigenous *Streptomyces* populations in a marine environment determined with a 16S rRNA probe. Applied Environ. Microbiol., 61: 3694-3700.

Okami, B. and A.K. Hotta, 1988. Search and Discovery of New Antibiotics. In: Actinomycetes in Biotechnology, Goodfellow, M., S.T. Williams and M. Mordarski (Eds.). Pergamon Press, Oxford, pp: 33-67.

Okazaki, T. and Y. Okami, 1975. Actinomycetes tolerant to increased NaCl concentration and their metabolite. J. Ferment. Technol., 53: 833-840.

Oldfield, C., N.T. Wood, S.C. Gilbert, F.D. Murray and F.R. Faure, 1998. Desulphurisation of benzothiophene and dibenzothiophene by actinomycete organisms belonging to the genus *Rhodococcus* and related taxa. Antonie Van Leeuwenhoek, 74: 119-132.

Rabah, F.L., E. Ali, S. Mahmoud, C. Bengraa and H. Hacene, 2007. Screening, isolation and characterization of a novel antimicrobial producing actinomycete, strain RAF10. Biotechnology, 6: 489-496.

Sembiring, L., A.C. Ward and M. Goodfellow, 2000. Selective isolation and characterisation of members of the *Streptomyces violaceusniger* clade associated with the roots of *Paraserianthes falcataria*. Antonie van Leeuwenhoek, 78: 353-366.

Strohl, W.R., 1997. Biotechnology of Antibiotics. 2nd Edn., Marcel Dekker, New York.

Takizawa, M., R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol., 59: 997-1002.

Tang, J.S., H. Gao, K. Hong, Y. Yu and M.M. Jiang *et al.*, 2007. Complete assignments of 1H and 13C NMR spectral data of nine surfactin isomers. Magn. Reson. Chem., 45: 792-796.

Thangapandian, V., P. Ponmurugan and K. Ponmurugan, 2007. Actinomycetes diversity in the rhizosphere soils of different medicinal plants in Kolly Hills-Tamilnadu, India, for secondary metabolite production. Asian J. Plant Sci., 6: 66-70.

Wang, B., S. Liang, W. Zhang and Q. Zan, 2003. Mangrove flora of the world. Acta Botanica Sinica, 45: 644-653.

Wu, J., Q. Xiao, J. Huang, Z. Xiao, S. Qi, Q. Li and S. Zhang, 2004. Xyloccensin O and P, unique 8,9,30-phragmalin ortho esters from *Xylocarpus granatum*. Org. Lett., 6: 1841-1844.

Xie, X.C., W.L. Mei, Y.X. Zhao, K. Hong and H.F. Dai, 2006. A new degraded sesquiterpene from marine actinomycete *Streptomyces* sp. 0616208. Chinese Chem. Lett., 17: 1463-1465.

Yan, B., K. Hong and Z. Yu, 2006. Archaeal communities in mangrove soil characterized by 16S rRNA gene colones. J. Microbiol., 44: 566-571.

Zengler, K., G. Toledo, M. Rappe, J. Elkins, E.J. Mathur, J.M. Short and M. Keller, 2002. Cultivating the uncultured. PNAS., 99: 15681-15686.

Zheng, Z., W. Zeng, Y. Huang, Z. Yang, J. Li, H. Cai and W. Su, 2000. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol. Lett., 188: 87-91.