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Isolation and Characterization of *Streptomycetes* with Plant Growth Promoting Potential from Mangrove Ecosystem

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Abstract

A total of 66 actinomycetes isolates were isolated from mangroves of Andhra Pradesh, India, using various enrichment techniques and pre-treatments. The samples were collected from Coringa mangrove ecosystem and pre-treated by enrichment with $CaCO_3$, sodium dodecyl sulphate and phenol, plated on the media supplemented with cycloheximide (50 mg/ml), nystatin (25 mg/ml) and nalidixic acid (50 mg/ml). The population count of actinomycetes fluctuated from 1.9×10^5 to 8.0×10^5/g soil. Out of the isolated 66 actinomycetes, 8 isolates possessing plant growth promoting potential were further studied and characterized by physiological and biochemical traits and identified by 16S rRNA gene sequencing as different species of *Streptomycetes* genera.

Key words: 16S rRNA, ARDRA, IAA, mangroves, PGPR, *Streptomycetes*

Introduction

Mangrove is one of the most productive ecosystems and a natural renewable resource (Kathiresan, 2003; Balachandran *et al*., 2009). These are a unique woody plant community of intertidal coasts in the tropical and subtropical zones, which are regarded as highly productive ecosystems and abode to unexplored microbial diversity including actinomycetes (Balagurunathan *et al*., 2010). Mangroves provide a unique ecological niche to a variety of microorganisms (Agate, 1991). About 125 species of microorganisms (bacteria, fungi, algae) have been identified from mangrove environment (Kathirvel, 1996). Exploitation of less/unexplored ecosystems for actinomycetes is highly necessary for the discovery of novel bioactive metabolites (Sahoo and Dhal, 2009).

Among the different mangrove locations Andhra Pradesh of India has dense mangrove vegetation found towards coast rather than on shore land because of the dense branching network of creeks, which exist towards the coast (RSAM, 1992). There is more mangrove vegetation on tidal flats on the western side of the Krishna delta than on its eastern side. Dense mangroves are also seen over recent sand / mud spits on the Nizampatnam bay (RSAM, 1992). Sparse mangroves are found on the eastern side of the Krishna delta.

Actinomycetes being gram-positive bacteria showing a filamentous growth like fungi. They are aerobic and group of organisms widely spread in nature, with high G+C content (60–70 mol %) and are important sources of antibiotics (> 50%) and enzymes (Edwards, 1993; Gharaibeh *et al*., 2003; Weber *et al*., 2003; Shantikumar *et al*., 2006).

Screening and isolation of promising actinomycetes from mangrove ecosystem with potential antimicrobial compounds is still a thrust area of research and it is suggested that the exploration of new areas and habitats played a vital role in the search for new microbes and novel metabolites (Horinouchi, 2002). The genus *Streptomyces* produce approximately 75% of commercially and medically useful antibiotics and 60% of antibiotics used in agriculture (Sanghvi *et al*., 2014). Seasonal variations of antagonistic actinomycetes have been determined in selected mangrove ecosystems and highest numbers of actinomycetes during monsoon have been recorded. The microbial interrelationship in mangrove sediments does exist between bacteria and

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actinomycetes, bacteria and fungi, and fungi and actinomycetes (Rathna Kala, 1995). However, across the globe, the world's mangroves are threatened. Mangrove habitats are being destroyed as rivers are dammed, their waters diverted and the intertidal zone extensively developed for agriculture or aquaculture. Previous study showed that actinomycetes isolated from Malaysian soil have the potential to inhibit the growth of plant pathogens (Jeffrey *et al*., 2007). Likewise actinomycetes isolated from Turkey's farming soil have the ability to inhibit *Erwinia amylovora* a bacteria that cause fireblight to apple and *Agrobacterium tumefaciens* a causal agent of Crown Gall disease (Oskay *et al*., 2004). Besides acting agents for control of plant pathogens they also possess the capability for plant growth promotion (Nassar *et al*., 2003; da Silva Sousa *et al*., 2008). This is due to their capacity to produce IAA, antibiotics, siderophores, enzymes that have antimicrobial activity, substances that promote plant growth, solubilization of phosphates and competition with plant pathogens for substratum and nutrients (Hamdali *et al*., 2008; da Silva Sousa *et al*., 2008).

The present study aims at isolation of actinomycetes from mangrove ecosystem of Andhra Pradesh, India and to evaluate their plant growth promoting potential by siderophore and IAA production assay. The study also focusses on the genetic diversity study of the plant growth promoting isolates on the basis of morpho-physiological, biochemical and molecular characteristics.

Experimental

Materials and Methods

Soil sampling. A total of 10 soil samples and one water sample were collected from the Mangrove ecosystem of Kakinada District, Coringa (latitude 16°44′ to 16°53′N and longitude 82°14′ to 82°22′E), Andhra Pradesh, India by systematic sampling method. Samples were collected from 15 cm depth and transported to the laboratory in sterile bags and air-dried at room temperature. The geographical locations, as well as physiochemical properties of samples were recorded (Table I).

Isolation of actinomycetes. Samples were subjected to various enrichment techniques like $CaCO_3$ (Tsao *et al*., 1960), SDS (Hayakawa and Nonomura, 1989), phenol (Hayakawa *et al*., 2004), and media such as, Starch Casein Agar (SCA), Actinomycetes Isolation Agar (AIA) and Soil Extract Agar (SEA), amended with antibiotics nystatin (25 mg/ml), cycloheximide (50 mg/ml) and nalidixic acid (50 mg/ml), were employed for the isolation of *Streptomycetes*. The air dried samples were incubated at 55°C for 5 min in an incubator. One gram of soil was dissolved in 100 ml of distilled water (10^{-2} dilution) and 1 ml of 10^{-3}, 10^{-4}, 10^{-5} serial dilutions were spread plated on media amended with antibiotics using dilution-plate technique and incubated at 30°C for 2–3 weeks. After incubation, actinomycetes colonies were selected and maintained by sub-culturing on ISP-2 agar (g/l: 4.0 g glucose; 4.0 g yeast extract; 10.0 g malt extract; 18.0 g agar) slants and stored at 4°C for further use.

Identification by polyphasic taxonomy. Out of the isolated actinomycetes eight isolates (AM2-2, AM2-3, AM2-4, AM2-7, AM2-8, AM2-10, AM2-11, AM5-16) were characterized by morpho-physiological, biochemical and molecular methods. Identification of actinomycetes was carried out, using standard methods of morphological and physiological traits (Shirling and Gottlieb, 1966) and Bergey's Manual of Systematic Bacteriology (Williams *et al*., 1989). Morphological methods consisted of macroscopic and microscopic studies. For chemotaxonomic studies, the colonies were grown in glucose yeast extract malt extract broth for 4–5 days and then filtered out and processed for determination

Table I
Details of sources of samples, pH and geographical Co-ordinates

Sample type	Source	Geographical Co-ordinates	pH	Cfu/g soil
Mangroves water sample	Nearby sea shore.	16°30"-17°N,82°10"-80°23"E	7.4	8.0×10^5
Rhizospheric soil	*Avicennia marina* (mangrove plant)	16° 56" N, 82° 13" E	7.2	2.25×10^5
Rhizospheric soil	*Avicennia officinalis* (mangrove plant)	16° 56" N, 82° 13" E	7.3	1.9×10^5
Rhizospheric soil	*Excoecaria agallocha* (mangrove plant)	16° 56" N, 82° 13" E	7.6	2.4×10^5
Rhizospheric soil	*Sonneratia apetala* (mangrove plant)	16° 56" N, 82° 13" E	7.2	3.06×10^5
Rhizospheric soil	*Aegicerale corniculatum* (mangrove plant)	16° 56" N, 82° 13" E	7.4	3.9×10^5
Rhizospheric soil	Grasses growing in mangrove	16° 56" N, 82° 13" E	7.0	2.86×10^5
Rhizospheric soil	*Ceriops decandra* (mangrove plant)	16° 56" N, 82° 13" E	7.2	3.93×10^5
Rhizospheric soil	*Rhizophora conjugata* (mangrove plant)	16° 56" N, 82° 13" E	7.5	3.61×10^5
Rhizospheric soil	*Hibiscus titraceous*	16° 56" N, 82° 13" E	7.1	4.05×10^5
Soil sample	Sample near the start of Coringa wild life sanctuary	16° 56" N, 82° 13" E	7.3	3.1×10^5

of the diaminopimelic acids (LL-DAP or meso-DAP) isomers and whole cell sugar patterns (Lechevalier and Lechevalier, 1980) using thin layer chromatography (TLC) on precoated cellulose plates (Merck, India).

Scanning electron microscopy. Spore surface ornamentation was evaluated by Scanning electron microscopy (SEM). Mycelia were taken (after 10 days of incubation) and washed in 0.1 M sodium cacodylate buffer (pH 7.4). They were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 4 h at 4°C followed by post-fixation with 1% osmium tetraoxide in 0.1 M sodium cacodylate buffer (pH 7.4) and dried in a critical point dryer (EMITECH model K850, Hitachi). The preparations were mounted onto aluminium holders, sputter-coated with 10 nm Au and observed by SEM (Hitachi model S3400 at 15–30 kV, 2–5.00 μm).

Physiological characterization, plant growth promoting (PGP) attributes and extracellular enzymes production. Physiological characterization such as the effect of pH (6–9), temperature (20–45°C), salinity and carbon source utilization were studied. Carbohydrate utilization was determined by growth on carbon utilization medium (ISP-2) (Pridham and Gottlieb, 1948) supplemented with 1% carbon source (Glucose, Arabinose, Rhamnose, Mannitol, Dulcitol, Raffinose, Fructose, Sucrose, Lactose, Inositol). The intrinsic resistance of actinomycetes to salinity was evaluated according to Yadav *et al.* (2009), by observing the growth at 28 ± 2°C in tryptose soya broth amended with different concentrations of NaCl (2, 4, 6, 8% w/v). For growth promoting attributes, phosphate solubilization (Mehta and Nautiyal, 2001), siderophore production (You *et al*., 2005), nitrate reduction (Glass *et al*., 1997), cyanogenesis (Schippers *et al*., 1990) and IAA production (Bano and Musarrat, 2003) were evaluated. The extracellular enzyme activity was assayed using standard methods such as amylase (Mishra and Behera, 2008), protease (Manachini *et al*., 1988), cellulase (Farkas *et al*., 1985), urease and gelatin degradation and hydrogen sulphide production were also studied following the protocol as reported by Cappuccino and Sherman (1992).

Molecular characterization

Extraction of actinomycetes DNA and PCR amplification. Genomic DNA was extracted from eight selected *Streptomycetes* isolates (having plant growth promoting properties) following the modified protocol of Boudjella *et al.* (2006). The purity check of DNA for analysing its quality and quantity was done by measuring the absorbance at 260 and 280 nm by spectrophotometer. The 16S rRNA gene fragment for the *Streptomycetes* was amplified by using two universal primer pair fD1 (5'-GAGTTTGATCCTGGCTCA-3') and Rp2 (5'-CGGCTACCTTGTTACGACTT-3'). The 16S rRNA was amplified by PCR using Promega kit. The final volume of reaction mixture of 50 μl contained 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 9.0 at 25°C), 1.5 mM $MgCl_2$, 200 mM of each dNTP, 1 mM of each primer, 0.25 U of *Taq* polymerase and 500 ng of template DNA. The amplification was performed on BioRad thermal cycler (initial denaturation step at 98°C for 3 min, after which *Taq* polymerase was added, followed by 30 amplification cycles of 94 C for 1 min, 52°C for 1 min, and 72°C for 10 min).

Amplified ribosomal DNA restriction analysis (ARDRA). For restriction analysis, amplified ribosomal DNA was digested with three restriction endonucleases *Taq* I, *Msp* I and *Hae* III (Promega, India) according to manufacturer's instructions and analyzed by horizontal electrophoresis in 2.5% agarose gels at 70 V for 2.5 h and documented on alpha-Imager gel documentation system (Alpha-Imager, USA). The restriction analysis profiles generated, were compared by calculating Jaccard's similarity coefficient for each pairwise comparison and dendrogram was constructed from the similarity matrix by the UPGMA. In order to test the goodness of fit of cluster analysis, co-phenetic value matrices were calculated and compared with the original similarity matrices that were UPGMA clustered by using the NTSYSpc analysis package (version 2.02e; Exeter Software, Setauket, NY). The amplified product of representative isolates from each clusters were purified by PCR purification kit (Promega, India) and sequenced directly with the Taq-mediated dideoxy chain terminator cycle sequencing in ABI 3130xl automated genetic analyser (Applied Biosystem, UK) according to manufacturer's instructions. The sequences were aligned by ClustalW and BlastN programme was used to compare the sequences deposited in public databases and the phylogenetic tree was constructed with the MEGA software version 4.1 (Saitou and Nei, 1987). Gaps were treated by pairwise deletions and bootstrap analysis was done by using 5,000 pseudo-replications.

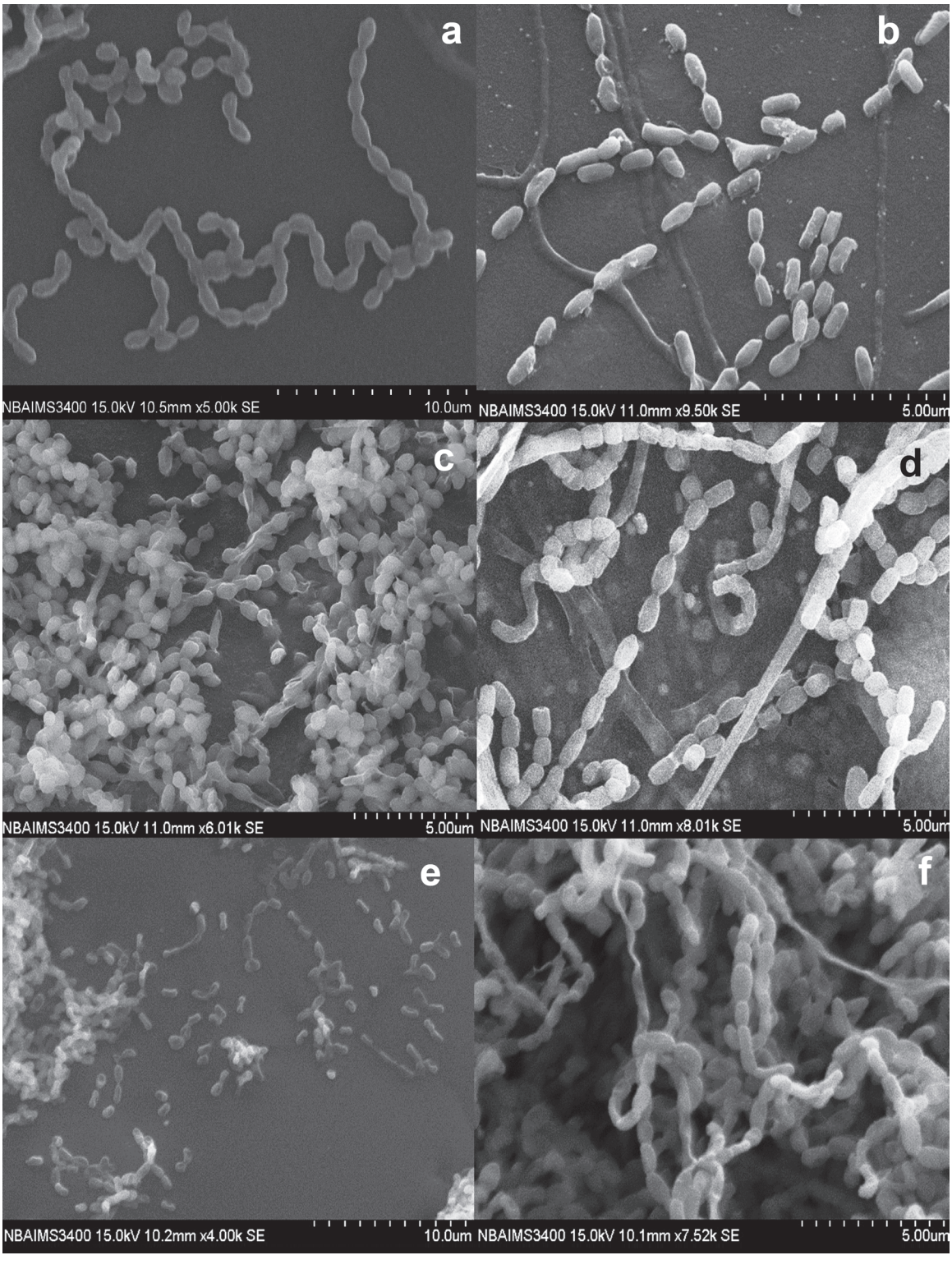
Accession numbers: A total of eight sequences of 16S rRNA gene were deposited in public databases (GenBank, NCBI) under the accession numbers from KC511801 to KC511808.

Results

Isolation and clustering of actinomycetes by polyphasic taxonomy. A total of 66 actinomycetes isolates were isolated from Coringa mangrove ecosystem, of these eight isolates showed considerable amount of variations in their colony morphology and possessing plant growth promoting attributes were chosen for further physiological and biochemical characterization. The population count of actinomycetes fluctuated from 19×10^4 to 80×10^4 g soil^{-1} (Table I). Most of the isolates

belonged to genus *Streptomycetes* and were tentatively identified by morphological characterization using aerial mycelial colour, substrate mycelial colour, pigments, arrangement of spores in chain, like straight chain, rectiflexibles *etc*. as revealed by scanning electron microscopy (Fig. 1). Cell wall composition analysis of actinomycetes using thin layer chromatography (TLC) revealed type I cell wall with LL-DAP isomers.

Physiological characterization of the isolated actinomycetes. Physiological tests are an important tool for classification of actinomycetes, influencing their growth and other properties. Physiological parameters like pH, temperature, NaCl concentration, carbon source utilization in the growth media were analysed. Growth of the selected 8 *Streptomycetes* isolates occurred in the pH range of 6–9 with optimum growth at pH 7.



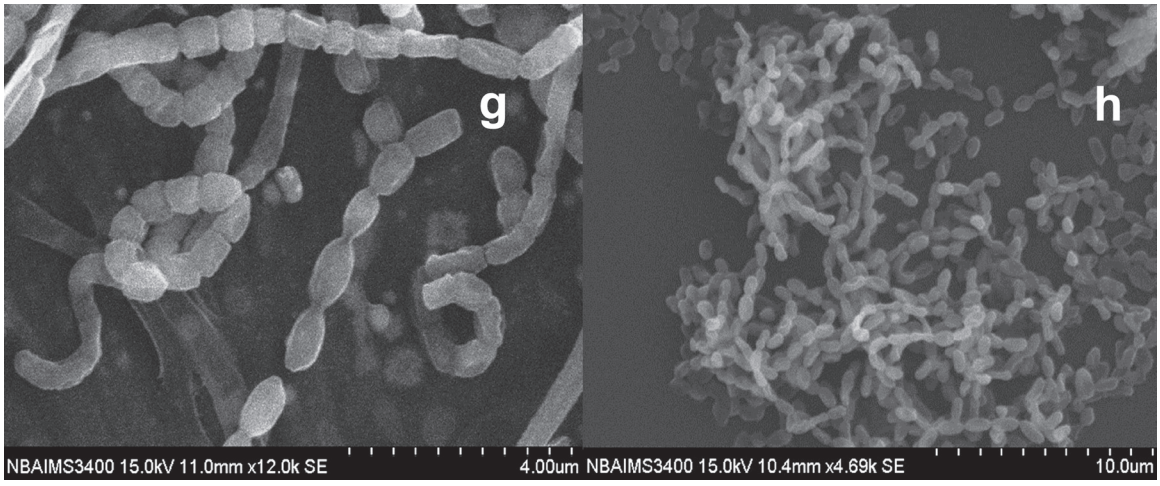


Fig. 1. Scanning electron microscopy (SEM) of *Streptomyces* isolates isolated from mangrove of Andhra Pradesh, India, showing variations in spore chain morphology.

a) AM2-2 (*Streptomyces globisporus*), (b) AM2-3 (*S. roseoviolaceus*), (c) AM2-4 (*S. cavourensis*), (d) AM2-10 (*S. celluloflavus*), (e) AM2-11 (*S. albogriseolus*) (f) AM2-7 (*S. spiralis*), (g) AM2-10 (*S. macrosporeus*) (h) AM5-16 (*S. rochei*)

The temperature range for growth was 20–45°C with the optimum temperature being 35°C. The isolates exhibited salt tolerance up to 8% with optimum growth at 6% NaCl; hence, the isolates could be placed in intermediate salt tolerance group. All the isolates were able to utilize 8–10 sugars as a carbon source out of 10 sugars being tested (Glucose, arabinose, rhamnose, mannitol, dulcitol, raffinose, fructose, sucrose, lactose and inositol).

Plant growth promoting attributes and extracellular enzyme production. Plant growth promoting and extracellular enzyme activity of the selected eight isolates revealed that all the 8 isolates (100%) were siderophore and IAA producers, 4 (50%) were phosphate solubilizers, 3 (37.5%) were H_2S and amylase producers, 5 (62.5%) were urease, protease and HCN producers, 6 75%) of the selected isolates were nitrate reducing and only 1 (12.5%) isolate produced the enzyme gelatinase (Table II).

Molecular characterization and phylogenetic analysis. The molecular characterization of the selected *Streptomycetes* isolated from mangroves were carried, based upon 16S rRNA gene amplification and its RFLP pattern with a set of three restriction enzymes *Taq*I, *Msp*I and *Hae*III (Fig. 2) and dendrogram (Fig. 3) was generated using NTSYSpc software. For More precise identification the isolates were sequenced by Sanger's di-deoxy nucleotide sequencing method and identified based on percentage similarity (>97% compared with public database sequences, NCBI), by BLAST homology (Table III). Further phylogenetic analysis of the isolates was carried out for their similarity to known actinobacteria aligned together with the sequences (closest representatives), available in public databases (GenBank, NCBI), of actinobacteria (Fig. 4). Three genetic groups were formed among the identified isolates (Table III). The 4 isolates included in group I showed 100% sequence identity compared with most closely related sequences in public database (*Streptomyces cavourensis*, *Streptomyces albogriseolus*, *Streptomyces spiralis*, *Streptomyces rochei*), followed by 2 isolates in group II with 99% similarity (*Streptomyces roseoviolaceus*, *Streptomyces celluloflavus*) and 2 isolates in group III showing <99% similarity (*Streptomyces globisporus*, *Streptomyces macrosporeus*).

Discussion

Actinomycetes population have been identified as one of the prominent group of soil microbes which differ with soil type, soil pH, geographical location and climatic condition (Arifuzzaman *et al*., 2010). The characterization of these microbes is as important as studying their existence in the natural environments (Hirsch and Valdes, 2010). Actinomycetes play a vital role in the soil such as mineralization of organic matters, immobilization of nutrients, antibiosis and production of plant growth promoters (Adegboye and Babalola, 2012). In our studies heat pre-treatments, enrichment techniques and selective isolation media resulted in considerable decrease in the unwanted bacterial population, which was similar as obtained by Hayakawa (2008). By various enrichment techniques and media used a total of 66 actinomycetes were isolated out of which 8 isolates with significant plant growth promotory attributes and growth under different pH and salinity levels, were characterised by 16S rRNA sequencing as belonging to *Streptomycetes* genera. This genus had also been previously evaluated for the characterization of plant growth promoting and other important traits as

Table II
Phenotypic characteristics of all selected *Streptomycetes* strains.

Properties	*S. globisporus* AM2-2	*S. roseoviolaceus* AM2-3	*S. cavourensis* AM2-4	*S. celluloflavus* AM2-10	*S. albogriseolus* AM2-11	*S. spiralis* AM2-7	*S. macrosporeus* AM2-8	*S. rochei* AM5-16
Morphological Characteristics								
Color of aerial mycelium	White	white	Ivory	White	White	White	White	Creamish white
Color of substrate mycelium	Yellow	Dark brown	Yellow brown	Light yellow	Yellow brown	Cream	Red brown	Yellow
Spore mass	Light brown	White	Ivory	Off white	Light grey	Cream	Creamish	Cream
Diffusible pigments	–	–	–	–	–	–	–	–
Carbon Source Utilization								
Glucose	+	+	+	–	+	+	+	+
Arabinose	–	+	+	+	–	+	+	+
Rhamnose	+	+	+	+	+	+	+	–
Mannitol	+	+	+	–	+	+	+	–
Dulcitol	–	+	+	+	–	+	+	+
Raffinose	+	+	+	+	+	+	+	+
Fructose	+	+	–	–	–	+	–	–
Sucrose	+	+	+	–	–	–	+	+
Lactose	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+
Chemotaxonomic characters								
Cell wall amino acid Analysis	L-DAP	L-DAP	L-DAP	L-DAP	L-DAP	L-DAP	L-DAP	L-DAP
PGP traits								
IAA Production	+	+	+	+	+	+	+	+
Phosphate Solubilization	–	+	+	–	–	+	–	+
Siderophore Production	+	+	+	+	+	+	+	+
HCN Production	–	+	–	–	+	+	+	+
Nitrate Reduction	+	+	–	+	+	–	+	+
Hydrolytic enzymes and biochemical characterization								
Urease	+	–	–	+	+	–	+	+
Protease	–	+	–	+	+	–	+	+
Gelatinase	–	–	+	–	–	–	–	–
Amylase	+	–	–	–	–	+	–	+
H_2S Production	–	–	+	+	–	–	+	–

Table III
Closest BLASTN matches for the full 16S rRNA sequences and their percentage similarity with the closest actinobacterial strains.

IsolateCode	Closest species	GenBank accessionnumber	Similarity (%)
AM2-2	*Streptomyces globisporus*	KC511801	98%
AM2-3	*Streptomyces roseoviolaceus*	KC511802	99%
AM2-4	*Streptomyces cavourensis*	KC511803	100%
AM2-10	*Streptomyces celluloflavus*	KC511804	99%
AM2-11	*Streptomyces albogriseolus*	KC511805	100%
AM2-7	*Streptomyces spiralis*	KC511806	100%
AM2-8	*Streptomyces macrosporeus*	KC511807	98%
AM5-16	*Streptomyces rochei*	KC511808	100%

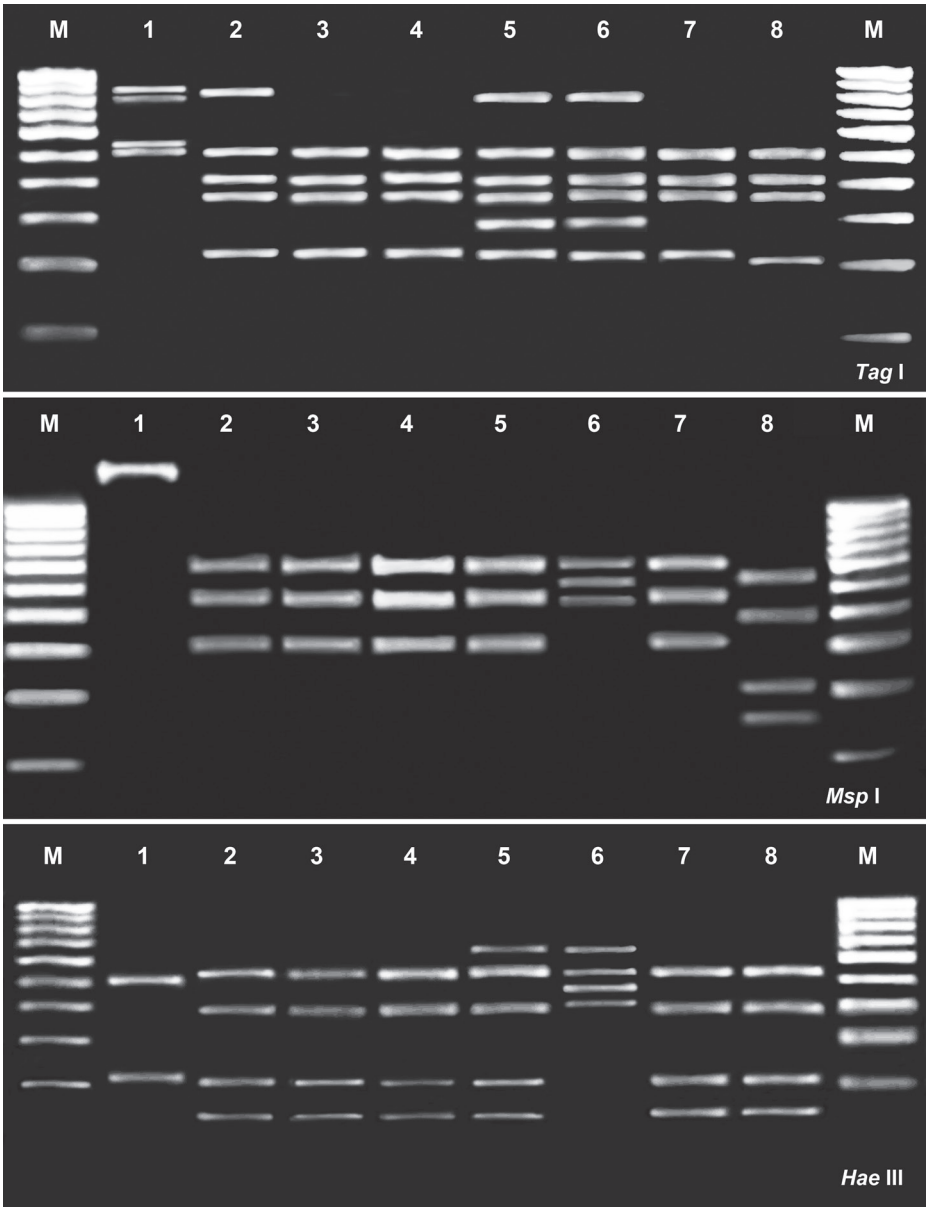


Fig. 2. Restriction digestion banding pattern of isolates amplified by 16S rDNA and digested by *Taq* I, *Msp* I and *Hae* III restriction enzymes.

Lane M: 100bp molecular weight marker (Genei, Bangalore), Lane 1: AM2-2, Lane 2: AM2-3, Lane 3: AM2-4, Lane 4: AM2-10, Lane 5: AM2-11, Lane 6: AM2-7, Lane 7: AM2-8 and Lane 8: AM5-16

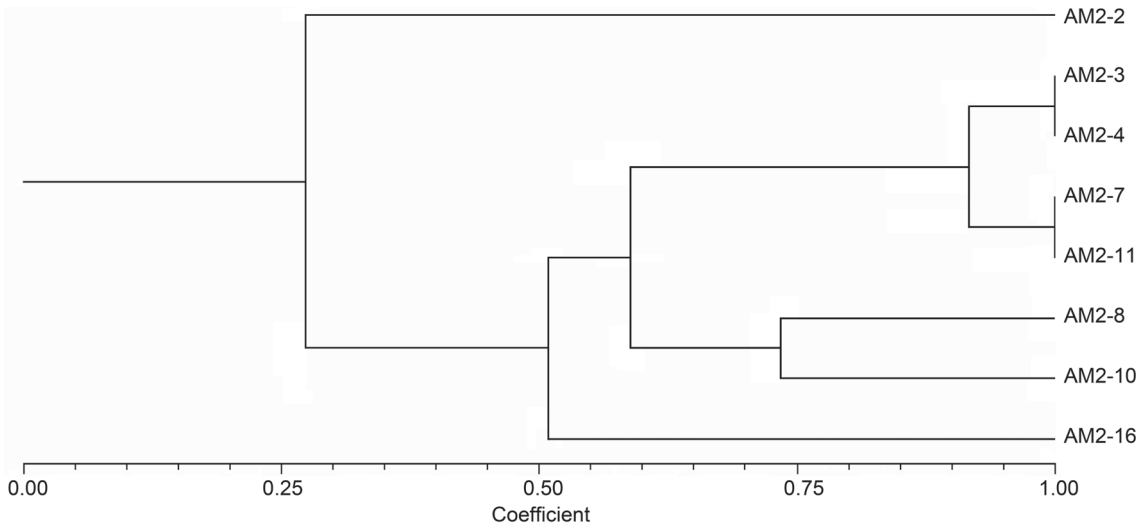


Fig. 3. UPGMA cluster analysis showing the genetic relationship among eight isolates of Actinomycetes, banding pattern based on restriction digestion by restriction enzyme *Taq* I, *Hae* III and *Msp* I

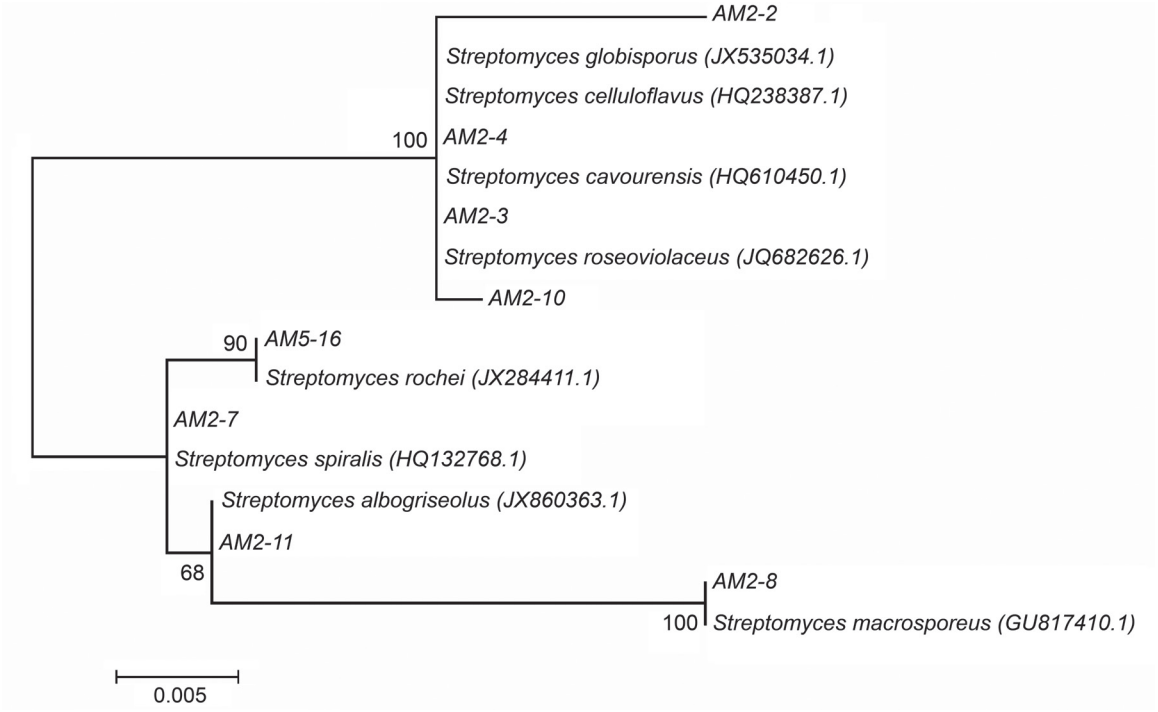


Fig. 4. NJ phylogenetic tree of full 16S rRNA sequences from selected isolates. The sequence data for several closely related actinobacterial type cultures were recovered from GenBank and included in the tree. The boot strap values from 5,000 pseudoreplications are shown at each of the branch points on the tree. Bar indicates % similarity

enough information available in the public databases (Zhang *et al*., 1998; Malviya *et al*., 2011; Yandigeri *et al*., 2012), but *Streptomycetes* isolated from stressed habitats with plant growth promoting potential have not been studied extensively. Bacteria isolated from different stressed habitats possess stress tolerance capacity alongwith plant growth-promoting traits. Salinity tolerance and the ability of *Streptomycetes* for the production of extracellular enzymes, plant growth promoting attributes and other activities were evaluated and it was reported that all the 8 (100%) *Streptomycetes* isolates from mangrove region could tolerate up to 8% NaCl concentration showing optimum growth at 6% NaCl concentration, hence the strain could be placed in intermediate salt tolerance group according to Tresner *et al*. (1968). The ability of *Streptomyces* strains to tolerate high concentrations of NaCl is well known (Waksman, 1959). Similar results were reported from the *Streptomycetes* isolated from Indo-Gangetic Plains (IGP) (Malviya *et al*., 2011), and halophilic *Streptomycetes* from India and their utilization in agricultural fields (Vasavada *et al*., 2006). Da Silva Sousa *et al*. (2008) studied six *Streptomycetes* isolated from rhizospheric soil of various crops and found out that they could tolerate a NaCl level up to 3%. Kavya *et al*. (2012), also reported salinity tolerance of *Streptomycetes* from Coringa mangrove ecosystem but their results indicated a tolerance of 3–4% NaCl concentration, which is less as compared to isolates under current study. Sadeghi *et al*. (2012) have reported the beneficial role of *Streptomyces* on PGP activity under salinity stress. Hence, it can be concluded that these strains may have the ability to survive in the harsh environments such as saline and acidic to alkaline pH soils. In our studies all the 8 *Streptomycetes* isolates were positive IAA and siderophore producers. There are many reports which demonstrated the ability of endophytic and rhizospheric soil *Streptomycetes* to produce indole acetic acid and thus promote plant growth (Khamna *et al*., 2010; Solans *et al*., 2011; Yandigeri *et al*., 2012; Kaur *et al*., 2013). In the rhizosphere soils, root exudates are the natural source of tryptophan for rhizosphere micro-organisms, which may enhance auxin (IAA) biosynthesis in the rhizosphere. Likewise Sadeghi *et al*. (2012) have also reported the production of IAA and siderophore by halotolerant *Streptomycetes* isolate. Siderophore production may be involved in the inhibition of pathogens and thus promote plant growth indirectly because *Streptomyces* species are known for the production of hydroxamate type siderophores, which inhibit phytopathogen growth by competing for iron in rhizosphere soils (Khamna *et al*., 2009; Kaur *et al*., 2013), thus our isolates could be involved in the inhibition of pathogens. Malviya *et al*. (2011), also reported production of siderophore by *Streptomycetes* isolated from IGP region but in their studies only 8% of the isolates from the total 145 isolates were positive siderophore producers but in our studies 75% of the selected *Streptomycetes* were nitrate

reducers, 50% were phosphate solubilizers and 62.5% were HCN producers. In agriculture, biological phosphate solubilisation as an alternative to natural phosphate utilisation plays an important role in efficient nutrient uptake of plants. Patil *et al.* (2010) isolated nine antagonistic actinomycetes from IGP region and found that out of them 8 were *Streptomycetes.* Out of these eight *Streptomycetes* isolates five were phosphate solubilizers and one produced HCN. Some actinomycetes are known to produce hydrolytic enzymes such as protease and amylase (Kaur *et al.*, 2013). These enzymes help in preventing the crops from plant pathogens and deleterious microbes by degrading their cell walls. In our study we reported that five of our isolates could produce enzyme protease and three isolates were amylase producers. Also it was reported that five isolates were producing urease, three H_2S producers and one isolate was able to show gelatinase activity. After studying the cultural and morphological characteristics of the potent plant growth promoting isolates, they were assigned under the genus *Streptomyces*. Concerning phenotypic characteristics, isolates produced varied colour aerial and substrate mycelia. Their chemotaxonomic characteristics further validate that they all belong to genus *Streptomyces* as their cell wall contain L-diaminopimelic acid (cell wall type-I). Thus, this study is in accordance with the previous reports that *Streptomycetes* are known to be predominant among actinomycetes with antagonistic and plant growth promoting potential and produce antifungal compounds (Ouhdouch *et al.*, 2001; Kaur *et al.*, 2013).

The reliable generic identification of members of this genus by the first approach was confirmed by DNA sequence analysis. Variations in 16S rDNA can also be assessed by analyzing restriction fragment length polymorphism (RFLP) of 16S rDNA sequences. PCR based markers such as RAPD, RFLP, DAPD and SSR have been used to discriminate bacterial strains and to analyse genetic diversity (Yadav *et al.*, 2013). In this study, we used 16S rDNA sequence-based phylogenetic analysis to investigate the species diversity of Streptomycetes. It is also known that microbes with 16S rRNA sequence similarity up to 97% identical should be considered as members of the same genus (Petit *et al.*, 1999; Malviya *et al.*, 2011). Since all the isolates were belonging to genus *Streptomyces*, we tried to study their diversity using ARDRA, which is the rapid and convenient method, and can be very useful in grouping actinobacterial isolates efficiently as well as effectively reduce the number of isolates by de-replicating the isolates during screening for diversity (Jiang *et al.*, 2010). In the current study, we used a set of three restriction endonucleases *Msp* I, *Hae* III and *Taq* I that specifically recognize the sequence 'CCGG', 'GGCC' and 'TCGA' respectively. The results of the different RFLP patterns obtained allowed us to effectively differentiate the strains into distinct groups of actinobacteria. In our study, a total of 8 representative isolates were identified by 16S rRNA gene sequence analysis as *S. globisporus* (KC511801), *S. roseoviolaceus* (KC511802), *S. cavourensis* (KC511803), *S. celluloflavus* (KC511804), *S. albogriseolus* (KC511805), *S. spiralis* (KC511806), *S. macrosporeus* (KC511807) and *S. rochei* (KC511808). All the isolates recorded 98–100% similarity with the representative isolates as shown in the Table III. Similar results were observed by Patil *et al.* (2010) and Malviya *et al.* (2011) the *Streptomyces* isolated by them also showed 98–100% similarity except one isolate in case of Malviya *et al.* 2011 which was 96% similar with the representative isolate.

The present study highlights that mangroves are the potential reservoirs for actinomycetes especially which can tolerate salt levels and biochemical and plant growth promotion assay of the isolates demonstrates their potential to be used as biocontrol agents and biofertilizers which may play important role in plant growth promotion either directly or indirectly and thereby increase crop yield.

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