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Distribution of actinobacteria in the near shore estuarine sediments along north coastal Andhra Pradesh, India

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Abstract

The distribution of actinobacteria in the near shore sediments of eight estuaries in the north coastal Andhra Pradesh was investigated in relation to physic-chemical parameters during May 2015 to May 2016. Eleven media were used to culture the actinobacteria. The study recorded 11 genera (*Actinomyces, Actinoplanes, Kibdellosprangium, Microbispora, Micromonospora, Microtetraspora, Saccharomonospora, Saccharopolyspora, Streptomyces, Streptosporangium* and *Thermomonospora*) of actinobactera. The study registered the occurrence of 143 species of *Streptomyces* of which, 79 species were recorded at Station 5 (Gosthani estuary). The species *S.albus, S.atroolivaceus, S.catenulae, S.griseus, S.olivaceous, S.bellus* and *S.psammoticus* are common species. Forty species were recorded only once during the study period. The mean densities of Actinobacteria and the genus *Streptomyces* at Station 5 (Gosthani estuary) were 36 x 10^2 cfu/g and 29 x 10^2 cfu/g respectively. The medium Glucose Asparagine Agar supported maximum number of (6) actionobacteria genera. The ISP 3 medium (Oat Meal Agar) harboured maximum number (27) of *Streptomyces* species. Majority (8) of the media supported *Streptomyces albus*. Species diversity indices (Simpson's Index D : 0.513 to 0.643; Shannon's Index: 0.755 to 1.3; Margalef's Index :0.303 to 1.058) values were calculated. Four species exhibited moderate antibacterial activity. The distribution of actinobacvteria is discussed in relation to the physicochemical parameters investigated.

***Key words*:** Actinobacteria, *Streptomyces*, estuaries, Andhra Pradesh,

Introduction

The Actinobacteria are phylogenetically related to high G + C group Gram positive bacteria of Eubacteria. They consist of a large group of branching , unicellular microorganisms and are found virtually on every natural substrate. Most of them are free-living saprophytes and are widely distributed in terrestrial and marine sediments. They show a marked chemical and morphological diversity to form a distinct evolutionary line of organisms. They are known for their significance in the production of secondary metabolites and have the capacity to synthesize wide range of biologically active compounds. Ramesh and William (2012) , reviewing the Marine Actinimycetes, quote that there are more than 22,000 known microbial secondary metabolites, 70% of which are produced by actinomycetes. Various approaches including classical chemo taxonomical, numerical taxonomic and molecular have been routinely employed for the identification of actinomycetes (Mukesh 2014). Earlier several researchers conducted investigations on the distribution of actinobacteria from different habitats of India and abroad. The important works include: Grein and Meyers (1958), Baam *et al* (1966), Postmaster and Freitas (1975), Lakshmanperumalsamy (1978), Lakshmanperumalsamy *et al* (1978), Manuel *et al* (1983), Ellaiah and Reddy (1987), Balagurunathan *et al* (1989), Jensen *et al* (1991 & 1995), Takizawa *et al* (1993), Balagurunathan (1992), Ratnakala and Chandrika (1995), Ghanem et al (2000), D'Souza *et al* (2000), Sivakumar (2001), Ellaiah *et al* (2002), Mathew and Philip (2003), Nathan *et al* (2004), Kokare *et al* (2004), Ismet *et al* (2004), Piza *et al* (2004) Ellaiah *et al* (2004), Sujatha et al (2005), Dhanasekaran et al (2005), Sivakumar *et al* (2005), Vijayakumar *et al* (2007), Bull and Stach (2007, review), Bredholdt *et al* (2007), Sivakumar *et al* (2007, review), Bredholdt *et al* (2008), Jacques *et al* (2008), Mitra *et al* (2008), Raghavendrudu (2008), Gupta *et al* (2009), Dhanasekaran *et al* (2009), Jiang (2009), Moldanado *et al* (2009), Suthindran & Kannabiran (2009), Hong *et al* (2009), Ramesh & Mathiavan (2009), Nithya and Pandyan (2010), Arifuzzaman *et al* (2010), Pugazhvendan *et al* (2010), Baskaran *et al* (2011), Santhi and Jebakumar (2011), Siva Kumar *et al* (2011), Krishnaraj and Narayanasamy (2011), Ramesh & William (2012, review), Sathya *et al* (2012), Sunil *et al* (2012), Sharma & David (2012), Rajkumar *et al* (2012), Mani (2012, review), Valli *et al* (2012), Saravanan *et al* (2013), Aparanji et al (2013), Gunasekaran and Thangavel (2013), Doralyn *et al* (2013), Panchanathan *et al* (2013, review), Deepthi *et al*

(2013), Sonashiya and Nandakumar (2013), Amayaly *et al* (2013), Lopamudra *et al* (2013), Mukesh (2014, review), Nazarian *et al* (2014, review) and Sengupta *et al* (2015).

The information available on actinobacteria, based on the earlier investigations, indicates paucity of data on the distribution of actinobacteria in the estuarine habitats of coastal Andhra Pradesh. Hence in the present study an attempt has been to study the distribution of actinobacteria in the near shore sediments with special attention to *Sytreptomyces* species in the Gosthani estuarine habitat (monthly sampling) and in other adjacent estuaries of coastal Andhra Pradesh (one-time sampling).

A :Actinobacteria Sampling Stations : St 1 to St 7

B :Actinobacteria Sampling Stations: St.8a to St.8e

Material and methods

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Sampling sites

Near-shore sediment samples for actinobacteria distribution were collected from twelve estuarine stations during 2015-2016. The location of sampling stations is presented in Plate 1a and 1b. The geographical location (Lat. and Long.) of these stations is given in Table 1. Regular monthly sediment samples for actinobacteria were collected from St. 5 (Gosthani estuary). One- time sampling was carried out at St. 1 (Mahendratanaya estuary), St. 2 (Vamsadhara estuary), St. 3 (Nagavali estuary), St. 4 (Champavathi estuary), St. 6 (Sarada estuary), St. 7 (Varaha estuary) and St. 8 (Gautami Godavari estuary: 8a: Etimoga, 8b: Chollangi, 8c: Matlapalem, 8d: Gaderu and 8e: Pillavarava) (Figs. 2.2 and 2.3). Except Gautami Godavri River, all other Rivers are seasonal in nature. Gautami Godavari estuary supports luxuriant growth of mangrove vegetation (dominant plants are: *Avicennia marina* and *Excoecaria agallocha*). The mud flats are covered with the grass *Porteresia coarctata*. Sarada and Varaha estuaries harbour dwarf mangrove plants (*Avicennia marina* and *Excoecaria agallocha)*. Gosthani estuary has no mangrove plants but salt marsh plants (*Suaeda maritima* and *Salicornia brachiata*) are present. Champavathi, Nagavali, Vamsadhara and Mahendratanaya do not possess any mangrove plants and salt marsh plants; but sparse grass vegetation is seen here and there. The lower reaches of all these estuaries support extensive brackish water aquaculture activities. In the study area the tides are semi-diurnal; tidal amplitude is 2 m; sediments are mainly clayey silt and shores are gradient at sampling stations. Seasonally, South West Monsoon (July-September) brings freshwater into the estuariesl. Low temperatures prevail during North East Monsoon (October to January). Summer season extends from February to June. . Table 2.1 provides the Latitudes and Longitudes of the Stations 1 to 8e.

Table 1 : Geographical location of sampling Stations 1 to 8e during study period.

S.NO	Stations	Latitude	Longitude
1	Mahendratanaya estuary (St 1)	84°35'41.27"E	18°52'39.45"N
2	Vamsadhara estuary (St 2)	84°07'29.11"E	18°20'26.81"N
3	Nagavali estuary (St 3)	83°56'34.49"E	18°17'51.86"N
4	Champavathi estuary (St 4)	83°33'59.27"E	18°00'49.57"N
5	Gosthani estuary (St 5)	83°27'07.93"E	17°54'07.00"N
6	Sarada estuary (St 6)	82°52'12.30"E	17°25'23.30"N
7	Varaha estuary (St7)	82°51'57.45"E	17°25'13.71"N
8	Etimoga (St 8a)	82°15'25.00"E	16°56'15.75"N
9	Chollangi (St 8b)	82°14'55.20"E	16°54'27.80"N
10	Matlapalem (St 8c)	82°15'25.61"E	16°53'11.36"N
12	Gaderu (St 8d)	82°18'51.61"E	16°50'51.28"N
13	Pillavarava (St 8e)	82°21'03.46"E	16°51'06.03"N

Sampling procedure and Processing

Five surface sediment samples, at each estuarine sampling station, were aseptically collected into sterile polythene bags during low tide period of Springs between 11 am and 3 pm on sampling days. The samples were kept cool until transported to laboratory for further processing. In addition, at each station samples/data were collected for physico-chemical parameters *i.e.* temperature, salinity, dissolved oxygen and pH. Temperature of sediment samples was measured using 0.1°C sensitivity hand-held mercury thermometer. Salinity and dissolved oxygen, of sediment water samples, were measured by Harvey method (Harvey, 1960) and Winkler's method (Schlieper, 1972) respectively. Water samples for dissolved oxygen were treated with Winkler's A and B reagents in the field. Sediment water pH was measured using a digital pH meter (Elico). Besides, surface sediment samples were collected into polythene bags for sedimentary organic matter studies. The sedimentary organic matter was estimated by chromic acid digestion method (Jackson 1967).

In the laboratory, the actinobacteria samples were processed using soil dilution technique (Haritha *et al* 2010). Each sediment sample (10g) was diluted with 100 ml of aged seawater (50% aged seawater and 50% deionized water) in a 250 ml conical flask. The conical flask was shaken on a rotary shaker for five minutes. The particulate matter was allowed to settle down. The suspension was used to inoculate the media plates (as spread plate technique) using 10-fold dilution. The sedimentary actinobacteria were cultured on 11 different agar media (HIMEDIA). They include: Yeast Malt Agar (ISP medium no. 2), Oat Meal Agar (ISP medium no. 3), Inorganic Salt Starch Agar (ISP medium no. 4), Glycerol Asparagine Agar (ISP medium no. 5), Peptone Yeast Extract Iron Agar (ISP medium no. 6), Tyrosine Agar (ISP medium no. 7), Kuster Agar, Glucose Asparagine Agar, Czapek's Agar, Starch Casein Agar and Glycine Glycerol Agar. These media were aseptically prepared using 50% sterile aged seawater. The media were supplemented with 5 µg/ml of rifampicin and 25 µg/ml of Nystatin (HIMEDIA) to minimize bacterial and fungal contaminations respectively. The inoculated plates were incubated for 7 to 14 days at 32°C (+/- 1°C) in a bacteriological incubator. The densities of actinobacteria were counted and expressed as Nos. x 10^2 cfu/g. Individual colonies were picked up and sub-cultured on Oat Meal Agar plates.. These cultures were checked for purity and maintained at 4°C. The purified isolates of actinobacteria were identified upto genus level using classical approach: cultural, morphological and biochemical characteristics (mycelium structure, colour and arrangement of conidiophores, spore chain morphology, reverse side pigment, temperature range of growth, pH tolerance, melanin production, various sugars. H2S production, coagulated serum, tyrosine reaction, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, milk coagulation and nitrate reduction) (Waksman 1953 & 1961; Krasilnikov 1964; Williams and Cross 1971, Nonomura 1974, Sneath *et al* 1987, Sneath and Williams 2002 and Goodfellow *et al* 2012). The Genus *Streptomyces* was identified up to species level as per International *Streptomyces* Project (Kuster 1972, Shrling

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and Gottlieb 1966, Locci 1989, Anderson & Wellington 2001, Kampfer 2012). Diversity indices (Simpson index, Shannon index and Margalef index) were calculated (Hammer *et al* 2001) for Actinobacteria genera and *Streptomyces* species at Station 5 (Gosthani estuary) only. The composition (g/1000 ml) of the actinobacteria media is: Yeast Malt Agar (ISP Medium No. 2; Peptic digest 5 g, Yeast extract 3 g, Malt Extract 3 g, Dextrose 10 g and Agar 20 g); Oat Meal Agar (ISP Medium No. 3; Oat meal 20 g, Agar 18 g and Trace salts solution 1 ml); Inorganic Salt Starch Agar (ISP Medium No. 4: :Starch solution 10 g, Dipotassium phosphate 1 g, Magnesium sulphate 1 g, Sodium chloride 1 g, Ammonium sulphate 2 g, Calcium carbonate 2 g and Agar 20 g); Glycerol Asparagine Agar (ISP Medium No. 5; Glycerol 10 ml, Asparagine 1 g, Sodium chloride 1 g, Ammonium lactate 1 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.12 g, Calcium chloride 0.05 g and Agar 20 g); Peptone Yeast Extract Iron Agar (ISP Medium No. 6; Peptone digest 15 g, Proteose peptone 5 g, Yeast extract 1 g, Ferric Ammonium citrate 0.5 g, Dipotassium phosphate 1 g, Sodium thiosulphate 0.08 g and Agar 15 g); Tyrosine Agar (ISP Medium No. 7; Tyrosine 0.5 g, Asparagine 1 g, Dipotassium phosphate 0.5 g, Magnesium sulphate 0.5 g, Sodium chloride 0.5, Ferrous sulphate 0.5 g, Glycerol 15 ml and Agar 20 g); Kuster Agar (Soluble starch 10 g, Casein hydrolysate 1 g, Sodium nitrate 1 g, Dipotassium phosphate 0.15 g, Magnesium carbonate 0.4 g, Calcium carbonate 2 g, Ferrous sulphate 10 mg and

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Agar 15 g); Glucose Asparagine Agar (Glucose 10 g, Asparagine 1 g, Dipotassium phosphate 1 g and Agar 15 g); Czapek's Agar (Sucrose 30 g, Sodium nitate 2 g, Dipotassium phosphate 0.5 g, Magnesium sulphate 0.5 g, Potassium chloride 0.5 g, Ferrous sulphate 0.01 g and Agar 15 g); Starch Casein Agar (Soluble starch 10 g, Casein hydrolysate 1 g, Dipotassium phosphate 0.5 g and Agar 15 g); Glycine Glycerol Agar (Glycerol 20 ml, Glycine 2 g, Dipotassium phosphate 1 g, Sodium chloride 2 g, Magnesium sulphate 0.5 g, Ferrous sulphate 0.1 g, Calcium carbonate 0.2 g and Agar 18 g). In the present study, a preliminary attempt has been made to study the antibacteriial activity of the pure cultures of *Streptomyces* spp., which showed antibacterial activity in the cultures. The antibacterial activity was tested on the five species of bacteria *i.e. Bacillus subtilis*, *Staphylocaccus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* which were obtained from King George Hospital, Visakhapatnam and were maintained in the laboratory during study period. The *Streptomyces* species were grown on Tryptone Yeast Extract (HIMEDIA) (ISP medium No. 1). Each *Streptomyces* species culture was inoculated separately in 100 ml of TYE medium in conical flasks. The inoculated flasks were incubated at room temperature for 14 days without agitation. After incubation, the bacterial colonies were filtered off and the filtrate was centrifuged at 5000 rpm to get cell-free and clear supernatant solution, which was taken for extraction. The active compounds were extracted in four

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solvents *i.e.* Chloroform, Ethyl ether, Ethyl acetate and Methanol. The culture filtrate and solvent were mixed in 1:3 ratio for extraction purpose. Then the solvent and culture filtrate mixture was shaken thoroughly for 15 minutes in a separating funnel and allowed to settle for another 15 minutes. After extraction with solvent, the solvent layer was separated and evaporated at 50 ± 2°C using a Rota Vapour. The residue was used for testing the antibacterial activity using test organisms by Agar Diffusion method (Casida 1968). The test organisms were grown on Muller-Hinton agar. The zone of inhibition (diameter) was measured after an incubation period of 24 hours.

Results and discussion

The distribution of environmental parameters for Station 5 and for Stations 1 to 4 and 6 to 8e is given in Tables 2a and 2b respectively. The distribution of actinobacteria genera at Stations 1 to 8e and at Station 5 (Gosthani estuary) are presented in Tables 3a and 3b respectively. The *Streptomyces* species recorded during the study period at Stations 1 to 8e and at Station 5 are provided in Tables 4a and 4b respectively. The diversity indices for actinobacteria genera and Streptomyces species are given Tables 5a and 5b respectively. The mean density distribution of actinobacteria genera and *Streptomyces* species of St 5 and Sts 1-4 & 6-8e are provided in Tables 6a and 6b respectively. Pearson correlation values between environmental parameters and actinobacterial densities are presented in Table 7. Table 8 presents the zones of inhibition recorded for four *Streptomyces* species against five test bacteria. An attempt has been made to to study the distribution of actinobacteria in relation to the different media used in the present study at Station 5 (Gosthani estuary) as the data are available for one year period at Station 5. The medium Glucose Asparagine Agar supported maximum number of (6) actionobacteria genera followed by the media ISP 6 (Peptone Yeast Extract Agar) (5 genera) and Starch Casein Agar (5 genera) during the present study period. The ISP 3 medium (Oat Meal Agar) harboured maximum number (25 species + 2 unidentified species) of *Streptomyces* spp. followed by ISP 3 medium (Tyrosine Agar) (22 species) and ISP 5 medium (Glycerol Asparagine Agar) (21 + 2 unknown species). Majority of the media supported *Streptomyces albus* (8 media) and *Streptomyces psammoticus* (5 media). The dominant species in the different media are as follows: ISP 2 (*S. catenulae*, *S. olivaceous* and *S. pyridomyceticus*), ISP 3 (*S.albus* and *S.psammoticus*), ISP 4 (*S.pristenaespiralis*, *S.griseolus*, *S.albus* and *S.macrosporeus*), ISP 5 (*S.bellus* and *S.orientalis*), ISP 6 (*S.albus* and *S,psammoticus*), ISP 7 (*S.psammoticus*, *S.candidus* and *S.gelaticus*), Czapek's Agar (*S.albus*, *S.alboniger* and *S.griseus*), Glucose Asparagine Agar (*S.psammoticus*, *S.albus* and *S.catenulae*), Glycine Glycerol Agar (*S.albus* and *S.catenulae*), Kuster Agar (*S.albus*) and Starch Casein Agar (*S.albus*, *S.griseus* and *S.psammoticus*). The mean density (Nos.x10^2 cfu/g) distribution of actinobacteria and the genus *Streptomyces* at St 5 is 37 and 32 respectively. The mean density (Nos.x10^2 cfu/g) distribution of actinobacteria and the genus *Streptomyces* at Sts 1-4 and 6-8e is 45 and 37 respectively. Station 8b registered maximum densities for actinobacteria (112) and the genus *Streptomyces* (105). Of the 143 species recorded,

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only four species exhibited moderate antibacterial activity. The Methanol (12 out of 20 observations showed antibacterial activity) and the Ethyl Alcohol (11 of 20) proved as good extraction agents when compared with Chloroform (6 of 20) and Ethyl Ether (6 of 20). But the inhibition zones (14 to 16 mm) observed are of moderate in size only.

Table 2a : Mean (n=5) distribution of environmental parameters at Station 5 (Gosthani estuary) sediments during June 2015 -May 2016.(Monthly sampling)

Date	T (°C)	D O (mg/l)	S (‰)	pH	OM (%)
14.06.2015	34.8	6.10	24.3	7.8	3.60
15.07.2015	30.1	6.60	17.0	7.6	4.10
15.08.2015	31.8	6.50	19.0	7.6	3.42
13.09.2015	34.1	5.40	23.0	8.4	3.90
14.10.2015	35.6	3.87	18.7	8.8	2.27
11.11.2015	30.6	3.43	21.4	8.1	2.90
10.12.2015	28.2	3.43	21.1	8.2	2.37
12.01.2016	31.7	5.33	19.3	7.7	3.22
08.02.2016	35.3	5.27	23.6	7.7	2.60
10.03.2016	30.1	4.20	20.1	8.1	3.10
04.04.2016	30.1	4.20	20.1	8.1	2.74
05.05.2016	31.3	5.17	17.2	7.8	2.44
Mean	**32.0**	**5.0**	**20.4**	**8.0**	**3.10**

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Table 2 b :Mean (n=5) distribution of Environmental Parameters at Stations 1 to 4 and Stations 6 to 8 sediment during June 2015-May 2016.(One time Sampling)

Stations	Date	T (°C)	D O (mg/l)	S (‰)	pH	OM (%)
St 1	9.12.2015	27.4	4.60	13.6	8.2	1.93
St 2	03.07.2015	32.2	4.73	13.5	7.7	3.10
St 3	11.10.2015	35.4	5.80	22.2	7.4	2.13
St 4	24.11.2015	29.8	3.73	26.6	8.1	2.33
St 6	24.09.2015	34.4	4.28	6.2	7.6	3.55
St 7	24.09.2016	33.6	4.28	4.3	7.8	3.60
St 8a	13.06.2015	34.6	5.00	32.7	7.6	3.27
St 8b	24.08.2015	33.7	4.77	6.2	8.5	4.06
St 8c	08.03.2016	28.4	4.25	9.2	7.7	4.25
St 8d	26.07.2016	29.2	6.20	11.0	8.0	5.10
St 8e	07.02.2016	28.4	4.10	30.2	8.2	4.30

Table 3a : Distribution of Actinobacteria genera in different months at Stations 1 to 8e during 2015 – 16

Genus	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8a	St 8b	St 8c	St 8d	St 8e
Actinomyces	+	+	-	+	+	+	-	+	+	+	+	+
Actinoplanes	+	+	-	+	+	-	+	-	-	-	+	-
Kibdelosporangium	-	-	-	-	+	-	-	-	-	-	-	-
Microbispora	-	-	-	-	+	-	-	-	-	-	-	-
Micromonospora	+	+	+	+	+	+	+	+	+	+	+	+

Microtetraspora	-	-	-	-	+	-	-	-	-	+	-	-
Saccharomonospora	-	-	-	+	-	+	-	-	-	+	-	-
Saccharopolyspora	-	-	+	+	+	-	-	-	+	-	-	-
Streptomyces	+	+	+	+	+	+	+	+	+	+	+	+
Streptosporangium	-	-	-	-	+	-	-	-	+	+	+	-
Thermomonospora	-	-	-	-	+	-	-	-	-	+	-	-
No. of Genera	4	4	3	6	10	4	3	3	5	7	5	3

Table 3b :Distribution of Actinobacteria genera a tStation 5 during 2015 – 16.

Genus	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Actinomyces	+	-	-	-	+	+	+	+	+	+	+	+
Actinoplanes	+	-	-	-	+	+	-	-	-	+	+	+
Kibdelosporangium	-	-	-	-	-	-	-	-	-	+	+	-
Microbispora	-	-	-	+	-	-	-	-	-	-	-	-
Micromonospora	+	+	-	+	+	+	+	+	+	+	+	+
Microtetraspora	-	-	-	+	-	-	-	-	-	-	-	+
Saccharopolyspora	-	-	-	-	-	+	-	-	-	+	-	+
Streptomyces	+	+	+	+	+	+	+	+	+	+	+	+
Streptosporangium	-	-	+	+	-	-	-	-	-	-	-	-
Thermomonospora	-	-	-	-	-	-	-	-	-	-	-	+
No. of Genera	4	2	2	5	4	5	3	3	3	6	5	7

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Generic Composition: The present study recorded 11 genera of actinobactera *i.e. Actinomyces,Actinoplanes, Kibdellosprangium, Microbispora, Micromonospora,Microtetraspora, Saccharomonospora, Saccharopolyspora, Streptomyces, Streptosporangium* and *Thermomonospora* in all the 12 stations. Of these, 10 genera are present at Station 5 (Gosthani estuary), which was sampled at monthly intervals. Summer months (March & May) supported maximum number (7) of genera. Flood period (July and August) harboured less number (2) of genera. At other Stations *i.e.* Sts 1-4 & 6-8e (which were sampled once only), some of these genera are only observed. Maximum number (7) genera are observed at Station 8c (Matlapalem), which was sampled in March (Summer month). Stations sampled during Flood Period i.e. Sts. 6 & 7 registered less number (3) of genera. The genera *Streptomyces* and *Micromonospora* are recorded at all 12 Stations indicating that they are the dominant and ubiquitous genera in the estuarine actinobacterial assemblages. The genus *Kibdellosporangium* is observed at Station 5 (Gosthani estuary) only during Summer months indicating its rareness. The genera *Microbispora, Microtetraspora, Sachharopolyspora, Streptosporangium* and *Thermomonospora* also exhibited restricted distribution by confining themselves to certain months. The diversity indices for actinobacteria genera (Fig. 3.65) further substantiate our observations that generic diversity is high in May and low August months. Sivakumar *et al* (2007), reviewing the research on marine actinobacteria in India, concludes: “Forty years of floristic inventory of marine actinobacteria in Indian Peninsula yielded 41 species belonging to 8 genera". Vijayakumar *et al* (2007) observed 68 isolates from the Palk Strait region of Bay of Bengal, which include *Streptomyces* (38), *Actinopolyspora*(10), *Saccharopolyspora (7)*, *Actinomadura* (4), *Nocardiopsis* (3), *Micromonospora* (2), *Actinoplanes* (1), *Microbispora* (1) and *Actinomyces*(1). The genera *Actonopolyspora*, *Actinomadura* and *Nocardiopsis* are not recorded in the present study, which may be attributed to habitat variation as the Palk Strait is a high saline habitat (> 50 ppt) when compared with present study of estuarine habitats. Bredholdt (2008) recorded *Streptomyces*-like actinobacteria and *Micromonospora* in the near shore shallow water sediments of the Trondheim fjord, Norway. Mitra *et al* (2008) examined the relationship between distribution of actinomycetes and antagonistic behavior with the physicochemical characteristics of the Sundarbans, India. They isolated the highest number of actinomycetes from an intertidal region having alluvial soil and the lowest from a site containing sandy sediments. In the present investigation also the low (25 x 10^2 cfu/g; St 3 Sandy shore) and high (112 x 10^2 cfu/g; St 8b Mangrove mudflat) densities of Actinobacteria are recorded in the sandy shore and alluvial Mangrove mudflat respectively. But at St 8d, which is also an alluvial Mangrove mudflat, recorded very low (21 x 10^2 cfu/g;) density of Actinobacteria. Moldanado *et al* (2009) recorded *Actinomadura*, *Nonomuraea*, *Saccharomonospora*, *Saccharopolyspora* *Streptomyces* and *Micromonospora* from Gulf of California and *Micromonospora* and *Streptomyces* from Gulf of Mexico sediments. The genera *Actinomadura*, *Nonomuraea* and

Saccharomonospora are not observed in the present study and their absence may be attributed to habitat variation. They suggested that Actinobacteria are an indigenous part of the microbial community in the marine ecosystem and the ''Micromonospora- Rhodococcus- Streptomyces'' grouping should definitively be revisited. They further stated that "there is indeed an urgent need to improve traditional selective isolation methods to recover the untapped majority of microbes not only from the sea but also from terrestrial samples but, at the same time, ''old'' culture/selective isolation media can still help us to recover putative novel species from one of the most important biotechnologically microbial groups studied worldwide". Suthindhran & Kannabiran (2009) recorded the genera *Streptomyces, Micromonospora, Actinopolyspora* and *Saccharopolyspora* from shallow water sediments of Marakkanam, south east coast of Tamilnadu. All these genera, except *Actinopolyspora* are observed in the present study. Hong *et al* (2009) recorded 12 genera *i.e. Actinoplanes*, *Actinomadura*, *Asanoa*, *Actinomadura*, *Gordonia*, *Microbispora*, *Micromonospora*, *Nocardia*, *Nonomuraea*, *Rhodococcus*, *Streptomyces* and *Verrucosispora* from mangrove soils and plants in China. Ramesh and Mathiavan (2009) collected 98 actinomycetes from marine sediments, five from seawater and nine from marine animals (star fishes, mollusks and sea urchins), 43 from Pulicat lake samples (30 from sediments and 13 from brackish water), 22 from mangroves (15 from sediments & 7 from mangrove rhizospheres), 18 from deep sea sediments, one strain from deep seawater, 7 seven from estuary sediments and 4 from estuary water samples, using SCA (Starch Casein Agar) as selective medium prepared in aged seawater. Among 208 isolates, 115, 79, 6, 7 and 1 isolates were grey, white, blue, pink and orange pigmented respectively. Interestingly, grey and white mycelial pigmented marine actinomycetes were prominent in the Bay of Bengal. Further, out of 208 isolates, 6 produced diffusible pigment on SCA agar and 58 isolates produced EPS. These pigments and EPS production could be protective mechanisms for actinomycetes to survive in the hostile marine environment. The present study also substantiates their observation. Grey (48), white (19), yellow (18), green (11), blue (9) and red (8) actinobacteria colonies are recorded in the present study, which indicate the dominance of grey and white colonies. However in the present study Glucose asparagine agar supported maximum number (6) genera. Arifuzzaman *et al* (2010) identified four genera *i.e. Actinomyces*, *Nocardia*, *Micromonospora* and *Streptomyces*, with dominance of *Actinomyces* in the the mangrove sediments of Karanjil area of Sundarbans (Bangladesh). All the genera are recorded in the present study except *Nocardia*. But in the present study the genera *Streptomyces and Micromonospora* are dominant than the genus *Actinomyces.* Grein and Meyers (1958), Lakshmanaperumalswamy, (1978), Sivakumar (2001) and Rajkumar et al (2012) also reported similar observations i.e. the dominance of Streptomyces (80%) with associated genera (*Sacchropolyspora* and *Micromonospora).* Sathya *et al* (2012) recorded 4 genera (*Streptomyces*, *Pseudonocardia*, *Actinoplanes* and , *Sporichthya*) from Muthupet mangrove sediments of Tamilnadu coast. Sharma and David (2012) recorded 3 genera *Streptomyces* (60%), *Actinopolyspora* (35%) and *Nocardiodes* (5%) contributed 35%

(104 isolates) and 5% (11 isolates) from Pulicat, Muthukad and Ennore estuaries. Of these, *Actinopolyspora* and *Nocardiodes* are not recorded in the present syudy. But the dominance of Streptomyces genus is noted in both (the present study and the 3 estuaries) habitats. The absence of the genera *i.e. Actinopolyspora* and *Nocardiodes* in the present study may be due to inadequate sampling at Stations 1-4 and 6-8e (one time sampling). Rajkumar *et al* (2012) isolated 116 actinobacterial colonies from 30 mangrove sediment samples of Bhitarkanikka, Orissa. Forty three isolates were assigned to the genera: *Streptomyces*, *Saccharopolyspora* (5), *Nocardiopsis* (5), *Micromonospora* (3), *Actinomadura* (5), *Actinomycetes* (1), (*Actinopolyspora* from the mangrove sediments of Bhitarkanikka, Orissa. Of these *Nocardiopsis*, *Actinomadura*, *Actinomycetes* and *Actinopolyspora* are not recorded in the present investigation. Their absence in the present study may be due to insuffient (one-time) sampling in the Godavari mangroves. Amayaly *et al* (2013) report 5 genera *i.e. Micromonospora*, *Saccharomonospora*, *Streptomyces*, *Verrucosispora* and *Actinomadura* in the off shore sediments of Gulf of California. Of these, only two genera (*Micromonospora* and *Streptomyces)* are observed in the present study. In the present study, insignificant correlations between environmental parameters and densities of actinobacteria are observed. The correlations between temperature, dissolved oxygen & organic matter and actinobacterial densities are only (insignificant) positive correlations. The correlation between salinity, pH and organic content of marine sediments and actinomycetes population has been reported by several workers(Jensen *et al* 1991, Ndonde *et al* 2000 and Vijayakumar

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2007). Ghanem *et al*. (2000) reported that the variation in temperature, pH and dissolved phosphate showed insignificant values, but variation in total nitrogen and organic matter was significant in the population in Alexandria. Hence it could be concluded that though actinomycetes are ubiquitous, their population dynamics is often influenced by the available nutrients and the physico-chemical conditions of the ecosystem.

***Streptomyces* species Composition:** The study registered the occurrence of 143 species of *Streptomyces*, which includes 30 unidentified species. Of these, 79 species were recorded at Station 5 (Gosthani estuary :12 month sampling) besides 17 unidentified species. Among these 79 species, 24 species were exclusively observed at Station 5. Further of these 79 species, 55 species are also observed in other (Sts. 1, 2, 3, 4, 6, 7 and 8a to 8e) stations in the present study. The species *S.albus* is observed throughout the year except in October month. The species *S.atroolivaceus, S.catenulae, S.griseus, S.olivaceous, S.bellus, S.psammoticus* are common at Station 5 occurring a minimum of six months during study period. Half (40 species) of the species are recorded only once during the study period. The *Streptomyces* species diversity indices (Simpson, Shannon and Margalef indices) are also high in July (Flood Period) and low in March (Summer Period) at this Station 5 (Gosthani estuary). Besides, it is observed in the present study the actinobacteria generic diversity and *Streptomyces* species diversity are inversely related. Whenever there is the dominance of *Streptomyces* species, it is associated with low numbers of actinobacteria genera. In Stations 1 to 4 and 6 to 8e (one time sampling stations), 89 species of *Streptomyces* were identified. In addition, there are 13 unidentified species. Of these species, 34 species were exclusively observed at specific stations (Sts 1-4 and 6-8e). Five species at Station 1, six species at Station 2, two species at Station 3, three species at Station 4, one species at Station 6, one species at Station 7, ten species at Station 8a, six species at Station 8b, eleven species at Station 8c, one species at Station 8d and one species at Station 8e are exclusively observed in those stations only. In one time sampling stations, Station 8a (Etimoga) harboured maximum number (39) of *Streptomyces* species, followed by Station 8b (Chollangi, 34 species). Both these stations 8a and 8b are part of the Gautami-Gofdavari estuarine system that supports the The Coringa Mangrove Ecosystem. The 89 species of *Streptomyces* reported in the present study at Stations 1-4 and 6-8e may not form the total species composition at these stations as they were sampled only once. Their actinobacterial diversity may be high as they are the estuarine (Sts. 6 and 7) and deltaic (Sts. 8a to 8e) mangrove ecosystems. Further investigations in these areas may yield more information on actinobacteria. Sahu *et al* (2007) recorded 6 species of *Streptomyces* i.e. *Streptomyces xantholiticus*, *S. aureofasciclus*, *S. galtieri*, *S. vastus*, *S. galbus*, and *S. rimosus* from surface water and sediment samples collected from eight stations off Little Andaman Island. Raghavendrudu (2008) recorded six species of actionobacteria from Meghadri mangrove sediments, Visakhapatnam. They include *Streptomyces albovinaceous*, *S. flavoviridis*, *S. griseus*, *S. lucitannous*, *S. nigrifaciens and S. parvulus.* Of these six species two species *i.e. Streptomyces flavoviridis* and *S. lucitannous* are not recorded in the present study. Gupta *et al* (2009) identified (on the basis of their morphology, biochemical characteristics, growth behavior, utilization of carbon and nitrogen, antibiotic susceptibility and special activity) 20 species i.e. *Streptomyces albidoflavus*, *S. atroolivaceus*, *S. auranticus*, *S. canus*, *S. chromofuscus*, *S. exfoliates*, *S. griseoluteus*, *S. helstedii*, *S. lavenduale*, *S. longisporoflavus*, *S. luridus*, *S. lydicus*, *S. nogalator*, *S. pactum*,*S. prasinosporus*, *S. purpureus*, *S. tubercidus*, *S. versoviensis*,*S. viridochromogenes* and *S. xanthochromogenes* from different locations and plant sources in Bhitarkanika mangroves, Orissa. *Streptomyces auranticus*, *S. exfoliates*, *S. griseoluteus*, *S. helstedii*, *S. lavenduale*, *S. longisporoflavus*, *S. luridus*, *S. lydicus*, *S. nogalator*, *S. pactum*,*S. prasinosporus*, *S. purpureus*, *S. versoviensis*, and *S. viridochromogenes* are not recorded in the present study. The absence of these species in the present study may be due to sampling limitations (one time sampling in the Godavari Mangrove estuary) and local variations in physico-chemical parameters. Nithya *et al* (2010) reported 4 species i.e. *Streptomyces albus*, *Streptomyces rangoonensis*, *Streptomyces gibsonii and Streptomyces flocculus* from sediments of Palk Bay. The two species *i.e. Streptomyces rangoonensis* and *Streptomyces gibsonii* are not observed in the present study. Sengupta *et al* (2015) recorded eight species of *Streptomyces* (*Streptomyces variabilis*, *Streptomyces erythrogriseus* , *Streptomyces atrovirens*, *Streptomyces albogriseolus* , *Streptomyces griseorubens*, *Streptomyces labedae* , *Streptomyces coelicoflavus* and *Streptomyces lusitanus*) from Sundarbans, West Bengal. They state that the high anthropogenic pressure (i.e., oil leakage, agricultural wastes, and commercial market) in the Gadkhali area of Sundarbans may be the reason for low diversity of actinomycetes in that area. As many of the researchers paid attention to antimicrobial activity than the diversity of *Streptomyces* species, the available literature on *Streptomyces* species diversity is limited. It may be concluded that the estuaries may support high diversity of *Streptomyces* species when compare with the other habitats like off shore sediments.

Density Distribution: At Station 5 (Gosthani estuary), low (20 x 10^2 cfu/g) and high (58 x 10^2 cfu/g) densities of actinobacteria are recorded during Flood Period and Summer Period. Since the genus *Streptomyces* is the dominant genus at this Station 5, its densities governed the density distribution of actinobacteria. The mean densities of Actinobacteria and the genus *Streptomyces* are 36 x 10^2 cfu/g and 29 x 10^2 cfu/g respectively. At Stations 1 to 8e, both the low (16 x 10^2 cfu/g, Station 8e) and high (112 x 10^2 cfu/g, Station 8b) densities of Actinobacteria are recorded in the mangrove ecosystem. The genus *Streptomyces* also exhibited similar trends with low (11x 10^2 cfu/g) and high (105x 10^2 cfu/g) densities in the mangrove stations with a mean value of 37x 10^2 cfu/g. Studies on the mean density distribution of of actinobacteria are meager. Takizawa *et al* (1993) investigating the shallow sediments of Chesapeake Bay, USA observed high densities (140.0 x 10^3 cfu/g) of *Actinoplanes.* Sahu *et al* (2007) records the densities of Actinibacteria from 12 x 10^2 cfu/g at Station 2 (Naval area) to 33 x 10^2 cfu/g at Station 6 (Buttler Bay) in Andaman Islands. These values are also relatively lower than the density values recorded in the present study. Raghavendrudu (2008) records the mean densities of actinobacteria that range from 7 x 10^2 cfu/g (Meghadri mangroves, Visakhapatnam and Godavari mangroves, Kakinada) to 13 x 10^2 cfu/g (Mollagunta-Krishna mangroves). These densities are relatively low when compared with the present study density values of actinobacteria. He attributed these low densities to low temperatures and low salinities prevailing at that time. Dhanasekaran *et al* (2009) observed high densities of *Streptomyces* in the coastal areas (2700 to 3000 x 10^2 cfu/g) than in the estuarine (400 to 800 x 10^2 cfu/g) sediments. Their *Streptomyces* densities are higher than the densities observed in the present study. In similar studies, actinomycete densities in sediment samples from marine ecosystems reached 0-1500 x 10^2 cfu/g (Cochin, India; Ratnakala and Chandrika, 1995) and 100-400 x 10^2 cfu/g (Pichavaram mangroves,Tamilnadu, India; Sivakumar et al., 2005).

Table 4a : Distribution of *Streptomyces* species at Stations 1 to 8e during 2015 – 16.

S.No	*Species*	St 1	St 2	St 3	St 4	St 5	St 6	St 7	St 8a	St 8b	St 8c	St 8d	St 8e
1	*S.aburaviensis*	-	-	-	-	+	-	-	+.	+.	-	+.	-
2	*S.achromogenes*	-	-	-	-	+	-	-	-	+.	-	-	-
3	*S.acrimycini*	-	-	-	-	+	+.	-	-	-	+.	-	+.
4	*S.albidoflavus*	-	-	-	-	+	-	-	+.	-	-	-	-
5	*S.albocyaneus*	-	-	-	-	+	-	-	-	-	-	-	-
6	*S.albofaciens*	-	-	-	-	+	-	-	-	-	-	-	+.
7	*S.albogriseolus*	-	-	-	-	+	-	-	+.	-	-	-	-
8	*S.alboniger*	+	+	-	-	+	-	-	-	+.	-	+.	-

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9	*S.albovinaceus*	-	-	-	-	+	-	-	-	-	-	-	-
10	*S.alboviridis*	-	-	-	+	+	-	-	-	-	-	-	-
11	*S.albus*	+	+	+	+	+	+.	+.	+.	+.	-	+.	+.
12	*S.amakusaensis*	-	-	+	-	-	-	-	-	-	-	-	-
13	*S.arabicus*	-	-	-	-	+	-	-	+.	+.	-	-	-
14	*S.argenteolus*	-	+	-	-	-	-	-	+.	-	+.	-	-
15	*S.atroolivaceus*	-	+	+	+	+	+.	-	+.	+.	+.	+.	-.
16	*S.auraviensis*	-	-	-	-	+	-	-	-	-	-	-	-
17	*S.aureocirculatus*	-	-	-	-	-	-	-	+.	-	-	-	-
18	*S.aureofasciculus*	-	-	-	-	+	-	-	-	-	-	-	-
19	*S.aureofaciens*	-	-	-	-	-	-	-	-	-	+.	-	-
20	*S.aureoverticillatus*	+	-	-	-	+	+.	-	-	-	-	-	-
21	*S.azureus*	-	+	-	-	-	-	-	+.	-	-	-	+.
22	*S.bellus*	+	+	-	-	+	-	-	-	-	-	-	+.
23	*S.bicolor*	-	-	-	-	+	-	-	+.	-	-	-	-
24	*S.bottropensis*	-	+	-	-	+	-	-	-	-	-	-	-
25	*S.bungoensis*	-	-	-	-	+	-	-	-	-	-	-	-
26	*S.candidus*	+	+	-	-	+	-	-	+.	+.	-	+.	-
27	*S.canus*	-	-	-	-	+	-	-	-	+.	-	-	-

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28	*S.carnescens*	-	-	-	-	-	-	-	+.	+.	-	+.	-
29	*S.catenulae*	+	+	+	+	+	+.	-	+.	+.	+.	-	+.
30	*S.chartreusis*	-	-	-	-	-	-	-	-	+.	-	-	-
31	*S.chromofuscus*	-	-	-	-	+	-	-	-	-	-	-	-
32	*S.citreofluorescens*	+	-	-	-	+	-	-	-	-	-	-	-
33	*S.citreus*	-	-	+.	-	+	+.	+.	+.	+.	-	-	-
34	*S.coeliatus*	-	-	-	-	+	-	-	-	-	-	-	-
35	*S.coelicolor*	-	-	-	-	+	-	-	-	-	-	-	-
36	*S.coeruleofuscus*	-	+	-	-	-	-	-	-	-	-	-	-
37	*S.collinus*	-	-	-	-	+	-	-	-	-	-	-	-
38	*S.craterifer*	-	-	-	-	+	-	-	+.	+.	-	-	-
39	*S.chrseus*	+	-	-	-	-	-	-	-	-	-	-	-
40	*S.curacoi*	-	-	-	-	+	-	-	-	-	-	-	-
41	*S.cyanoalbus*	-	-	-	-	-	-	-	-	-	+.	-	-
42	*S.cyanocolor*	-	-	-	-	+	-	-	-	-	-	-	-
43	*S.filipinensis*	-	-	-	-	+	-	-	-	-	-	-	-
44	*S.flaveolus*	-	-	-	-	+	-	-	+.	+.	+.	-	-
45	*S.flavogriseus*	-	-	-	-	+	+.	+.	+.	+.	-	-	-
46	*S.flavovirens*	-	+	-	-	+	-	-	-	-	-	-	-

47	*S.flocculus*	-	-	-	-	+	-	-	+.	-	-	-	-
48	*S.flulvoviridis*	-	+	-	+	+	-	+	+	+	-	+	-
49	*S.galbus*	-	-	-	-	-	-	-	-	-	+.	-	-
50	*S.gelaticus*	-	-	-	-	+	-	-	-	-	-	-	-
51	*S.glaucescens*	-	-	-	-	+	-	-	-	-	+.	-	-
52	*S.griseoflavus*	-	-	-	-	+	-	-	+.	-	-	-	-
53	*S.griseoloalbus*	-	+	-	-	+	-	-	-	-	-	-	-
54	*S.griseolus*	+	+	-	-	+	-	+.	+.	+.	-	-	-
55	*S.griseomycini*	-	-	-	-	+	-	-	-	-	-	-	-
56	*S.griseoplanus*	+	-	-	-	+	-	-	+.	-	-	-	-
57	*S.griseoruber*	-	-	-	-	-	-	+.	-	-	-	-	-
58	*S.griseostramineus*	-	-	-	-	+	-	-	-	-	-	-	-
59	*S.griseoubeus*	-	-	-	-	+	-	-	-	-	-	-	-
60	*S.griseoviridis*	-	-	-	-	+	-	-	-	-	-	-	-
61	*S.griseus*	+	-	-	-	+	+.	+.	+.	-	-	-	-
62	*S.heimi*	-	-	-	-	+	-	-	-	+.	-	-	-
63	*S.herbaricolor*	-	-	+.	+.	-	-	-	+.	+.	-	+.	-
64	*S.humidus*	-	-	-	-	-	-	-	-	-	+.	-	-
65	*S.indigoferus*	-	-	-	+.	-	-	-	-	-	-	-	-

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66	*S.ipomea*	-	-	-	-	+	-	-	-	-	-	-	-
67	*S.janthinus*	-	+	-	-	+	-	-	-	-	-	-	-
68	*S.levoris*	-	-	-	+.	-	-	-	-	-	-	-	-
69	*S.litmocidini*	-	-	-	-	-	-	-	-	-	-	+.	-
70	*S.luteofluorescens*	+	-	-	-	-	-	-	-	-	-	-	-
71	*S.macrosporeus*	+	-	-	-	+	-	-	-	-	-	-	-
72	*S.microflavus*	-	-	-	-	+	-	-	-	-	+.	-	-
73	*S.minoensis*	-	+	-	+	+	-	+.	+.	+.	-	+.	-
74	*S.mutabilis*	-	-	-	-	+	-	-	-	+.	-	-	+.
75	*S.naganishii*	-	-	-	-	+	-	-	+.	-	-	-	-
76	*S.nigrifaciens*	-	+	-	-	+	-	-	+.	+.	+.	-	+.
77	*S.nitrosporeus*	-	+	-	+	+	-	-	+.	-	-	-	-
78	*S.olivaceoviridis*	+	-	-	-	-	-	-	-	-	-	-	-
79	*S.olivaceus*	+	+	+	+	+	+.	+.	+.	+.	+.	+.	+.
80	*S.olivoverticillatus*	-	-	-	-	+	-	-	-	-	-	-	-
81	*S.olivoviridis*	-	-	-	-	+	-	+.	-	-	-	-	-
82	*S.orientalis*	+	+	-	+	+	-	-	-	+.	-	-	-
83	*S.parvulus*	-	-	-	+.	-	+.	-	-	-	-	-	-
84	*S.pilosus*	-	-	+.	-	-	-	-	+.	-	-	-	-

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85	*S.pluricolonescens*	-	-	-	-	+	-	-	-	-	-	-	-
86	*S.prasinopilosus*	-	-	-	-	-	-	-	-	-	+.	-	-
87	*S.prasinus*	-	-	-	-	+	-	-	-	+.	-	-	+.
88	*S.pristinaespiralis*	-	-	-	-	+	-	-	-	-	-	-	-
89	*S.psammoticus*	+	+	-	+	+	-	-	+.	+.	-	+.	+.
90	*S.pseudogriseolus*	-	-	-	-	-	-	-	+.	+.	-	-	-
91	*S.puniceus*	-	-	-	-	+	-	-	+.	+.	-	-	-
92	*S.purpeofuscus*	-	-	-	-	+	-	-	-	+.	-	-	-
93	*S.pyridomyceticus*	-	-	-	-	+	-	-	+.	+.	-	-	-
94	*S.recifensis*	-	-	-	-	+	+.	-	-	-	-	-	-
95	*S.rimosus*	-	-	+.	-	+	-	-	-	-	-	-	-
96	*S.rochei*	-	-	-	-	-	-	-	-	-	+.	-	-
97	*S.roseochromogenus*	-	-	-	-	-	-	-	+.	-	-	-	-
98	*S.roseofulvus*	-	-	-	-	+	-	-	-	-	-	-	-
99	*S.roseus*	-	+	-	-	+	-	-	+.	+.	-	-	-
100	*S.rubiginosohelvolus*	-	-	-	-	+	-	-	-	-	+.	-	-
101	*S.rubiginosus*	-	+	-	-	-	-	-	-	+.	+.	+.	-
102	*S.scabies*	+	+	-	+	+	-	+.	-	+.	-	-	-
103	*S.setonii*	-	-	-	-	+	-	-	-	-	-	-	-

104	*S.sparsogenes*	-	-	-	-	-	-	-	+.	-	-	-	-
105	*S.spiroverticillatus*	-	+	-	-	-	-	-	-	-	-	-	-
106	*S.sprasinus*	-	-	-	-	+	-	-	-	-	-	-	-
107	*S.tendae*	-	-	-	-	-	-	-	-	+.	-	-	-
108	*S.thermovulgaris*	-	-	-	-	-	-	-	-	-	+.	-	-
119	*S.tubercidicus*	-	-	-	-	+	-	-	-	-	-	-	-
110	*S.umbrinus*	-	-	-	-	-	-	-	-	-	+.	-	-
111	*S.varsoviensis*	-	-	+.	-	-	-	-	+.	-	-	-	-
112	*S.vastus*	-	-	-	-	-	-	-	+.	-	-	-	-
113	*S.xanthochromogenus*	-	+	-	-	-	-	+.	-	-	-	-	-
114	*S.sp01*	-	-	-	-	+	-	-	-	-	-	-	-
115	*S.sp02*	-	-	-	-	+	-	-	-	-	-	-	-
116	*S.sp03*	-	-	-	-	+	-	-	-	-	-	-	-
117	*S.sp04*	-	-	-	-	+	-	-	-	-	-	-	-
118	*S.sp05*	-	-	-	-	+	-	-	-	-	-	-	-
119	*S.sp06*	-	-	-	-	+	-	-	-	-	-	-	-
120	*S.sp07*	-	-	-	-	+	-	-	-	-	-	-	-
121	*S.sp08*	-	-	-	-	+	-	-	-	-	-	-	-
122	*S.sp09*	-	-	-	-	+	-	-	-	-	-	-	-

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123	*S.sp10*	-	-	-	-	+	-	-	-	-	-	-	-
124	*S.sp11*	-	-	-	-	+	-	-	-	-	-	-	-
125	*S.sp12*	-	-	-	-	+	-	-	-	-	-	-	-
126	*S.sp13*	-	-	-	-	+	-	-	-	-	-	-	-
127	*S.sp14*	-	-	-	-	+	-	-	-	-	-	-	-
128	*S.sp15*	-	-	-	-	+	-	-	-	-	-	-	-
129	*S.sp16*	-	-	-	-	+	-	-	-	-	-	-	-
130	*S.sp17*	-	-	-	-	+	-	-	-	-	-	-	-
131	*S.sp 18*	+	-	-	-	-	-	-	-	-	-	-	-
132	*S.sp 19*	-	-	-	-	-	-		-	+	-	-	-
133	*S.sp 20*	-	-	-	-	-	-	-	-	+	-	-	-
134	*S.sp 21*	-	-	-	-	-	-	-	-	+	-	-	-
135	*S.sp 22*	-	-	-	-	-	-	-	-	+	-	-	-
136	*S.sp 23*	-	-	-	-	-	-	-	-	+	-	-	-
137	*S.sp 24*	-	-	-	-	-	-	-	-	+	-	-	-
138	*S.sp 25*	-	-	-	-	-	-	-	-	-	-	-	+
139	*S.sp 26*	-	-	-	+	-	-	-	-	-	-	-	-
140	*S.sp 27*	-	+	-	-	-	-	-	-	-	-	-	-
141	*S.sp 28*	-	-	-	-	-	-	-	-	+	-	-	-
142	*S.sp 29*	+	-	-	-	-	-	-	-	-	-	-	-
143	*S.sp 30*	-	-	-	-	-	-	-	-	-	-	+	-

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Total known	18	26	10	15	79	11	12	39	34	19	13	11
Total Unknown	2	1	0	1	17	0	0	0	7	0	1	1

Table 4b :Monthly distribution of *Streptomyces* species at Station 5 (Gosthani estuary) during June 2015 – May 2016.

Species	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
S.aburaviensis	-	-	+	+	-	-	-	-	-	-	-	-
S.acrimycini	-	-	-	-	-	-	+	+	-	+	-	-
S.achromogenes	-	+	-	-	-	-	-	-	-	-	-	+
S.albidoflavus	-	-	-	-	-	-	-	-	+	-	-	-
S.albocyaneus	-	-	-	-	-	-	+	-	-	-	-	+
S.albofaciens	-	-	-	-	-	-	+	-	-	+	-	-
S.albogriseolus	-	-	-	-	+	-	-	-	-	-	-	-
S.alboniger	+	-	+	-	-	-	+	-	-	+	+	-

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S.albovinaceus	-	-	-	-	-	-	-	+	+	+	-	-
S.alboviridis	-	-	-	+	-	-	-	-	-	-	-	-
S.albus	+	+	+	+	-	+	+	+	+	+	+	+
S.arabicus	-	-	-	-	-	-	-	-	-	-	+	-
S.atroolivaceus	+	+	+	+	-	-	-	+	+	-	-	+
S.auraviensis	-	-	-	-	-	-	-	+	-	-	-	-
S.aureofasciculus	-	+	-	-	-	-	-	-	-	-	-	-
S.aureoverticillatus	-	-	-	-	-	-	+	-	-	-	-	-
S.bellus	+	+	+	-	+	-	-	-	-	-	+	+
S.bicolor	-	-	-	-	+	-	-	-	-	-	+	-
S.bottropensis	-	-	-	-	-	-	-	-	-	+	-	-
S.bungoensis	-	+	-	-	-	-	-	-	-	-	-	-
S.candidus	+	-	-	-	-	-	-	+	+	+	-	-
S.canus	+	-	-	-	-	-	-	-	-	-	-	-
S.catenulae	+	+	-	+	-	+	-	+	+	-	+	-
S.chromofuscus	+	-	-	-	-	-	-	-	-	-	-	-
S.citreofluorescens	-	-	-	-	+	-	-	-	-	-	-	-
S.citreus	+	-	-	-	-	-	-	-	+	-	-	-
S.coeliatus	-	-	+	-	-	-	-	-	-	-	-	-
S.coelicolor	+	-	-	-	-	-	-	-	-	-	-	-
S.collinus	-	+	-	-	-	-	-	-	-	-	-	-

S.craterifer	-	-	+	-	-	-	-	-	-	-	+	-
S.curacoi	-	-	+	-	-	-	-	-	-	-	-	-
S.cyanocolor	-	-	-	-	-	-	-	+	-	-	-	-
S.filipinensis	-	-	-	-	+	+	-	-	+	-	-	-
S.flaveolus	-	-	-	-	+	+	-	-	-	-	-	-
S.flavogriseus	-	+	-	-	-	-	-	-	+	-	-	-
S.flavovirens	-	-	-	-	-	-	-	+	-	-	-	-
S.flocculus	-	-	-	-	-	-	+	-	-	-	-	-
S.flulvoviridis	-	-	-	-	-	-	+	-	-	-	-	-
S.gelaticus	+	-	-	-	-	+	-	-	-	-	+	-
S.glaucescens	-	-	-	-	-	-	+	-	-	-	-	-
S.griseoflavus	+	+	-	-	-	-	-	-	+	-	-	-
S.griseoloalbus	-	-	-	-	-	+	-	-	+	-	-	-
S.griseolus	+	+	-	-	-	-	-	-	+	-	+	-
S.griseomycini	-	-	-	-	+	-	-	-	-	-	-	-
S.griseoplanus	-	-	+	-	-	-	-	-	-	-	-	-
S.griseostramineus	-	-	-	+	-	+	-	-	-	-	-	-
S.griseorubeus	-	-	-	-	-	-	-	-	-	-	-	+
S.griseoviridis	+	-	-	-	-	-	-	-	-	-	-	-
S.griseus	+	-	-	-	-	+	+	+	+	+	+	-
S.heimi	+	-	-	-	-	-	-	-	-	-	-	-

S.ipomea	-	-	+	+	+	-	-	-	-	-	-	+
S.janthinus	-	-	-	-	-	-	-	+	-	-	-	-
S.macrosporeus	+	-	+	-	-	-	-	+	-	-	+	+
S.microflavus	-	-	-	-	+	-	-	-	-	-	-	-
S.minoensis	-	-	-	-	-	-	-	+	+	-	-	-
S.mutabilis	-	+	-	+	-	-	-	+	-	-	-	-
S.naganishii	-	+	-	-	-	-	-	-	-	-	-	-
S.nigrifaciens	-	+	-	-	+	-	-	-	-	-	-	-
S.nitrosporeus	-	-	+	-	-	-	+	-	+	-	-	-
S.olivaceus	+	+	+	+	-	+	-	+	-	-	+	-
S.olivoverticillatus	-	-	-	-	+	-	-	-	-	-	-	-
S.olivoviridis	-	-	+	-	-	-	-	-	-	-	-	-
S.orientalis	-	+	+	-	-	-	+	+	-	+	+	-
S.pluricolonescens	+	-	-	-	-	-	-	-	-	-	-	-
S.prasinus	+	-	+	-	-	-	-	-	-	-	-	-
S.pristinaespiralis	-	-	+	-	-	-	-	+	-	-	-	-
S.psammoticus	+	+	+	-	-	+	-	-	+	-	+	-
S.puniceus	-	-	-	-	-	+	-	-	-	-	-	-
S.purpeofuscus	-	-	-	+	-	-	-	-	-	-	-	-
S.pyridomyceticus	-	+	-	-	-	-	-	-	-	-	-	-
S.recifensis	-	-	-	+	-	-	-	-	-	-	-	-

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S.rimosus	-	-	-	-	-	-	-	-	-	+	-	-
S.roseofulvus	-	+	+	+	-	-	-	-	-	-	-	-
S.roseus	-	-	-	-	+	+	-	-	+	+	+	-
S.rubiginosohelvolus	-	-	-	-	-	-	+	+	-	-	-	-
S.scabies	-	+	-	-	-	-	-	-	-	-	+	-
S.setonii	-	+	-	-	-	-	-	-	-	-	-	-
S.sprasinus	-	-	-	-	-	-	+	-	-	-	-	-
S.tubercidicus	+	-	-	-	-	-	-	-	-	-	-	-
S.sp01	-	+	-	-	-	-	-	-	-	-	-	-
S.sp02	-	-	-	+	-	-	-	-	-	-	-	-
S.sp03	-	+	-	-	-	-	-	-	-	-	-	-
S.sp04	-	-	-	-	-	-	-	-	-	-	-	+
S.sp05	-	-	-	-	+	-	-	-	-	-	-	-
S.sp06	-	-	-	-	-	-	-	-	-	-	-	+
S.sp07	-	-	-	-	-	-	-	-	-	-	-	+
S.sp08	-	+	-	-	-	-	-	-	-	-	-	-
S.sp09	-	-	-	+	-	-	-	-	-	-	-	-
S.sp10	-	-	-	-	-	+	-	-	-	-	-	-
S.sp11	-	+	-	-	-	-	-	-	-	-	-	-
S.sp12	-	+	-	-	-	-	-	-	-	-	-	-
S.sp13	-	-	-	-	-	-	-	-	-	-	-	+

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S.sp14	-	-	-	-	+	-	-	-	-	-	-	-
S.sp15	-	-	-	+	-	-	-	-	-	-	-	-
S.sp16	-	-	-	-	-	-	-	-	-	-	-	+
S.sp17	-	-	-	-	-	-	-	-	+	-	-	-
Total Known	21	21	19	12	12	12	14	18	17	11	16	8
Total unknown	0	5	0	3	2	1	0	0	1	0	0	5

Table 5a : Diversity indices of Actinobacteria genera at Station 5 during 2015-16.

	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Taxa_S	5	3	3	6	5	6	4	4	4	7	6	8
Simpson_1-D	0.558	0.533	0.513	0.59	0.589	0.632	0.57	0.574	0.608	0.626	0.57	0.643
Shannon_H	0.91	0.822	0.755	1.056	1.04	1.231	0.961	0.981	1.07	1.24	0.975	1.3
Margalef	0.559	0.282	0.303	0.819	0.651	0.739	0.474	0.435	0.456	0.923	0.742	1.058

Table 5b : Diversity indices of *Streptomyces* species at Station 5 during 2015-16.

	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Taxa_S	22	26	19	15	14	13	14	18	18	11	16	13
Simpson_1-D	0.922	0.931	0.922	0.902	0.902	0.859	0.847	0.923	0.923	0.755	0.894	0.909

Shannon_H	2.825	2.919	2.672	2.472	2.446	2.256	2.273	2.684	2.694	1.8	2.498	2.481
Margalef	3.32	3.954	3.057	2.708	2.493	2.12	2.379	2.828	3.057	1.84	2.55	2.19

Table 6a : Mean (n=12) density distribution (Nos.x10^2 cfu/g) of actinobacteria and the genus *Streptomyces* at Station 5 (Gosthani estuary) during study period.

Month	Actinobacteria	*Streptomyces*
Jun 2015	58	51
Jul	55	52
Aug	37	36
Sep	20	20
Oct	21	17
Nov	39	26
Dec	26	21
Jan 2016	49	45
Feb	33	24
Mar	30	25
Apr	38	32
May	34	33
Mean	**37**	**32**

Table 6b : Mean (n=11) density distribution (Nos.x10^2 cfu/g) of actinobacteria and the genus *Streptomyces* at Stations 1-4 and 6-8e during study period.

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Station	Actinobacteria	*Streptomyces*
Station 1	95	56
Station 2	58	52
Station 3	25	23
Station 4	30	20
Station 6	25	17
Station 7	28	24
Station 8a	65	64
Station 8b	112	105
Station 8c	27	23
Station 8d	21	15
Station 8e	16	11
Mean	**45**	**37**

Table 7 :Pearson correlation values between environmental parameters and Actinobacteria genera and *Streptomyces* species densities in the sediment during 2015-2016 at Station 5 (Gosthani estuary).

Parameter	Actinobacteria genera	Streptomyces species
Sediment Temperature	-0.10182	- 0.1016
Sediment Dissolved Oxygen	0.557155	0.712118

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Salinity	-0.05197	0.19144
pH	-0.72404	-0.73883
Organic matter	0.496774	0.594159

Antibacterial activity: Eventhough, antibacterial activity study is not the main objective of the present study, the present study made a preliminary attempt with crude extracts of *Streptomyces* species, which exhibited antagonistic nature in cultures. Of the 143 species recorded in the present study, only four species exhibited antagonistic activity in cultures. The Actinobacterial (*Streptomyces glaucescens, S.humidus, S.griseus* and *S.bottropensis*) extracts obtained from methanol and ethyl alcohol showed moderate antibacterial activity (zone of inhibition: 14-16 mm) against test pathogens of bacteria. Sivakumar *et al* (2007) reviewed the research on Indian marine actinobacteria, which have potential antimicrobial activity. Several researchers carried out investigations on antimicrobial potential actinobacteria species from different types of marine habitats and achieved different rates of success. Majority of them opined that the nature of culture medium ingredients govern their antimicrobial nature. The main works include: Kathiresan *et al* 2005, Arifuzzaman *et al* 2010 {13 isolates; Sundarbans}, Krishnanraj *et al* 2011, Sathiyaseelan and Stella 2011, Sivakumar *et al* 2011, Sunil *et al* 2012, Aparanji *et al* 2013, Gunasekaran & Tangawel 2013, Doralyn *et al* 2013 and Sengupta *et al* 2015. Sahu *et al* (2007) opined that the occurrence of antagonistic actinomycetes may be due to continuous fluctuations of physico-chemical parameters in the coastal environment, which enhance production of antagonistic substances in organisms to enable them to survive. Mitra *et al* (2008) opined that the two factors: 1. soils having more nitrogen in comparison to carbon and 2. sites influenced by tides may play role in the high antagonistic potential of actinobacteria in marine ecosystems. Sunil *et al* (2012) and Gunasekaran and Tangawel (2013) observed that Starch Casein Agar is an ideal culture medium to have more antagonistic actinobacteria and the latter authors found that ethyl acetate is a good pressure (i.e., oil leakage, agricultural wastes, and commercial market) in the Gadkhali area may be the reason for low diversity of actinomycetes in that area. It may be concluded that, based on the earlier works and the present study, the ingredients of the culture medium besides other physico-chemical parameters play an important role in determining the antagonistic nature of actinobacteria. Based on the present observations, it may be mentioned that the estuarine and deltaic mangrove ecosystems are the ideal habitats to explore for new actinobacteria.

Table 8 :Antibacterial activity of four *Streptomyces* species against bacterial pathogens (Zone of inhibition in mm)

	S.glaucescens				*S.humidus*				*S.griseus*				*S.bottropensis*			
Species	C	EA	EE	M	C	EA	EE	M	C	EA	EE	M	C	EA	EE	M
Bacillus subtilis	14	0	0	14	0	16	16	16	16	16	12	14	0	14	0	0

Escherichia coli	16	16	0	0	0	0	0	14	16	0	0	16	0	14	0	0
Klebsiella pneumoniae	14	16	0	16	0	14	14	14	0	0	14	14	0	16	0	0
Pseudomonas aeruginosa	0	0	0	0	0	14	14	14	14	0	16	0	0	14	16	16
Staphylococcus aureus	0	14	16	14	0	16	16	0	0	16	14	16	0	0	0	0

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