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# Actinobacterial Diversity of Machilipatnam Coast (India) with an Emphasis on Novel Preparation of Salinispora Actinobacterial Probiotics in Sustainable Aquaculture

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**ABSTRACT**

Marine microbes serve as an important source for commercial bioactive compounds. The present research is focussed on the Actinobacterial diversity of Machilipatnam coast. Actinobacteria are Gram positive bacteria that resemble Fungi in having filaments forming mycelia colonies. Owing to their morphological and cultural characteristics Actinobacteria are considered a group other than Bacteria. The different Actinobacterial Strains were studied having an ability to utilize the various carbon compounds as source of energy. 27 isolates of Actinobacteria including white, green, grey, orange and pink with different morphological types were isolated from Station I and II. Among them 19 isolates were from Pedapatnam and 27 from Polatitippa. The 27 identified species were falling under 10 genera including *Actinobispora, Actinomadura, Actinomyces, Microbispora, Nocardis, Nocardiopsis, Saccharomonospora, Streptomyces, Streptosporangium and Thermomonospora. Streptomyces* was the most dominant genus. For the evaluation of antibacterial activity, clinical strains of bacteria such as Gram positive *Staphylococcus aureus,* and *Streptococcus faecalis* and Gram – negative *Proteus vulgaris* and *Salmonella typhi* were used. *Streptomyces alboniger, S.coelicolor* and *S.griseus* were selected to study their antagonistic activity against the above mentioned clinical bacteria.

***Key Words:*** *Mangroves, Actinomycetes, Streptomycetes, Salinispora*

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## INTRODUCTION:

Actinobacteria represents a phyllogenetically coherent lineage of high G+C Gram positive bacteria. The best known of this is the Sub Class Actinobacteridae, which includes the order Actinomycetales. Various methods exist for estimating the diversity of organisms in an environment, of which richness estimators are most appropriate for microbial studies (Hughes *et al*., 2001). Marine habitats due to their ecological pressure, including the competition of space and predation and physical properties such as salinity, pressure and temperature variations have led marine microorganisms to develop secondary metabolite with various biological activities to survive in this highly demanding ecosystem (Donia *et al*., 2003). Some of the species were found to produce unique compounds such as salinosporamides that are now in clinical trials as potent anticancer agents (Feling *et al*., 2003). Another recently characterized genus *Salinibacterium* can tolerate up to 10% NaCl but does not have a salt requirement for growth (Han *et al*., 2003). Environmental conditions of the sea are extremely different from terrestrial conditions (Kojjoa and Sawangwong, 2004).

The first application of such estimators of deep sea Actinomycetes revealed a remarkable diversity of organisms in bathyal sediment: approximately 1400 phenotypes of which 90% are predicted to represent new species and genera (Stach *et al*., 2005). Recently reported Verrucosispora strain AB-18-032 (Reidlinger *et al*., 2004) qualifies as an indigeneous marine Actinobacterium. More recently, cultivation efforts have revealed considerable Actinomycetes diversity within the marine species (Jensen et al., 2005). And there is evidence that Actinomycetes are capable of growth in the marine environment (Mincer et al., 2005).

Preliminary evidence for bio geographic distribution patterns of marine Actinomycetes has been advances for strains of *Salinispora tropica* (Jensen *et al*., 2005). The recent description of three marine genera (Maldonado *et al*., 2005) provides strong support for the concept of marine specific Actinomycetes. The application of molecular techniques to the field of marine ecology has provided a new perspective on the diversity of Actinobacteria in the sea (Ward and Bora, 2006). Hence culture independent studies have shown that indigenous marine Actinomycetes certainly exist in the marine oceans (Ward and Bora, 2006).

Their ability to form stable populations in different habitats and produce novel compounds with various biological activities (Prudhomme *et al*., 2008) illustrate that indigenous marine Actinomycetes indeed exist in the oceans and are an important source of secondary metabolites. In a prior study of sediment samples collected off the coast of California,

culture dependent Actinomycetes diversity was assessed between near shore and off shore sites (Davo *et al*.,2008). Marine Actinomycetes have different characteristics from terrestrial Actinomycetes and produce novel bioactive compounds and new antibiotics (Mathivanam, 2009).Currently the phylum Actinobacteria especially Actinomycetes represents the most prominent group of microorganisms for the production of bioactive compounds notably antibiotics and anti tumour agents (Good Fellow and Fielder, 2010). Investigations focussed on marine Actinobacterial isolates from Chile have been rather scarce (Jiang *et al*., 2010). Two of the four new classes of antibiotics discovered in recent years have been derived from Actinobacterial strains (Hardesty and Juang, 2011). Many vitamins, antibiotics, enzymes, siderophores obtained by Actinomycetes have pharmaceutical, veterinary, agricultural and clinical applications (Naine *et al*., 2011) in addition to anti tumour and wound healing properties (Janardhan *et al*., 2012).

40% of all microbial bioactive secondary metabolites derive from Actinobacteria, where approximately 80% of them are produced by the genus *Streptomyces* (Berdy, 2012). Actinobacteria act as symbionts in marine sponges (Henstschel *et al*., 2012). A novel compound dominated theinodolin with a unique mechanism of action has been isolated from *Streptomyces* strain derived from marine sediments in Valparaiso (Park *et al*., 2013). This can be exemplified by marine sediments, which are nutrient rich habitats, harbouring a considerable Actinobacterial biodiversity with metabolic and genetic potential to develop secondary metabolites (Duncan et al., 2014).

Many researchers discovered that the poorly explored mangrove environments contain high populations of novel Actinobacteria as demonstrated by *Streptomyces xiamensis* (Yan *et al*.,2006) : *Asano iriomotensis* (Han *et al*.,2007) : *Nonomuraca mahesh kaliensis* (Ara *et al*.,2007). Of the 9 maritime states in Indian Peninsula only very few states have been extensively covered for the study of marine Actinobacteria for antagonistic properties against different pathogens (Sivakumar *et al*., 2007). Several reports are available on antibacterial and anti fungal activity of marine actinomycetes (Bredholt *et al*., 2008). Antifungal secondary metabolites have been isolated from *Nocardia sp. ALAA 2008* (Gindy *et al*., 2008), marine *Streptomyces sp. DPTB16* (Dhanasekaran *et al*.,2008). The capacity of Actinomycetes to produce promising new compounds will certainly be unsurpassed for a long time and they are still responsible for producing the majority of clinically applied antibiotics (Anzai *et al*., 2008).

The constant changes in the environmental factors such as tidal gradient and salinity in the mangrove environments are understood to be the driving force for metabolic pathway adaptations. Hence increasing exploitation of the mangrove microorganism's resources (Hong *et al*., 2009) is seen. Furthermore, many strains are also prolific producers of useful antibiotics (Xu *et al*., 2009). Actinobacteria also have the ability to synthesize anti fungal (Zarandi *et al*.,2009) and insecticidal compounds (Pimentel *et al*.,2009). Researchers are focussing on screening programs of microorganisms, primarily Actinomycetes for the production of antibiotics and increased productivity of such agents has gained importance (Selvameenal *et al*., 2009).

The secondary metabolites especially antibiotics derived from Actinomycetes are being used as therapeutic drugs for the treatment of various ailments in humans and animals (Prabavathy *et al*., 2009). Marine derived metabolites become prototypes for the development of new substances with a putative insecticidal and anti microbial potential which make them excellent candidates for their use as agro chemicals (Newman and Cragg., 2010 : Blunt *et al*., 2011). One clear example is the case of Kasugamycin, a systemic fungicide against *Magnaporthe grisea* and bactericide against *Burkhloderia glumae* (Yoshi *et al*., 2012). This bioactive compound was isolated from marine strains of *Streptomyces rutgersensis subsp. Gulangyuensis* (Kim, 2013).

According to a report of (Thenmozhi and Kannabiran, 2012) ethyl acetate extract of *Streptomyces species VITSTK7* isolated from the marine environment of Bay of Bengal exhibited 43.2% DPPH scavenging activity. Studies on Actinomycetes isolation from marine environment have been reported with varied anti microbial potency against pathogens as well (Valli *et al*., 2012). Marine representatives of the phyla Actinobacteria are recognized as one of the most important groups with biotechnological potential (Manivasagan *et al*., 2013). Findings on antimicrobial agents from Actinomycetes remains hope for proper anti microbial treatment of infectious diseases which has been challenged by growing multi drug resistant pathogens reported from everywhere attributing high morbity and mortality (Padalkar and Reshme, 2013). Compounds derived from marine microorganisms have also been evaluated for their role as quorum quenchers, suitable for acting as anti pathogenic compounds through interruption of pathogenic bacterial communication, this interruption reduces damage in the host (Teasdale *et al*., 2013 : Kabir, 2013).

Actinobacteria also have the ability to synthesize anti oxidant compounds (Janardhan *et al*.,2014). (Lee *et al*.,2014) isolated and identified the Actinobacteria from the Tanjung Lumpur mangrove forest located on the east coast of Peninsular Malaysia and screened them to discover potential sources for antimicrobial secondary metabolism.(Nagaseshu *et al*., 2016) reported anti oxidant activity of methanol extracts of Actinobacteria isolated from the marine sediments collected from the Kakinada coast. They also correlated the anti oxidant activity of the extract with cytotoxic and anti proliferative activities. Vishwanathan and Rebecca, 2017) isolated a total of 114 strains of Actinomycetes from the coastal region of Chennai beach out of which 22 strains showed anti microbial activity. (Kapur *et al*., 2018) studied antimicrobial activity of Actinomycetes of the sea and beach soils of Colombo and Havelock and Carbon islands of Andaman and Nicobar islands. (Ramachandran *et al*., 2018) investigated the antibacterial activity of the endophytic Actinomycetes isolated from the mangrove plant of *Avicennia marina* in Muthupet mangrove region of the South east coast of Tamil Nadu.

The most frequently encountered acidophilic Actinomycetes belongs to the genus *Streptomyces* which in general dominates the fungal complexes in all types of soils (Zvyaginstev *et al*., 2001). Under representation of commonly cultured Actinomycetes in 16sRNA sequence libraries may be due to insufficient cell lysis and DNA extraction within a complex substrate, such as sediment and primer bias due to high GC content of Actinomycetes

(Schiwientek *et al*., 2001). Biodiversity of Actinomycetes plays an important role in degradation of waste material and as an integral part of the recycling of materials in nature (Kulkarni and Deshmukh, 2002). Many studies have shown that exceptional potential of *Streptomyces* species for production of bio-active compounds (Magarvey *et al*., 2004).

Actinomycetes accounts for > 45% of all bioactive metabolites discovered in nature (Janos Berdy, 2005). Actinobacteria from marine sediments have been reported to vary from 0% to 17%, Bull, 2005). Bonafide Actinomycetes not only exist in the oceans but are distributed in different marine ecosystems (Kim, 2006). These communities commonly include taxa like *Planctomycetales, Firmicutes* and *Verrucomicrobiales* (Musat *et al*., 2006). Culture independent studies can be done by selectively culturing a diverse library of filamentous Actinomycetes from sediment samples by inhibiting the growth of Gram –ve bacteria and selecting for the recovery of spore forming and slow growing Actinomycetes (Newmann and Hill, 2006). Ocean sediments harbour microbial cell counts that can exceed those of sea water by three orders of magnitude with diversity estimates consistently among the highest of all studied environments (Louzupone and Knight, 2007).

Antibiotic production in Actinomycetes has been linked to nutrient sensing and morphological differentiation (Rigali *et al*., 2008).Abundance have also been observed in culture independent studies with the Actinobacteris component of bacterial communities accounting for 12.7% in forest soils, 21-30% in soils varying land use, 10% in Arctic deep sediments and 2-4% in polar sea waters. The low detection of *Streptomyces* species using culture independent techniques despite an abundance of cultured isolates has been studied (Babalola *et al*., 2009). A study to establish effective methods for selective isolation of acidophilic filamentous Actinomycetes from acidic soils was performed employing four pre-treatment and five media supplemented with antibiotics (Ding *et al*., 2009). Although species specific secondary metabolite production has been observed for marine *Salinispora sp,* this might not be a case for *Streptomyces* (Jensen *et al*., 2010).

The marine Actinomycetes genus *Salinispora* provides a useful model to address the ecological roles of bacterial secondary metabolites, it is comprised of three species: *Salinispora aerinocola, Salinispora tropica* and *Salinispora pacifica* which are well delineated despite sharing 99% 16sRNA gene sequence identity (Freel *et al*., 2013). Bacteria in the order Actinomycetales constitute a minor component of sediment communities, yet decades of culturing efforts have shown they persist in most well sampled sediments (Dalisay *et al*., 2013).

Recent assessments have been done on the isolation of Actinomycetes from marine sediments: *Salinibacterium* (Han *et al*., 2003) : *Aeromicrobium* (Bruns *et al*., 2003) :*Williamsia* (Stach *et al*., 2004) : *Solwaraspora* (Magarvey *et al*., 2004) : *Marinospora* (Jensen *et al*., 2005) : *Salinispora* (Jensen *et al*., 2005 : Mincer *et al*., 2005 : Maldonada *et al*., 2005) : *Camerjespora* (Fortman *et al*., 2005) : *Marinactinospora* and *Sciscionella* (Tain *et al*., 2009) : *Serinicoccus* (Xiao *et al*., 2011). The rare Actinomycetes produce diverse and unique, unprecedented sometimes very complicated compounds exhibiting excellent bioactive potency and usually low toxicity (Kurtboke, 2012). Different physical and chemical characteristics, prevailing in the mangrove environment, may influence the population density and diversity of Actinobacteria to a greater extent. This is in agreement to the total of 21 Actinomycetes isolates recorded including different locations in marine soils of Pallaverkadu, Tamil Nadu (Kartikeyan *et al*., 2014).Therefore, the present work was undertaken to isolate and identify the Actinobacteria from two different areas of Machilipatnam, situated along the southeast coast of India.

**Materials and Methods:**

**Isolation of Actinobacteria**

The sediment samples were collected from the study sites (two stations) of Machilipatnam coast with the sterile spatula. The collected samples were transferred to sterile polythene bags and transported immediately to the laboratory. After arrival to the laboratory, the samples were air-dried aseptically for one week. Air-dried sediment samples were incubated at 55°C for 5 min (Balagurunathan, 1992) and then 10-fold serial dilutions of the sediment samples were prepared using filtered and sterilized 50% seawater. Serially diluted samples were placed on the Actinobacterial isolation agar medium in duplicate petriplates.

To minimize bacterial and fungal contamination, all the agar plates were supplemented with 20 mg/l of nystatin and 100 mg/l of cycloheximide (Kathiresan *et al*., 2005). The Actinobacteria colonies that appeared on the petriplates were counted from 5$^{th}$ day onwards, up to 28$^{th}$ day using a colony counter. All the colonies that grew on the petriplates were separately streaked in petriplates, sub cultured, ensured for their auxenicity and maintained in slants.

**Identification (Genus and species affiliation)**

Based on the morphological, biochemical, physiological and molecular properties, seashore isolates of Actinobacteria was identified. The identity of the species was also confirmed by Bergey's Manual of Systematic Bacteriology (William *et al*., 1989).

Through the preliminary studies, the potential enzyme producing strains were selected for analysis of their cell wall components to conclude their cell wall type with the following procedure.

**Hydrolysis**

Hydrolysis of the strains was done for releasing amino acids. Harvested cells of each strain weighing 20 mg (fresh) were placed in a screw capped test tube, to which 1 ml of 6N HCL was added and sealed with alcohol. The samples were kept at 121°C for 20 hrs in a sand bath. The bottles were cooled by keeping them at room temperature.

Hydrolysis was also done separately for releasing sugars. Harvested cells of each strain weighing 50 mg (fresh) were placed in an ambo bottle to which 1ml of 5N H2SO4 was added and sealed with alcohol. The samples were kept at110°C for 12 hrs. The bottles were then cooled by keeping them at room temperature.

**Thin Layer Chromatography (TLC) for amino acids**

Spotting of the whole cell hydrolysis was made carefully on silica coated TLC plate (Merck, Pvt. Ltd. Kolkata) using a micropipette. Each sample (10µl) was applied on the base

line of silica TLC plate (20 cm x 20 cm). Adjacent to this, 3µl of DL-diaminopimelic acid (an authentic material mixture of DAP isomers) and 3µl of amino acetic acid (glycine) were spotted as standards. TLC plate was developed with the solvent system containing methanol: pyridine: glacial acetic acid and water (5:0.5:0.125:2.5 V/V). It took approximately more than 2 h for development. The spots were visualized by spraying with 0.4% Ninhydrin solution in water-saturated-n-Butanol, followed by heating at 100°C for 5 min. The sample spots were immediately compared with the spots of the standard.

**Cultural characteristics**

Cultural characteristics of the isolates were studied including the following aspects:

**Aerial mass colour:** The colour of the mature sporulating aerial mycelium was recorded in a simple way (white, grey, red, green, blue and violet). When the aerial mass colour fell between two colours series, both the colours were recorded. If the aerial mass colour of a strain to be studied showed intermediate tints, then also, both the colour series were noted. The media used for the purpose were yeast extract-malt extract agar (ISP2) and inorganic-salt starch agar (ISP4).

**Melanoid pigments**

The grouping was made on the production of melanoid pigments (i.e. greenish brown, brownish black, distinct brown and the pigments modified by other colours) on the medium. The strains were grouped as melanoid pigments produced (+) and not produced (-). This test was carried out on tyrosine agar as recommended by International *Streptomyces* Project (Shirling and Gottlieb, 1996; Sivakumar, 2001).

**Reverse side pigments**

The strains were divided into two groups, according to their ability to produce characteristic pigments on the reverse side of the colony, namely, distinctive (+) and not distinctive or none (-). In case, a colour with low Chroma such as pale yellow, olive or yellowish brown occurred, it was included in the latter group (-). This test was carried out with the yeast extract-malt extract agar (ISP2) medium.

**Soluble pigments**

The strains were divided into groups by their ability to produce soluble pigments other than melanin: namely, produced (+) and not produced (-). The colours (red, orange, green, yellow, blue and violet) were considered as soluble pigments present. This test was carried out with Tyrosine agar (ISP7) medium.

**Spore chain morphology:**

Spore morphological characters of the isolates were studied by inserting 3-4 sterile cover slips at an angle of 45°C in the ISP-2 medium. Then, the isolates were inoculated at the point of insertion of cover slip in the medium and it was incubated at 55°C. After 7 days interval cover slip was removed with the help of sterile forceps and observed under a light microscope for the formation of aerial mycelium, sporophore structure and spore morphology under high power of magnification (400X). In addition to this, scanning electron microscope (Hitachis-450-SEM) pictures were also taken for studying the surface of the spores.

**Spore surface**

Spore morphology and its surface features were observed under the scanning electron microscope. The electron grid was cleaned and adhesive tape was placed on the surface of the grid. The mature spores of the strain were carefully placed on the surface of the adhesive tape and gold coating was applied for half-an-hour. Then the specimens were examined under the scanning electron microscope with different magnifications. The spore morphology was characterized as smooth, spiny, hairy, and warty.

**Assimilation of carbon source**

Different Actinobacterial strains having an ability to utilize various carbon compounds as source of energy were studied adopting the following method. Chemically pure carbon sources, certified to be free of and mixture with other carbohydrates or contaminating materials were used for this purpose. Carbon sources viz. arabinose, xylose, inositol, mannitol, fructose, rhamnose, sucrose and raffinose were sterilized following the procedure as given below. Dry carbon sources were weighed and spread as a shallow layer in a pre-sterilized flask fitted with a loose cotton plug. Sufficient diethyl ether was added to over the carbohydrate and it was allowed to evaporate at room temperature under a ventilated fume hood overnight. After evaporation of ether, sterilized distilled water was added aseptically to make a 10% w/v solution of the carbon source. Then, each sterilized carbon sources were added to the sterilized basal mineral salts medium to give a final concentration of 1%. After that, the mixture was agitated and 20 ml of the medium was poured on each petriplate. Medium without carbon source was treated as negative control and glucose added medium as positive control. After pouring the medium, the plates were kept in room temperature for one day to dry the surface of the medium. Then the strain was inoculated into the plates containing different carbon sources; incubated at room temperature for 7-10 days and the growth on different plates was compared.

For each of the carbon sources, utilization is expressed as i) strongly positive (++), when growth on tested carbon in basal medium was equal to or greater than growth on basal medium plus glucose, ii) positive (+),when growth on tested carbon was significantly better than on basal medium without carbon, but somewhat less than on glucose, iii) doubtful (±), when growth and significantly less than with glucose, iv) negative (-), when growth was similar to or less than the growth on basal medium without carbon.

**Correlation Studies**

The relationship between the meteorological and physico-chemical parameters with that of the Actinobacterial population was studied by Pearson-correlation co-efficient method.

**Results:**

The lowest population density (18 X$10^4$ CFU/g) was observed during December, 2017, at both stations; while the highest during January 2018 at station I (33 X $10^4$ CFU/g) and June 2017 at station II (35X$10^4$ CFU/g). Totally 27 Actinobacterial isolates including white, green, grey, orange and pink coloured colonies with different morphological types were isolated from two different sampling stations (Table-1). Among them, 19 isolates from Pedapatnam and 27 from mangrove soil of Poalatitippa were recorded (Table-2).

Common Actinobacterial species at both stations were recorded.

The identified species (27) were falling under 10 genera including *Actinobispora, Actinomadura, Actinomyces, Microbispora, Nocardia*, *Nocardiopsis*, *Saccharomonospora, Streptomyces, Streptosporangium* and *Thermomonospora.*The Pearson correlation studies revealed that the actinobacterial population at station I showed a significant positive correlation at 0.01 level(P>0.01) for atmospheric temperature, surface water temperature, pH, salinity and organic matter. On the other hand, nutrients such as nitrogen, potassium, zinc, copper, iron, manganese and phosphorous registered a significant negative correlation at 0.01 level (P>0.01). The positive correlation recorded for electric conductivity and negative correlation recorded for rainfall and dissolved oxygen did not show any statistical significance in the present study (Table-3). At station II, the total Actinobacterial population was positively correlated with atmospheric temperature, water temperature, pH and salinity showed statistical significance at 0.01 level (P>0.01) and electric conductivity at 0.05level (P>0.05) while the organic matter showed significant negative correlation at0.05 level (P>0.05) and nutrients such as potassium, zinc, iron and manganese registered a significant negative correlation at 0.01 level (P>0.01). On the other hand negatively correlated rainfall, nitrogen and phosphorus did not show any statistical significance in the present investigation (Table-4).

**Discussion**

In the present study, totally 27 species of Actinobacteria were isolated from two soil types (sandy and clay) from two locations namely Pedapatnam (seashore) and Polatitippa (mangrove soil). The vast majority of Actinobacteria have originated from soil (Stach and Bull, 2005). Number of isolates of Actinobacteria was high in mangrove soil (27) followed by seashore soil (19).However, the diversity of Actinobacterial isolates may perhaps be increased due to the nutritive status of the respective soil. The first report on marine Actinobacteria was made by Nadson (1903) from the salt mud. Actinomycetes have also been found on decomposing plant litter of streams (Otoguro *et al*., 2001). Actinobacteria have been reported from a complete spectrum of extreme ecosystems in addition to terrestrial, marine and even fresh water forms. Early evidences supporting the existence of marine Actinobacteria comes from the description of *Rhodococcus marinonascene*, the first marine Actinomycstes species to be characterized (Sahu *et al*., 2007). There are several reports on inhabitancy of microorganisms including Actinomycetes in salt mines (Chen *et al*., 2007) : brine wells (Xiang *et al*., 2008) : solar salterns (Sabet *et al*., 2009) : salt lakes (Swan *et al*., 2010). Actinobacteria exist in a various habitats including exotic locations such as the Antarctic soils (Moncheva *et al*., 2012). Actinomycetes isolation was done in volcanic zones and hyper arid and glaciers (Hameedi *et al*., 2013). The existence of acid tolerant, alkaliphilic, psychro-tolerant, thermo-tolerant, halo-tolerant, alkali-tolerant, halo alkali-tolerant and xerophoilous Actinobacteria have been reported (Lubsanova *et al*., 2014). Further support has come from the discovery that some strains display specific marine adaptations whereas others appear to be metabolically active in marine sediments. Isolation of Actinomycetes from agriculture soils was done by (Stephen, 2014).

As evident by the isolation of various genera like *Agrococcus, Arthobacter, Dietzia, Gordonia, Mycobacterium, Pseudocardia, Rhodococci, Streptomyces* etc (Claverias *et al*., 2015), Actinobacteria are common in marine habitats (Behie *et al*., 2017 : Betancur *et al*., 2017). Among the genera recorded, in the present study, *Streptomyces* was the most predominant genus. The dominance of *Streptomyces* among the Actinobacteria especially in soils has been reported by many workers (Moncheva *et al*., 2002: Mansour, 2003 : You *et al*., 2005 : Peela *et al*., 2005). Besides *Streptomyces*, the genera most frequently appeared on media were *Actinomadura, Actinobispora, Nocardia, Nocardiopsis, Saccharomonospora, Streptosporangium,Thermomonospora* and *Actinobispora.*

In spite of the fact that the Actinobacteria have wide distribution they show variation in their population dynamics. In the present investigation, it was found that there was correlation co-efficient between Physico-Chemical properties of sediment and total Actinobacteria population (TAP). It revealed that the significant positive correlation between TAP and copper (r=0.706; P<0.05%) and between electric conductivity and pH (r=0.798; P<0.05%). Similar type of study was reported by (Mansour, 2003).

Lakshmanaperumalsamy *et al*.,1986 and Jiang and Xu, 1990). (Saadoun and Al-Momani, 1996) have studied the pH, moisture, organic matter, nitrogen and phosphorus content of the soils and correlated with Actinobacterial population. The correlation between salinity, pH and organic content of marine sediments and Actinobacterial population has been reported by several workers. (Jensen *et al*.,1991) reported that there was no correlation between the percentage of organic content of marine sediment and Actinobacterial population.(Ghanen *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.

(Lee and Hwang, 2002) reported that the soil pH (5.1-6.5), moisture (9.1-13 MHC) and organic matter (9.1-11%) influenced the dominance of *Streptomyces* in the agricultural field soils of Western part of Korea. Hence it could be concluded that Actinobacteria are ubiquitous, their population dynamics are often influenced by the available nutrients and the physico-chemical conditions of the ecosystem. The present study clearly demonstrates that the physico-chemical parameters influenced the biodiversity and distribution of Actinobacteria in two different marine soils. The marine Actinobacteria are of significance in the marine ecology as well as in the marine biotechnology as a source of high value products like antibiotics. It is necessary to review our contribution towards understanding marine Actinobacteria especially *Streptomyces* and their antimicrobial activity.

In the present study, totally 27 species of Actinobacteria were isolated from two soil types (sandy and clay) from two locations namely Pedapatnam (seashore) and Politippa (mangrove soil). The vast majority of Actinobacteria have originated from soil (Stach and Bull, 2005). Number of isolates of Actinobacteria was high in mangrove soil (27) followed by seashore soil (19).However, the diversity of Actinobacterial isolates may perhaps be increased due to the nutritive status of the respective soil.

**Table1. Cultural and biochemical characteristics of Actinobacteria isolated from marine and mangrove sediments**

| S. No | Name of the actinobacteria | Aerail mass colour | Melanoid pigment | Reverse side pigment | Soluble pigment | Spore chain | Spore surface | Carbon source | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Arabinose | Xylose | Inositol | Mannitol | Fructose | Rhamnose | Sucrose | Raffinose |
| 1 | Actinobispora sp. | White | - | + | - | Bispores | Smooth | + | + | + | + | + | + | - | - |
| 2 | Actinomadura atramentaria | White | + | + | + | Bispores | Smooth | - | - | - | - | - | - | - | - |
| 3 | A. cremea | White | + | - | - | Spiral | Warty | + | + | - | + | + | + | + | - |
| 4 | A. echinospora | Yellow-pink | - | - | + | Bispores | Spiny | + | + | - | + | + | - | + | - |
| 5 | A. libanotica | Pale pink | - | - | - | Hooks | Warty | + | + | - | - | + | + | + | - |
| 6 | A. rugatobispora | Green | - | - | - | Bispores | Rough | - | - | - | - | - | - | - | - |
| 7 | A. spadix | Grayish yellow | - | - | + | Pseudospores | Smooth | + | + | + | + | + | + | + | + |
| 8 | A. virdis | Green | - | - | - | Bispores | Rough | (+) | - | - | + | + | + | (+) | - |
| 9 | Actinomyces sp. | White | - | + | - | Straight-rectus | Smooth | + | + | + | + | + | - | - | + |
| 10 | Microbispora sp. | Pale pink | - | + | + | Bispores | Smooth | + | + | - | + | + | + | + | - |
| 11 | Nocardia sp. | Pink-Gray | - | - | + | Spiral | Smooth | - | - | - | + | + | - | + | + |
| 12 | Nocardiopsis sp. | White | - | - | - | Oblong | Smooth | + | + | + | + | + | + | + | + |
| 13 | Saccharomonospora sp. | Dark green | + | + | - | Single | Warty | + | - | - | - | + | + | + | + |
| 14 | Streptomyces actuosus | Gray | + | - | - | RF | smooth | + | + | + | + | + | + | + | + |
| 15 | S.alboniger | White | - | - | + | RF | Smooth | + | ± | + | + | ± | – | – | ± |
| 16 | S.aureofasciculus | White | + | + | - | RF | *Smooth* | + | + | + | + | + | + | ± | + |
| 17 | S. avermitilis | Gray | + | | + | Spiral | smooth | + | + | - | + | + | + | + | + |
| 18 | S.coelicolor | Yellow | - | + | + | RF | smooth | + | + | - | + | + | - | - | - |
| 19 | S.finlayii | Gray | - | + | - | RF | Hairy | + | - | - | - | + | + | - | - |
| 20 | S.griseus | Yellow | - | - | - | RF | smooth | - | + | - | + | + | - | - | - |
| 21 | S.hygroscopius | Dull white | - | + | - | Spiral | Rough | + | + | + | + | + | + | - | + |
| 22 | S. noursei | Light gray | - | | + | Spiral | Spiny | + | - | + | + | - | - | - | + |
| 23 | Streptomyces sp. | White | + | + | - | Spiral | Wrinkled | + | - | - | - | + | - | - | - |
| 24 | Streptomyces sp. | Gray | + | + | + | Spiral | Smooth | + | + | + | + | + | - | + | - |
| 25 | S. roseum | Pink | - | + | + | Globular | Smooth | + | + | + | - | + | + | + | + |
| 26 | Streptosporangium nondiastaticum | Pink | - | - | - | Globular | Smooth | + | - | - | + | - | - | - | - |
| 27 | Thermomonospora sp. | Light orange | - | + | - | single | Warty | - | - | - | + | - | - | + | - |

RF Rectiflexible; ± Denotes doubtful; (+) weakly positive

**Table2. Actinobacterial species recorded in the sediment samples of station I & II collected during January 2017-January 2018**

| S. No | Name of the actinobacteria | Station – I | | | | Station – II | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre-monsoon | Monsoon | Post-monsoon | Summer | Pre-monsoon | Monsoon | Post-monsoon | Summer |
| 1 | Actinobispora sp. | - | - | - | - | - | + | + | + |
| 2 | Actinomadura atramentaria | + | + | + | + | - | + | - | + |
| 3 | A. cremea | - | - | - | - | + | - | + | + |
| 4 | A. echinospora | + | + | + | + | + | + | + | + |
| 5 | A. libanotica | - | - | - | - | - | - | + | - |
| 6 | A. rugatobispora | - | - | - | - | - | + | - | + |
| 7 | A. spadix | - | - | - | - | - | + | + | + |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 8 | A. virdis | - | - | + | + | - | - | - | + |
| 9 | Actinomyces sp. | - | - | - | - | - | - | - | + |
| 10 | Microbispora sp. | - | + | - | + | - | - | - | + |
| 11 | Nocardia sp. | - | - | + | + | + | - | + | - |
| 12 | Nocardiopsis sp. | - | - | + | - | - | - | + | + |
| 13 | Saccharomonospora virdis | + | - | + | + | + | - | - | + |
| 14 | Streptomyces actuosus | + | + | + | + | - | + | + | + |
| 15 | S.alboniger | + | + | + | + | + | + | + | + |
| 16 | S.aureofasciculus | + | + | + | + | + | + | + | + |
| 17 | S. avermitilis | - | - | + | + | - | + | + | - |
| 18 | S.coelicolor | + | + | + | + | - | + | - | + |
| 19 | S.finlayii | + | + | + | + | - | + | - | + |
| 20 | S.griseus | + | + | + | + | + | + | + | + |
| 21 | S.hygroscopius | + | - | + | - | + | + | + | - |
| 22 | S. noursei | + | + | + | + | - | - | + | + |
| 23 | Streptomyces sp. | + | + | + | + | + | - | - | + |
| 24 | Streptomyces sp. | - | - | - | - | + | - | - | - |
| 25 | Streptosporangium roseum | - | + | + | + | + | - | + | + |
| 26 | S. nondiastaticum | - | - | - | - | + | - | + | + |
| 27 | Thermomonospora sp. | - | - | - | + | + | - | - | + |
| | **Total** | **12** | **12** | **17** | **17** | **13** | **13** | **16** | **22** |

+ Species present, - Species absent

**Table3. Pearson correlation coefficient (r) values for meteorological, physical and chemical parameters and Actinobacterial population at station I**

| | Total actino bacterial diversity | RF | AT | SWT | pH | S | DO | EC | OM | N | K | Zn | Cu | Fe | Mn | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total actino bacterial diversity | 1 | | | | | | | | | | | | | | | |
| RF | -.179 | 1 | | | | | | | | | | | | | | |
| AT | .867(**) | -.228 | 1 | | | | | | | | | | | | | |
| ST | .755(**) | -.044 | .909(**) | 1 | | | | | | | | | | | | |
| pH | .909(**) | -.270 | .776(**) | .619(*) | 1 | | | | | | | | | | | |
| S | .830(**) | -.334 | .741(**) | .516 | .875(**) | 1 | | | | | | | | | | |
| DO | -.321 | .938(**) | -.387 | -.220 | -.462 | -.416 | 1 | | | | | | | | | |
| EC | .573 | -.248 | .746(**) | .773(**) | .680(*) | .499 | -.476 | 1 | | | | | | | | |
| OM | .791(**) | -.385 | .668(*) | .411 | .854(**) | .847(**) | -.481 | .458 | 1 | | | | | | | |
| N | -.857(**) | .209 | -.684(*) | -.421 | -.845(**) | -.862(**) | .289 | -.342 | -.952(**) | 1 | | | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | -.813 (**) | .297 | -.650 (*) | -.370 | -.827 (**) | -.854 (**) | .360 | -.335 | -.976 (**) | .991 (**) | 1 | | | | | |
| Zn | -.813 (**) | .237 | -.838 (**) | -.655 (*) | -.829 (**) | -.804 (**) | .364 | -.725 (**) | -.734 (**) | .766 (**) | .737 (**) | 1 | | | | |
| Cu | -.769 (**) | .260 | -.528 | -.249 | -.765 (**) | -.784 (**) | .311 | -.163 | -.931 (**) | .968 (**) | .972 (**) | .613 (*) | 1 | | | |
| Fe | -.865 (**) | .153 | -.808 (**) | -.668 (*) | -.948 (**) | -.880 (**) | .337 | -.730 (**) | -.857 (**) | .836 (**) | .822 (**) | .825 (**) | .715 (**) | 1 | | |
| Mn | -.826 (**) | .427 | -.744 (**) | -.573 | -.902 (**) | -.868 (**) | .578(*) | -.597 (*) | -.929 (**) | .846 (**) | .861 (**) | .721 (**) | .815 (**) | .877 (**) | 1 | |
| P | -.729 (**) | .309 | -.487 | -.281 | -.789 (**) | -.712 (**) | .405 | -.276 | -.848 (**) | .812 (**) | .830 (**) | .483 | .853 (**) | .738 (**) | .838 (**) | 1 |

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

**Table4. Pearson correlation co-efficient(r) values for meteorological, physical and chemical parameters and Actinobacterial population at station II**

| | Total actino bacterial diversity | RF | AT | SWT | pH | S | DO | EC | OM | N | K | Zn | Cu | Fe | Mn | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total actino bacterial diversity | 1 | | | | | | | | | | | | | | | |
| RF | -.115 | 1 | | | | | | | | | | | | | | |
| AT | .805 (**) | -.227 | 1 | | | | | | | | | | | | | |
| ST | .692 (*) | -.044 | .909 (**) | 1 | | | | | | | | | | | | |
| pH | .870 (**) | -.128 | .779 (**) | .613 (*) | 1 | | | | | | | | | | | |
| S | .844 (**) | -.411 | .824 (**) | .615 (*) | .915 (**) | 1 | | | | | | | | | | |
| DO | -.209 | .953 (**) | -.340 | -.161 | -.225 | -.505 | 1 | | | | | | | | | |
| EC | .704 (*) | -.100 | .702 (*) | .592 (*) | .818 (**) | .685 (*) | -.227 | 1 | | | | | | | | |
| OM | -.690 (*) | -.009 | -.518 | -.255 | -.789 (**) | -.705 (*) | -.021 | -.526 | 1 | | | | | | | |
| N | -.555 | .346 | -.313 | -.029 | -.569 | -.702 (*) | .318 | -.221 | .697 (*) | 1 | | | | | | |
| K | -.789 (**) | .285 | -.630 (*) | -.347 | -.864 (**) | -.889 (**) | .326 | -.627 (*) | .886 (**) | .857 (**) | 1 | | | | | |
| Zn | -.729 (**) | .429 | -.857 (**) | -.754 (**) | -.791 (**) | -.842 (**) | .585 (*) | -.688 (*) | .378 | .353 | .635 (*) | 1 | | | | |
| Cu | -.474 | .355 | -.273 | -.080 | -.540 | -.670 (*) | .357 | -.208 | .496 | .924 (**) | .761 (**) | .438 | 1 | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fe | -.825 (**) | .068 | -.699 (*) | -.450 | -.866 (**) | -.765 (**) | .090 | -.751 (**) | .901 (**) | .553 | .839 (**) | .540 | .356 | 1 | |
| Mn | -.721 (**) | .107 | -.807 (**) | -.778 (**) | -.758 (**) | -.760 (**) | .261 | -.663 (*) | .415 | .405 | .619 (*) | .832 (**) | .494 | .494 | 1 |
| P | -.532 | .427 | -.426 | -.220 | -.698 (*) | -.774 (**) | .502 | -.442 | .466 | .773 (**) | .747 (**) | .674 (*) | .864 (**) | .431 | .695 (*) | 1 |

** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

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