

Research on marine actinobacteria in India

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Received: 19 May 2007 / Final revision: 29 May 2007 / Accepted: 2 June 2007

Abstract Marine actinobacteriology is one of the major emerging areas of research in tropics. Marine actinobacteria occur on the sediments and in water and also other biomass (mangrove) and substrates (animal). These organisms are gaining importance not only for their taxonomic and ecological perspectives, but also for their unique metabolites and enzymes. Many earlier studies on these organisms were confined only to the temperate regions. In tropical environment, investigations on them have gained importance only in the last two decades. So far, from the Indian peninsula, 41 species of actinobacteria belonging to 8 genera have been recorded. The genus, *Streptomyces* of marine origin has been more frequently recorded. Of 9 maritime states of India, only 4 have been extensively covered for the study of marine actinobacteria. Most of the studies conducted pertain to isolation, identification and maintenance of these organisms in different culture media. Further, attention has been focused on studying their antagonistic properties against different pathogens. Their biotechnological potentials are yet to be fully explored.

Keywords Marine actinobacteria · Diversity · Antibiotics · Anticancer compounds · Enzymes.

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Introduction

Actinobacteria have been looked upon as potential sources of bioactive compounds, and the work done earlier has shown that these microbes are the richest sources of secondary metabolites. They hold a prominent position as targets in screening programs due to their diversity and their proven ability to produce novel metabolites and other molecules of pharmaceutical importance¹. Since the discovery of actinomycin², actinobacteria have been found to produce many commercially bioactive compounds and antitumor agents in addition to enzymes of industrial interest³. Approximately, two-thirds of the thousands of naturally occurring antibiotics have been isolated from these organisms⁴. Of them, many have been obtained from *Streptomyces*⁵ and these natural products have been an extraordinary source for lead structures in the development of new drugs.

The terrestrial soils have been the predominant and widely exploited source, and investigations on marine actinobacteria are a few and inconclusive, though they are the important sources for new bioactive compounds⁶. In recent years, there has been a growing awareness of the potential value of marine sediments as sources of actinobacteria that produce useful bioactive metabolic products⁷. Goodfellow & Hayens⁷ reviewed the literature on the isolation of actinobacteria from marine sediments and suggested that these sources may be valuable for the isolation of novel actinobacteria with the potential to yield useful new products. However, it has not yet been resolved whether the actinobacteria form part of the autochthonous marine microbial community of sediment samples, originated from terrestrial habitats or they are simply carried to the sea in the form of resistant spores.

However, actinobacteria isolated from marine environment have recently been screened for novel metabolites, and there is evidence that actinobacteria usually make up only a small portion of the bacterial flora of marine habitats with absolute numbers of actinobacteria much lower than the terrestrial habitats⁸. The consequence is that it should be more difficult to obtain large numbers of isolates from the marine samples for screening purposes. Some microbiologists have investigated the distribution and biological characteristics of the aquatic actinobacteria and their distribution is expected to be different from that of the soil actinobacteria⁹. Research on the biodiversity of marine actinobacteria is not only important for the basic studies but also necessary for their exploitation. Still now, there are no comprehensive documents on the marine actinobacterial research in India, and hence, the present attempt has been made to review available literature on various aspects of marine actinobacteria of India.

Role of actinobacteria in the marine environment

Actinobacteria have a profound role in the marine environment. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms. There is a speculation that the increase or decrease of a particular enzyme-producing microorganism may indicate the concentration of natural substrate and conditions of the environment¹⁰. The cellulolytic^{11–13}, proteolytic^{1,14}, amylolytic^{1,15}, lipolytic¹, chitinolytic¹⁶, phosphate-solubilizing¹⁷ activities of marine actinobacteria were reported. Actinobacteria are also reported to contribute to the breakdown and recycling of organic compounds¹⁸.

Biotechnological importance of marine actinobacteria

Antibiotics Marine actinobacteria constitute an important and potential source of novel bioactive compounds¹⁹. Since environmental conditions of the sea are extremely different from terrestrial conditions, they produce different types of antibiotics. Several antibiotics have been isolated from marine actinobacteria by many researchers^{20–30}. The isolated antibiotics are entirely new and unique when compared to those from the terrestrial ones³¹.

Enzymes Marine actinobacteria have a diverse range of enzyme activities and are capable of catalyzing various biochemical reactions¹⁰. Different commercial enzymes viz. L-glutaminase³², α -galactosidase³³, amylase³⁴, cellulase¹³, protease¹⁴, L-asparaginase^{35,36} have also been obtained from the marine actinobacteria.

Enzyme inhibitors Enzyme inhibitors have received increasing attention as useful tools, not only for the study

of enzyme structures and reaction mechanisms but also for potential utilization in pharmacology³⁷. Marine actinobacteria are the potential source for production of enzyme inhibitors^{38,39}. Imade³⁹ reported different types of enzyme inhibitors viz. β -glucosidase, N-acetyl- β -D-glucosaminidase, pyroglutamyl peptidase, α -amylase inhibitors from marine actinobacteria.

Anticancer compounds Cancer is a term that refers to a large group of over a hundred different diseases that arise when defects in physiological regulation cause unrestrained proliferation of abnormal cells⁴⁰. In most cases, these clonal cells accumulate and multiply, forming tumors that may compress, invade and destroy normal tissue, weakening the vital functions of the body with devastating consequences including loss of quality of life and mortality. Nowadays, cancer is the second cause of death in the developed world, affecting one out of three individuals and resulting in one out of five deaths world wide⁴⁰. Diversified groups of marine actinobacteria are known to produce different types to anticancer compounds. Several kinds of cytotoxic compounds have been reported from marine actinobacteria^{41–48}. The isolated compounds showed significant activity against different cancer cell lines.

Single cell protein Actinobacteria are known to produce secondary metabolites that enhance the growth of juvenile fish, shrimp and prawn. Some of the secondary metabolites are organometallic compounds such as ferrioxamines, magnesidin with bleomycin, beron containing compounds such as boromycin & aplasmomycin⁴⁹ and unusual amino acids such as alanosine, amino dichlobutyric acid, azaleucine, 4-oxalysine etc.⁵⁰. Juveniles of prawn and shrimp fed on actinobacteria incorporated feed showed improved growth, food conversion efficiency and higher protein content⁵¹. Hence, among unconventional protein sources, single cell protein (SCP) of microbial origin appear to be a promising substitute for fishmeal, which can replace up to 25–50% fishmeal in aquaculture operations.

Research on marine actinobacteria in India

Maharashtra State Early work on marine actinobacteria in India was by Baam *et al.*⁵² who isolated two antagonistic *Streptomyces* species from Bombay waters and both the species exhibited antibacterial activity. Postmaster & Freitas⁵³ reported *Streptomyces* spp. from the marsh sediments of Bombay, of which seven showed antibiotic activity. Sharma & Pant⁵⁴ isolated an actinobacteria from a chronically oil-polluted coastal region near Mumbai harbour and it was identified as *Rhodococcus* sp. The isolate degraded the aliphatic and aromatic compounds, but not the

asphaltene fractions of three different crude oils. Under optimized conditions [70mM nitrogen as urea, 0.1 mM phosphorous as K_2HPO_4 , pH 8.0 at 30°C and 150 rpm on a laboratory shaker for 72 h], 72%, 60% and 35% of the aliphatic fractions of Bombay High, Assam and Gujarat crude oils were degraded, respectively. Although *Rhodococcus* sp. was isolated from the sea water, it grew optimally a 0.4M NaCl, tolerating up to 1.7M NaCl and was also able to grow on nutrient broth made in distilled water, suggesting that it is a facultative halophile. It may, therefore, be important in the biodegradation of hydrocarbon contaminated soils and aquatic systems, both marine and fresh water.

A halophilic *Actinopolyspora* species AHI was isolated from the sediments of Alibag coast of Maharashtra⁵⁵. This strain exhibited good antagonistic activity against gram-positive bacteria viz. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and fungi such as *Aspergillus niger*, *A. umigatus*, *A. flavus*, *Fusarium oxysporum*, *Penicillium* sp. and *Trichoderma* sp. It did not show any antibacterial activity against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes* and against the fungi, *Candida albicans* and *Cryptococcus* sp. The strain showed resistance to clindamycin, vancomycin, nalixidic acid and streptomycin.

Kerala State Kala & Chandrika⁵⁶ used different media for isolating and maintaining actinobacteria collected from mangrove sediments. Out of many recommended media for selective isolation of actinobacteria from soil, Glucose asparaginase agar, Grein and Meyer's agar, Oatmeal agar and Kuster's agar were found suitable for the isolation of actinobacteria from mangrove sediments. The best media which allowed good development of actinobacteria, while suppressing bacterial growth, were those containing starch or glucose as the carbon source with casein and asparagine or nitrate as the nitrogen source. Seawater-agar was also tried for isolation and maintenance, and found extremely good as a maintenance medium. Bacteriostatic and fungistatic compounds such as calcium carbonate, phenol, lactic acid and acetic acid were used for selective isolation of actinobacteria.

Mathew *et al.*⁵⁷ isolated *Streptomyces* spp. from *Vilvorita cyprinoids* and checked their antagonistic activity against *V. anguillarum*, *S. aureus*, *C. albicans*, *A. niger* and *S. cerevisiae*. The isolated strains were also checked for their L-asparaginase activity and growth at different pH, temperatures, sodium chloride concentrations, carbon compounds, nitrogen compounds and amino acids. Manju & Dhevendaran⁵¹ studied the effect of bacteria and actinobacteria as single cell protein feed on the growth of the juveniles of *Macrobrachium idella*. Improved growth, food conversion efficiency and high protein content in *M. idella*

were observed when actinobacteria were incorporated into the feed. Dhevendaran & Annie⁵⁸ isolated *Streptomyces* sp. from the shellfishes and the sediments of Veli estuarine lake and studied their L-asparaginase activity. Out of sixteen strains, one strain isolated from the *Fenneropenaeus indicus* showed maximum L-asparaginase activity at pH 7, 37°C and 2-5% of NaCl.

Dhevendaran & Anithakumari⁵⁹ reported 250 species of streptomycetes from the gut of the fish, *Therapon jarbua* and shellfish, *Vilvorita cyprinoids* and sediments of Veli lake. Among them, 16 isolates were randomly selected to check their antagonistic activity against the *Vibrio anguillarum*, *V. alginolyticus*, *V. parahaemolyticus*, *S. aureus*, *A. niger*, *C. albicans* and *S. cerevisiae*. Of these, 12 isolates inhibited the growth of *V. anguillarum*, while 10 isolates inhibited *S. aureus*. None of the isolates inhibited the growth of *V. alginolyticus*, *V. parahaemolyticus*, *A. niger*, *C. albicans* and *S. cerevisiae*. The strains exhibited L-asparaginase activity under growing conditions in a liquid broth with the optimum enzyme activity and growth at 37°C and pH with 7 with 2-5% NaCl.

Mathew & Philip⁶⁰ isolated six strains of actinobacteria from the sediments of the Arabian sea. The isolated strains were tested for the production of antibiotic on 14 different media. Of the 14 media used, only M₁₃ and M₁₄ media supported antibiotic production. Dhevendaran *et al.*⁶¹ isolated streptomycetes from *Perna viridis*, *Grapsus strigosus*, *Ulva fasciata* and *Sargassum wightii* collected from Kovalam coast. The distribution pattern of the microorganisms with special emphasis on streptomycetes was carried out using special microbiological media. Streptomycetes isolated from the visceral mass of *P. viridis* and *G. strigosus* showed maximum colonization in Actinomycete agar medium, whereas streptomycetes associated with the fauna and seaweed showed a high diversity in pigmentation. *Streptomyces* harboured in the visceral mass of *P. viridis* exhibited antagonism against *Aeromonas* sp.

Tamil Nadu State The nature of cellulose production in chemically defined media was investigated using 15 *Streptomyces* spp. collected from the Bay of Bengal¹¹. The culture filtrate was used as the source of cellulase. All the isolates examined were capable of elaborating extra-cellular cellulase to varying degrees, thus suggesting that the marine actinobacteria can play an active role in the degradation of cellulosic substrates in the marine environment. Laksmanaperumalsamy *et al.*⁶² isolated 518 *Streptomyces* strains from the sediments of estuarine, backwater, marine, freshwater and mangrove environment of Porto Novo using Grein and Meyer's agar, Kuster's agar and Glucose asparagine agar. These isolates were checked for both antibacterial and antifungal activities against *B. circulans*, *S. aureus*,

E. coli, *P. aeruginosa*, *S. cerevisiae* and *F. oxysporum*. It was found that, 27.03% of the strains elaborated one or more types of antibiotics and 59.27% were active against *B. circulans*, 47.01% against *S. aureus*, 30% against *E. coli*, 53.59% against *S. cerevisiae* and 39.3% against *F. oxysporum*. Majority of the isolates (46.43%) showed combined antibacterial and antifungal activity and 25% showed only antibacterial activity.

Three strains of *Streptomyces* were isolated from the digestive tract of *Barnea birmanica* collected from the mangrove region near Porto Novo⁶³. The isolates were checked for their cellulase activity at different pH and sodium chloride concentrations. Vanajakumar *et al.*⁶⁴ isolated 386 strains of actinobacteria from five marine molluscs *viz.* *Crassostrea madrasensis*, *Meretrix casta*, *Anadara rhombea*, *Telescopium telescopium* and *Bullia vittata* from the shell surface, mantle and gut of all the five molluscs. Mantle tissue harboured the most antagonistic strains (84%), followed by gut (77%) and shell surface (69%). When these strains were screened for the production of antibiotics by cross-streak method, 290 strains (75%) exhibited antagonistic properties. Combined antibacterial and fungal properties were found in 46.4% of the antagonistic actinobacteria. Cultures exhibiting only antifungal properties were found in 2.4% of the actinobacteria, while antibacterial activities were seen in 42%. In the colour-series tests, 99 (84%) of the grey-series, 63 (82%) of the 77 yellow series, 11 (73%) of the 15 red series and 117 (66%) of the 176 white series showed antagonistic properties.

Balagurunathan *et al.*⁶⁵ studied the antagonistic actinobacteria isolated from the littoral sediments of Parangipettai. Among the 51 strains, only 11 strains showed good antibiotic activity and they were identified as *Streptomyces* spp. and *Nocardia* spp. Y-lactone type of antibiotic was extracted from *Streptomyces griseobrunneus*⁶⁶. This antibiotic was tested against fish pathogens *viz.* species of *Vibrio*, *Aeromonas*, *Pseudomonas*, *Bacillus* and *Fusarium* and it inhibited all these pathogenic organisms with inhibition zones ranging from 10–30 mm. The *Vibrio* sp. and *Pseudomonas* sp. were more sensitive than the other bacterial species tested.

Sivakumar^{67,68} isolated actinobacteria from the Pitchavaram mangrove environment. The 16S rRNA genes of the isolated two strains were partially sequenced and he proposed them as new species (*Actinopolyspora indiensis* and *Streptomyces kathirae*) to the science. The sequence of the two new species were deposited in the Gen Bank, National Centre for Biotechnological Information, USA under the sequence of the accession numbers AY015427 and AY015428.

Partil *et al.*⁶⁹ reported 133 strains of actinobacteria from 129 marine samples collected from various stations along

the Tuticorin coast. Of the 104 strains of actinobacteria screened for the inhibitory activity against bacterial pathogens associated with fish diseases (*Aeromonas hydrophila*, *Aeromonas sobria* and *Edwardsiella tarda*), 77 isolates were inhibitory to at least one of the pathogens. The highest incidence of inhibitory isolates was noticed in the sediment samples and all the isolates of antagonistic marine actinobacteria were of *Streptomyces* spp. Balagurunathan & Subramanian⁷⁰ isolated 51 strains of *Streptomyces* from the littoral sediments of Parangipettai coastal waters. Out of these, only 8 strains showed very promising antibiotic activity against bacteria and fungi. These strains exhibited higher activity against gram-positive bacteria than the gram-negative bacteria. The strains also showed chitinase, protease and cellulase activities. Patil *et al.*⁷¹ isolated 20 actinobacterial strains from water and sediment samples of mangrove area of Tuticorin. The average actinobacterial load in the water and sediments was 4.79×10^4 CFU/ml and 5.03×10^4 CFU/g, respectively. The strains were checked for their antagonistic activity against seven shrimp bacterial pathogens. Among them, 83% showed good antagonistic activity against all the tested pathogens.

Dhevendaran & Praseetha⁷² isolated pigment producing streptomycetes from 14 different species of seaweeds of Cape-Comarin, using different culture media *viz.* Actinomycetes agar, Kuster's agar and Glyceroal asparagine agar media and more number of streptomycetes were observed in Glyceroal asparagine agar. Sahu *et al.*⁷³ isolated 40 strains of actinobacteria from the gut contents of three estuarine fishes *viz.* *Chanos chanos*, *Etroplus suratensis* and *Lates calcarifer*. The isolated strains were tested for their antagonistic activity against six bacterial species *viz.* *B. subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *S. aureus*, *Shigella flexneri* and *Vibrio cholerae*. Among them, only 10 strains of actinobacteria (30%) showed moderate antagonistic activity against all the tested pathogens.

Kathiresan *et al.*⁷⁴ isolated 160 strains from the sediments of mangrove, estuary, sand dune and industrially polluted marine environment of Cuddalore. Of these, mangrove sediments were the rich sources for actinobacteria. When these isolates were tested against phytopathogenic fungi *viz.* *Rhizoctonia solani*, *Pyricularia oryzae*, *Helminthosporium oryzae* and *Colletotrichum falcatum*, about 51% of the isolates were effective against *P. oryzae* and *H. oryzae*, 31% against *R. solani* and 12.5% against *C. falcatum*. Of the 160 isolates, 10 showed potent activity against all the fungi tested. These isolates produced high antifungal compounds at 120 h of incubation period in the production medium culture. Glucose and Soyabean meal were the best carbon and nitrogen sources respectively and 17.5 ppt was the best salinity level for maximum antibiotic production.

Dhanasekaran *et al.*⁷⁵ reported 107 strains of actinobacteria from 16 different marine soil samples and studied their antifungal activity against five test fungi *viz.* *A. niger*, *Curvularia palescens*, *C. albicans*, *Candida tropicalis* and *S. cerevisiae*. Out of these, only 22 isolates (21.2%) which were grown in Starch Casein agar produced diffusible antifungal substances in varying quantities. Potency of the culture filtrate was estimated by agar cup assay method using *C. albicans*. The antifungal activity was also tested by agar overlay method using *C. albicans* and *S. cerevisiae* as test organisms. Six isolates showed strong antifungal action in both agar cup and agar overlay assays. Sivakumar *et al.*⁷⁶ reported 91 strains of actinobacteria from different stations of the Pitchavaram mangrove ecosystem. The isolated strains were tested for their antagonistic activity against the potential human pathogens such as *B. subtilis*, *P. vulgaris*, *S. flexneri*, *K. pneumoniae*, *V. cholerae* and *S. aureus*. Out of the 91 strains, only 6 strains showed good activity and they were identified upto species level (Table 1). Sivakumar *et al.*⁷⁷ isolated actinobacteria colonies from different stations of the Pitchavaram mangrove ecosystem using three different media. Consistently a higher number of populations were isolated on Kuster's agar and the higher population density recorded was 4×10^4 CFU/g. This led them to conclude that for enumerating the actinobacterial populations from the mangrove environment, Kuster's agar medium is suitable. Sivakumar *et al.*⁷⁸ also isolated actinobacteria from different other stations of the Pitchavaram mangrove ecosystem. The isolated strains were tested for their antagonistic activity against various human pathogens. Among them, only one strain showed very prominent activity against *C. albicans*, *P. vulgaris*, *S. aureus* and *K. pneumoniae* and it was identified as *Streptomyces roseolilacinus*.

Sahu *et al.*⁷⁹ studied actinobacterial population density from different samples *viz.* water, sediments, seaweeds, molluscs and finfishes of the Vellar estuary. The sediment samples harboured higher population density compared to the water samples. Biological samples *viz.* seaweeds, molluscs and finfishes were also analysed for actinobacterial population. Among them, molluscs recorded higher population density in shell surface region than the gut contents, while the finfishes recorded higher population in gut contents followed by gills and skin. Seaweed samples also recorded considerable actinobacterial populations. Sahu *et al.*⁸⁰ studied the extra-cellular enzyme (amylase, lipase, protease, cellulase and chitinase) activities of actinobacteria isolated from the sediment and molluscan samples of the Vellar estuary. The study indicated that the actinobacteria are the potential sources for extra-cellular enzymes, which play a role in biodegradation of organic matter, thereby enhancing the productivity of the marine environment.

Umamaheswary *et al.*⁸¹ isolated 40 strains of actinobacteria from the estuarine fish, *Mugil cephalus* using Kuster's agar medium. Out of 40 strains tested, only the strain *S. galbus* showed good L-glutaminase activity. Various process parameters which influenced L-glutaminase production by the *S. galbus* were optimized. Maximal enzyme production (18.93IU/ml) was attained at pH 9, 36^o C, and glucose and malt-extract as carbon sources after 72 h of incubation.

Senthilkumar *et al.*⁸² isolated 41 halophilic actinobacterial strains from the salt marsh area of the Vellar estuary using four different media. SC agar medium was the best for the isolation of halophilic actinobacteria. Among the isolated strains, the strain SH-9 showed greater resistance towards mercuric chloride in agar diffusion assay. The strain was classified as *Actinopolyspora* sp. by its morphological and chemotaxonomical characters. Sivakumar *et al.*³² isolated actinobacterial strains from skin, gills and gut contents of the estuarine fish, *Chanos chanos*. Out of 20 strains tested, *Streptomyces rimosus* showed L-glutaminase activity. Optimum production of L-glutaminase (18.93IU/ml) was observed after 96 h at 27^o C, pH 9 with glucose and malt extract. Sahu *et al.*⁸³ also reported a total number of 40 strains of actinobacteria from the sediments of the Vellar estuary and checked their antagonistic activity against the human bacterial pathogens (*B. subtilis*, *Pseudomonas vulgaris*, *Shigella flexneri*, *K. pneumoniae*, *V. cholerae* and *S. aureus*). Among them, 9 strains (22.5%) showed activity against the tested pathogens and 5 strains which showed good activity were identified upto species level (Table 1).

Muthurayar *et al.*⁸⁴ isolated a total of 18 actinobacterial strains from an estuarine fish, *Chanos chanos* and studied their antagonistic activity against human bacterial pathogens. Out of 18 strains, only five strains (A-1, AA-5, AA-10, AA-13 and AA-17) showed moderate activity against all the tested pathogens. These strains were mutated using physical (UV radiation) and chemical (NTG) mutagens. The study suggested that mutation is one of the good methods for strain development to increase the efficiency of the actinobacteria for antibacterial production. Kundu *et al.*⁸⁵ isolated 39 strains of actinobacteria from different parts *viz.* foregut, midgut and hindgut of the alimentary canal of estuarine fishes. The isolated strains were tested for their extra-cellular enzyme (amylase, protease, cellulase and lipase) activities. Among thirty nine, six strains which exhibited prominent activities were identified upto species level (Table 1).

Murugan *et al.*¹³ isolated actinobacteria (35 strains) from the gut contents of the estuarine finfish *Mugil cephalus* and were examined for their cellulase activity. The strain *Streptomyces actuosus* showed maximum cellulase activity at pH 7, temperature 35^oC, NaCl concentration 1-2%,

Table 1 List of marine actinobacteria reported from the Indian Peninsula.

Sl. no.	Species	Habitat	Location	Reference
1.	<i>Actinomyces</i> sp.	Sediments	Managalavanam, Kerala	56
2.	<i>Micromonospora</i> sp.	Sediments	Visakhapatnam coast, A.P.	87
3.	<i>Micropolyspora</i> sp.	Sediments	Visakhapatnam coast, A.P.	87
4.	<i>Nocardia</i> sp.	Sediments	Visakhapatnam coast, A.P.	87
5.	<i>Rhodococcus</i> sp.	Oil polluted coastal region	Mumbai harbour, Mumbai	54
6.	<i>Streptomyces albidoflavus</i>	Sediments	Pitchavaram mangrove, T.N.	76
7.	<i>S. alboniger</i>	Sediments	Vellar estuary, T.N.	70
8.	<i>S. albovinaceus</i>	Sediments	Vellar estuary, T.N.	70
9.	<i>S. albus</i>	Different parts of fishes	Vellar estuary, T.N.	85
10.	<i>S. aureocirculatus</i>	Sediments	Pitchavaram mangrove, T.N.	76
11.	<i>S. aureofasciculus</i>	Sediments	Vellar estuary, T.N.	83
12.	<i>S. baarnensis</i>	Sediments	Vellar estuary, T.N.	70
13.	<i>S. californicus</i>	Sediments	Arabian Sea	60
14.	<i>S. canus</i>	Different parts of fishes	Vellar estuary, T.N.	36
15.	<i>S. chattanogensis</i>	Different parts of fishes	Vellar estuary, T.N.	35
16.	<i>S. clavifer</i>	Sediments	Pitchavaram mangrove, T.N.	76
17.	<i>S. fradiae</i>	Sediments	Arabian Sea	60
18.	<i>S. galbus</i>	Alimentary canal of fishes	Vellar estuary, T.N.	81
19.	<i>S. galtieri</i>	Sediments	Pitchavaram mangrove, T.N.	76
20.	<i>S. gibsonii</i>	Sediments	Pitchavaram mangrove, T.N.	76
21.	<i>S. griseobrunneus</i>	Sediments	Vellar estuary, T.N.	70
22.	<i>S. griseoflavus</i>	Sediments	Arabian Sea	60
23.	<i>S. griseorubiginosus</i>	Sediments	Vellar estuary, T.N.	70
24.	<i>S. hawaiiensis</i>	Different parts of fishes	Vellar estuary, T.N.	35
25.	<i>S. kanamyceticus</i>	Sediments	Pitchavaram mangrove, T.N.	76
26.	<i>S. marinensis</i>	Water	Visakhapatnam coast, T.N.	86
27.	<i>S. moderatus</i>	Sediments	Vellar estuary, T.N.	70
28.	<i>S. nigrifaciens</i>	Sediments	Vellar estuary, T.N.	70
29.	<i>S. olivoviridis</i>	Different parts of fishes	Vellar estuary, T.N.	35
30.	<i>S. orientalis</i>	Different parts of fishes	Vellar estuary, T.N.	35
31.	<i>S. palveraceus</i>	Sediments	Arabian Sea	60
32.	<i>S. plicatus</i>	Alimentary canal of fish	Veli Lake, Kerala	83
33.	<i>S. rimosus</i>	Gut contents of fish	Vellar estuary, T.N.	32
34.	<i>S. roseolilacinus</i>	Sediments	Pitchavaram mangrove, T.N.	78
35.	<i>S. scabies</i>	Different parts of fishes	Vellar estuary, T.N.	85
36.	<i>S. subflavus</i>	Sediments	Vellar estuary, T.N.	70
37.	<i>S. vastus</i>	Sediments	Vellar estuary, T.N.	83
38.	<i>S. violaceus</i>	Sediments	Vellar estuary, T.N.	83
39.	<i>S. xantholiticus</i>	Sediments	Pitchavaram mangrove, T.N.	76
40.	<i>Streptosporangium</i> sp.	Sediments	Visakhapatnam coast, A.P.	87
41.	<i>Streptoverticillium</i> sp.	Sediments	Visakhapatnam coast, A.P.	87

carbon compound viz. sucrose and without addition of any amino acids. The molecular weight of the cellulase on SDS-PAGE was 110 kDa. Sahu *et al.*¹⁴ screened actinobacterial strains from gut contents of the tiger shrimp, *Penaeus monodon*. Out of the 17 strains tested, the strain *Streptomyces galbus* showed protease activity. Optimum production of protease (14.52 IU/ml) was observed after 72 h of incubation at pH 9, temperature 39°C with starch and casein as carbon and nitrogen sources, respectively. Sahu *et al.*³⁵ isolated 40 species of actinobacteria from the different parts viz. skin, gills and gut contents of three species of fishes viz. *Mugil cephalus*, *Chanos chanos* and *Eetroplus suratensis* from the Vellar estuary. The strains were tested for their L-asparaginase activity and among them, only six strains showed significant L-asparaginase activity. Impact of various physical and chemical factors such as pH, temperature, sodium chloride concentration, carbon sources and amino acids on the growth of actinobacteria and L-asparaginase production was also studied. Optimum growth and enzyme activity was noticed at pH 7 to 8, 37°C, 1–2% sodium chloride concentration, sucrose as carbon source. Sahu *et al.*³⁶ also partially purified L-asparaginase enzyme from *Streptomyces canus* and studied the anti-leukemic activity in mice. Sahu *et al.*¹⁷ studied total actinobacteria and phosphate solubilizing actinobacterial population density from the different sediment samples of the Vellar estuary. Phosphatase activity in the sediments was also investigated. Consistently, a higher number of actinobacteria, phosphate solubilizing actinobacteria and phosphatase activities were recorded from the clay sediments than the sandy sediments at all the stations. In all, 7 strains showed phosphatase activity. Among them, one strain PS-3, which was tentatively identified as *Streptomyces galbus*, exhibited good activity. The phosphate solubilizing activity was high at pH 6–7 in 13 days.

Andhra Pradesh State A new antagonistic species *Streptomyces marinensis* producing neomycin (B&C) complex, was reported by Sambamurthy & Ellaiah⁸⁶ from the Visakhapatnam coast. Growth of this species was moderate to good in almost all the media as flat to low convex the colonies. Aerial mycelium was pink to dull pink in colour. It exhibited a strong amyolytic activity. The species completely coagulated and peptonized milk with an alkaline reaction, liquefied gelatin and coagulated serum, haemolysed blood, reduced nitrate strongly and showed catalase activity. Ellaiah & Reddy⁸⁷ isolated 140 strains of actinobacteria from the marine sediments of Visakhapatnam coast and identified them upto genus level (Table 1). Out of these 140 strains, only 18% exhibited anti-microbial activity against bacteria and fungi. Ellaiah *et al.*⁸⁸ isolated actinobacteria from the sediments off the Bay of Bengal and the strains

which showed good antagonistic activity were identified upto species level. Ellaiah *et al.*⁸⁹ isolated 80 strains of actinobacteria from the sediments off the Bay of Bengal near Machilipatnam by plating on starch casein agar medium. Of these, 7 isolates exhibited broad-spectrum antimicrobial activity, 68 showed proteolytic activity and 62 showed amyolytic activity. Ellaiah *et al.*¹ have isolated 60 actinobacteria from the Bay of Bengal near Kakinada coast with distinct characteristics, by plating on Starch Casein agar medium. Among them, 11 isolates exhibited antibacterial (18.3%), 10 isolates showed antifungal (16.6%) while 2 isolates showed both antibacterial and antifungal (3.3%) activities. All 60 isolates were also tested for enzymatic activities; 49 (81.6%) and 51 isolates (85%) exhibited amyolytic and proteolytic activities, respectively.

Andaman and Nicobar group of islands Kerkar⁹⁰ isolated *Streptomyces* sp. from the intertidal sediments, collected from the Carbyns cove and it showed a broad range of inhibitory activity against non-marine and marine cultures. Optimum conditions for its growth and production of antibiotics were studied. Production of antibiotics was mediated by two plasmids (3.38 Kb and 7.58 Kb). Antibiotic activity of this species was high at pH 5 and at 28±2°C and it was unaffected by the variations in sodium chloride concentrations. The partially purified antibiotic was stable at 4°C even after 15 days, whereas it was inactivated after 2 days at 37°C. Sahu *et al.*⁹¹ assessed the population density of actinobacteria from eight different stations of the Little Andaman island. Mean population density of actinobacteria recorded from the water samples varied from 0.29 to 0.45 ×10³ CFU/ml with the minimum of 0.29 ×10³ CFU/ml at Navel Area and the maximum of 0.45 ×10³ CFU/ml at Chandra Nallah. In the case of sediment samples, population density ranged from 1.21 to 3.29×10³ CFU/g with a minimum of 1.21 ×10³ CFU/g at Navel Area and a maximum of 3.29 ×10³ CFU/g at Butler Bay. During the investigation, a total of 41 strains were isolated and tested for their antagonistic activity against the bacteria that are highly pathogenic to shrimps such as *Vibrio alginolyticus*, *V. harveyi* and *V. parahaemolyticus*. More than 61% of the strains (26) exhibited varying degree of antagonistic activity. Among them, 6 strains showed good activity and they were tentatively identified. The results suggest that the actinobacteria from the marine environment can be used as bio-control agents in shrimp culture systems to control diseases caused by bacterial pathogens.

Summary and conclusions

In summary, forty years of floristic inventory of marine actinobacteria in Indian Peninsula yielded 41 species belonging

to 8 genera. Majority of the surveys have been conducted in the coastal areas, collecting the littoral sediments from the states of Maharashtra, Kerala, Tamil Nadu and Andhra Pradesh. Studies covering the Gujarat, Goa, Karnataka, Orissa, West Bengal and Andaman and Nicobar islands are scanty. Recently, in India, attention is being focused to isolate the novel strains of actinobacteria from different biological samples such as fish, molluscs, mangroves, seaweeds, and sea grasses, besides seawater and sediments. Results are very encouraging and have opened up new areas for exploring the biotechnological potentials of these organisms in India.

Acknowledgements Authors thank Prof. T. Balasubramanian, Director, Centre of Advanced Study in Marine Biology and the authorities of Annamalai University for providing with necessary facilities. Authors are also thankful to all the authors who's papers have been used for this review. One of the authors (M.K.S.) is thankful to the Ministry of Environment and Forests, Government of India for the fellowship.

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