Marine actinobacteria: A concise account for young researchers

Rajagopal Gobalakrishnan1*, Palaniappan Sivasankar2 & Kannan Sivakumar3

1District Institution of Education and Training, Kalaiyarkoil- 630 551, Sivagangai District, Tamilnadu, India.
2Department of Environmental Science, Periyar University, Periyar Palkalai Nagar, Salem, 636 011, Tamil Nadu, India.
3Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamilnadu, India

*[E-mail: gobalaldy@gmail.com; sivasankar.ps@gmail.com]

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Present article consists the marine actinobacteria with reference to their occurrence, potentiality, culture media, pretreatment and identification. This information is important as it would pave way for future microbiologists to elucidate the potential of the marine actinobacteria for various economical applications.

[Key words: Marine actinobacteria, occurrence, potentiality, pretreatments, identification]

Introduction

Microorganisms live in every corner of the ocean and their habitats are diverse; they are distributed in open waters, sediments, bodies of marine macro and microorganisms, estuaries and hydrothermal vents1. Marine environment represents a largely less tapped source for the isolation of new microorganisms including actinobacteria2. These microbes are always involved in the important processes of the sea in promoting organic material transformation and mineralization in the sediments and overlying waters3.

Among the microbes, actinobacteria (actinomycetes) are one of the largest taxonomic units within the Bacterial domain4,5. They are versatile aerobic gram-positive bacteria with a higher amount of guanine plus cytosine (>50mol% G+C) in their DNA (deoxyribo-nucleic acid). Their morphology ranges from coccoid (e.g. Micrococcus), rod-coccoid (e.g. Arthrobacter), fragmenting hyphal forms (e.g. Nocardia) to those with a highly differentiated branched mycelium (e.g. Streptomyces). In the case of filamentous actinobacteria, hyphae that branch repeatedly become attached on the surface of the agar to form tough, leathery and velvety colonies, which highly resemble fungi6.

The first actinobacterium isolated from the oceanic sediments was not considered as a marine form. Scientists believed that it came from the spores of terrestrial bacteria that had simply blown into the oceans and remained dormant. But, further investigations showed that many actinobacteria isolated from the ocean sediments were true marine forms7,8. However, Maldonado et al.9 reported a real marine form in the genus Salinispora under the family, Micromonosporaceae. In the recent years, actinobacteria from the marine environments have aroused keen interest in the minds of researchers due to their rich species diversity. Several studies have shown that actinobacteria can be isolated from the mangrove swamps, other coastal environments and even from the ocean sediments10. It has also been demonstrated that a good assemblage of actinobacteria is present in the marine environment, including marine
sediments\textsuperscript{11,12}, especially deep sea sediments\textsuperscript{13}, as well as marine sponges\textsuperscript{14,15}. They are also associated with extreme environments such as acidic thermal springs, Antarctic regions and gamma and UV irradiated biotopes\textsuperscript{16}.

**Occurrence**

As actinobacteria possess valuable secondary metabolites, microbiologists have started isolating actinobacteria throughout the world from different environments. Since the greatest biodiversity was found in the oceanic ecosystems\textsuperscript{17}, the marine derived actinobacteria have become recognized as a rich source for biotechnology works\textsuperscript{18}, as they might produce different types of bioactive compounds\textsuperscript{19}. So, marine actinobacteria were isolated from various marine associated environments including hydrothermal vents at the mid-ocean ridges\textsuperscript{20}, deep-sea floor\textsuperscript{21,22}, mangroves\textsuperscript{23}, seagrasses\textsuperscript{24}, coral reefs\textsuperscript{24,25}, hyper saline beaches\textsuperscript{26}, mud volcano\textsuperscript{27} and the polar region\textsuperscript{28}. Marine plants and animals\textsuperscript{29}, polychaetes\textsuperscript{30}, mangrove sediments\textsuperscript{10}, marine sponges\textsuperscript{14}, estuarine fishes\textsuperscript{31}, living seaweeds\textsuperscript{32}, sea anemones\textsuperscript{33}, marine crabs\textsuperscript{34} and seagrasses\textsuperscript{35} are the other several sources, studied for the isolation of marine actinobacteria.

Among the different marine habitats, mangrove environment acts as a major nutrient transformation system, responsible for more microbial activity including that of actinobacteria\textsuperscript{35}. Further, microbial colonies can appear after the mangrove litter fall, grow quickly and reach very high densities\textsuperscript{36}, taking part in biomineralization and biotransformation of minerals\textsuperscript{37}. Sediment nutrients especially total organic carbon can significantly influence the actinobacterial population density\textsuperscript{38} and the actinobacteria can survive well as they are saprophytic, depending on the availability of carbon\textsuperscript{39}. In addition, recently, the mangrove ecosystems have been found to be the potential spots for embracing a good actinobacterial community\textsuperscript{40-42}.

**Potentiallyality**

Actinobacteria are one of the most efficient prokaryotes which can be economically and biotechnologically exploited for their production of about half of the discovered bioactive secondary metabolites\textsuperscript{33}. Among the various genera, *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes* are the major producers of commercially important biomolecules. They are the efficient producers of new secondary metabolites that show a range of biological activities notably antibacterial, antifungal, anticancer, insecticidal and antioxidant activities, immunosuppressive agents, antitumor agents, pigments, enzymes, enzyme inhibitors, biofuel activity and bioelectricity production\textsuperscript{8,25,44-47}.

Actinobacteria can metabolize many different compounds including sugars, alcohols and amino acids. Additionally, many of them (e.g. *Streptomyces* and *Rhodococcus*) produce extracellular hydrolytic enzymes to obtain nutrients from cellulose, hemicellulose, proteins and fats\textsuperscript{8}. Furthermore, some strains are degrading compounds of macromolecules (lignin, cellulose, chitin, in part starch and aromatic hydrocarbons). Therefore, actinobacteria often occur in materials where organic matter is degraded\textsuperscript{48,49}, such as soils, organic compost heaps and building materials. Their metabolic diversity is due to their extremely large genome which has hundreds of thousands of transcription factors that control gene expression, allowing them to respond to specific needs\textsuperscript{6}.

Actinobacteria are unsurpassed in their ability to produce many compounds that have pharmaceutically useful properties\textsuperscript{6}. Bioactive compounds from marine actinobacteria possess distinct chemical structures that may form the basis for the synthesis of new drugs that could be used to combat resistant pathogens\textsuperscript{50}. Since the discovery of actinomycin in 1940, over 10,000 metabolites have been obtained from these microbes. These metabolites are not limited to antibiotics alone as actinobacteria can also produce industrially important extracellular enzymes and herbicides in addition to antifungals, antivirals and anticancer drugs. Majority of these substances (approximately 75%) have been isolated from the genus *Streptomyces* and some strains produce more than one antibiotic. Furthermore, the same antibiotic may be produced by different species distributed in different parts of the world.

*Streptomyces* alone produces a large number of bioactive molecules\textsuperscript{51}. It has an enormous biosynthetic potential that remains unchallenged without a potential competitor among other microbial groups. Above 500 species of *Streptomyces* account for 70-80% of relevant secondary metabolites with small contributions from other genera such as *Saccharopolyspora*, *Micromonospora*, *Amycolatopsis* and *Actinoplanes*. Secondary metabolites produced by the marine actinobacteria can be classified on the basis of their structure\textsuperscript{52}. Notably, most of the bioactive molecules from the marine actinobacteria have been discovered in the
representatives of *Streptomyces* which belong to different chemical classes, showing diverse bioactivity.73

**Culture media**

Microorganisms in the natural world do not live in pure cultures; they exist as part of complex ecosystems comprising numerous other organisms. The first step in the cultivation of microorganisms is therefore the creation of a pure culture. A key development for the production of pure cultures was the ability to grow microorganisms on a solid medium. Robert Koch had noticed that when a nutrient surface such as cut potato was exposed to air, individual microbial colonies grew up, and he inferred from this that these had arisen from the numerous divisions of single cells. Whilst media such as nutrient agar are used to support the growth of a wide range of organisms, others are specifically designed for the isolation and identification of particular types.74 Generally, actinobacteriologists used various suitable media and isolated the marine actinobacteria. Initially, Starch ammonium sulphate agar,55 Nutrient glucose agar,56 Grein and Meyers agar,57 Glucose asparagine agar,58 Kuster’s agar,59 Starch casein agar,60 Glycerol glycine agar and Maltose yeast extract agar were used; subsequently, HV agar,62 AV agar,63 M1, M2, M3, M4 and M586 and Actinomycetes isolation agar68 were used. Whereas, ISP (International Streptomycetes Project) media were used for the characterization of actinobacteria66 for melanoid, soluble and reverse side pigment studies and the Starch Casein agar and Kuster’s agar were found to produce desirable level of actinobacterial colonies in the marine samples.

Especially, Kuster’s agar, supports the isolation of various types of actinobacteria.65,68 Saadoun and Al-Momani69 found the dominance of the gray series *Streptomyces*, in Jordan soils and the authors isolated 105 gray pigmented species of *Streptomyces*, using Kuster’s agar medium. Similarly, Sahu et al.70 isolated maximum number of actinobacteria using this medium from the Vellar estuary. Raghavendrudu and Kondalarao71 studied the distribution of actinobacteria in the Gaderu mangroves of Gautami Godavari estuarine system, east coast of India. They used five different agar media for the isolation and among them, Kuster’s agar was found to be more suitable for the isolation of *Streptomyces*. Baskaran et al.72 also reported that Kuster’s agar is a well supporting medium for marine actinobacteria. Recently, Sethubathi et al.80 have reported that Kuster’s agar medium has yielded higher counts of actinobacterial colonies and Mohseni et al.73 have isolated 44 actinobacterial strains from the sediments of the Caspian Sea, using Kuster’s agar as one of the media. Similarly, Gobalakrishnan and Sivakumar109 have reported potential cellulolytic marine actinobacteria from the sediment sample of the Havelock island, using Kuster’s agar.

**Pretreatment**

Actinobacteria are ubiquitous in the marine environment and there are several techniques for their isolation. In the conventional techniques, many factors contribute mainly for the isolation of actinobacteria, especially, pretreatments of sediment samples and adding of antibiotic molecules. Soil pretreatments are done for inhibiting or eliminating the unwanted microorganisms.74 Some of the researchers have employed pretreatments of soil by drying and heating to stimulate the isolation of rare actinobacteria and an alternative approach would be to make the isolation procedure more selective by adding chemicals such as phenol to the sediment suspension.80

Spores of some rare actinobacterial genera including *Streptosporangium* and *Microbispora* can withstand treatments with sporicidal chemicals like phenol, benzenthonium chloride and chlorhexidine gluconate. On the other hand, spores of the common actinobacteria succumb to the same treatment. Most of the actinobacterial genera and their aerial spores have been found to resist desiccation and show higher resistance to wet or dry heat than the vegetative hyphae. Subsequently, employing pretreatments of soil by drying and heating stimulated the isolation of rare actinobacteria.75 Addition of calcium carbonate and chitin to the growth medium has also been known to enhance actinobacterial growth.86

Pretreatment techniques begin with air dried sediment samples that are sieved to exclude large mineral and organic matter particles and debris and then ground in a Pestle and Mortar aseptically. Then samples are treated in varied dry heat conditions at 55 °C for 5 min, 55 °C for 60 min, 70 °C for 15 min and 100 °C for 1 h.75,77,78 and 120 °C for 60 min79 and wet heat in sterilized sea water (50 °C, 15 min)80. Treatments with chemical agents such as Chloramine-T81, phenol (1.5 %, 30 min at 30 °C)79 (Pisano et al. 1986), Sodium dodecyl sulphate (0.05 % ) and yeast extract (6 % ) at 40 °C, 200 rpm, 30 min79, sterile calcium carbonate (v/v)82, sodium propionate (0.4% w/v)83 (Crook et al., 1950), phenol
Many actinobacteria have shown multi-resistance to a wide range of antibiotics included in the growth media to minimize the growth of unwanted microbes, bacteria and fungi, while supporting the growth and proliferation of actinobacteria. Actinobacteriologists add of antibacterial and antifungal antibiotics such as anisomycin, cicloheximide, gentamicin, kanamycin, nalidixic acid, novobiocin, nystatin, penicillin, primaricin, polymyxin, rifampicin, streptomycin, tunicamycin, vancomycin, benlate, secnidazole and cycloserine, in various concentrations, to the isolation media to enhance the growth of members of the actinobacteria.

Identification

The strains isolated are to be identified correctly to classify them and the identification process is an important step in classification and it involves several methods starting from conventional to molecular levels.

When working with the actinobacteria, using the conventional methods, it is essential to study their morphological characteristics like spor arrangement or spor chain morphology, spor surface ornamentation and cultural characteristics like aerial mycelial colour, melanoid pigmentation, substrate mycelial pigmentation or reverse side pigmentation and soluble pigments. Besides, physiological characterization like carbon source utilization is necessary for the determination of species. Chemical characterization is also a prerequisite to distinguish the morphologically analogous actinobacterial strains ever since they are diverging in their chemical configuration. Determination of cell wall type by cell wall amino acid analysis and whole cell sugar analysis is also a prime factor in the chemical characterization of actinobacteria, using reliable approaches like conventional and recent procedures. In addition, other chemical factors are also to be considered for working out the taxonomy of actinobacteria.

Menaquinones are the characteristic constituents of isoprenoidquinones present in the membranes of actinobacteria, which are playing a key role in the taxonomic classification of actinobacteria. Similarly, phospholipid fatty acids are also good taxonomic markers to identify the actinobacterial strains up to the species level. Identification of actinobacteria for taxonomical understanding starts from analyzing the complete 16S rRNA gene sequence to assess the phylogenetic position of the strains. In such molecular analysis, primers of 27F and 1492R are to be prominent in delivering the complete sequence of the 16S region and the sequence analysis is very important to know the sequence quality as well as the similarity pattern. This can be achieved by using the following programs: ARB, EzTaxon, jPHYDIT and RDP as per the recommendations of Tindall et al. using the algorithms viz. Neighbor-joining, Maximum-likelihood and Maximum-parsimony. Strains with <98.7% sequence similarity is a substantial hint that the strain may perhaps represent a new species.

Once a strain is confirmed for novelty, it could be further studied for further genotypic characterizations like DDH (DNA-DNA Hybridization) and G+C content analysis. G+C composition is a key factor which greatly influences the pairing in hybridization process. The moles percent of G+C or DNA base ratio may vary from 24 to 76%, between the bacterial groups and this would help distinguish morphologically similar strains. G+C % and DDH are inter linked with the high G+C content organisms (actinobacteria) which show more and strong pairing during DDH. DDH can be performed only when the 16S rRNA gene sequence similarity is above 98-99% to weigh the uniqueness of the strains.

Conclusion

To conclude, this article of compilation of information on the occurrence, potentiality, culture media, pretreatment and identification of actinobacteria would deferential help the young microbiologists, wanting to begin their actinobacterial research, for exploring novel and potential species for accomplishing many biotechnological applications, for the benefit of humans.

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