## Original Research Article

# Occurrence of Marine Actinobacteria in the Subtropical front Sea water

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**Abstract:** Marine environment is the largest environment on the planet and the oceans serve as the host for huge microbial populations whose diversity is still challenging. Among the ocean microbial populations, actinobacterial diversity is of paramount importance for natural products synthesis. Studies on marine actinobacteria from the marine sediments is well known. However, reports of actinobacterial diversity from sea water are only few. Present study is one of such initiative works to investigate the actinobacterial diversity from sub-tropical sea waters. Seawater samples from DCM (Deep Chlorophyll Maximum) region of the sub tropical front waters of the Southern Ocean were collected using Niskin (5L) water sampler during the Southern Ocean Expedition 6. Actinobacterial counts reached $1.2x10^{-2}$ CFU/ml of sea water. Among the isolates, four showed distinct morphological and physiological properties. Cultural characteristics were determined after 3-4 weeks according to the methods given in the International Streptomyces Project. Established procedures were used to determine the diagnostic isomers of DAP and the whole cell sugars. These isolates were identified upto genus level on the molecular (16S rDNA sequence analysis) basis and the occurrence of species of *Streptomyces* and *Saccharopolyspora* was observed. Present study, reporting the two genera *Streptomyces* and *Saccharopolyspora* from the subtropical sea waters, signifies that there is no dearth of the microbes in the sea water and it would be worth attempting to explore their diversity, using metagenomics. Such culture independent studies may reveal and include rare and novel forms of planktonic actinobacteria from the marine realm.

**Key words:** Marine Actinobacteria, Saccharopolyspora, Streptomyces, Sub tropical front sea,

## Introduction

Marine environment is the largest environment on the planet and the oceans serve as the host for huge microbial populations whose diversity is still challenging. Among the ocean microbial populations, actinobacterial diversity is of paramount importance for natural products synthesis. Earlier works have reported the existence of marine actinobacterial genera: *Solwaraspora* (Magarvey *et al*., 2004), *Serinicoccus* (Yi *et al*., 2004), *Salinispora* (Maldonado *et al*., 2005), *Marinispora* (Kwon *et al*., 2006), *Demequina* (Yi *et al*., 2007) and still others. Present work describes the cultural and physiological characters besides the 16S rDNA sequence analysis of the selected actinobacterial strains.

## Materials and methods

Seawater samples from DCM (Deep Chlorophyll Maximum) region of the sub tropical front waters of the Southern Ocean were collected using Niskin (5L) water sampler during the Southern Ocean Expedition 6.

### Isolation and enumeration of actinobacteria

Seawater samples were subjected to serial dilution. For the isolation, serially diluted samples were spread on Starch Casein Agar medium, prepared with an addition of 20 mg/l of nystatin and cycloheximide (100 mg/l), respectively to minimize bacterial and fungal contaminations (Kathiresan *et al*., 2005).

The plates were incubated at 18°C for 15 days. The strains were sub-cultured on the Starch Casein Agar slants (medium with 100% sea water) for further investigation.

### Phenotypic and biochemical characterisitics

Cultural characteristics were determined after 3-4 weeks according to the methods given in the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966). All the media used were supplemented with 100% sterile seawater. Colours of the substrate and aerial mycelia and any soluble, reverse side pigment production were examined. Characteristics of the spore bearing hyphae and spore chains were determined using direct microscopic examination of the culture surface by using cover-slip culture method. Adequate magnification (400X) was used to establish the presence or absence of spore chains and to observe the nature of sporophores. Biochemical characteristics were determined by the ability of utilization of sole carbon sources by the strains, following the methods recommended in *International Streptomyces Project* (ISP).

### Chemotaxonomy

Established procedures were used to determine the diagnostic isomers of DAP and the whole cell sugars (Cummins and Harris 1956; Lechevalier and Lechevalier 1970).

### 16S rRNA gene sequencing

Genomic DNA was extracted from the isolates following the procedures described by Ausubel *et al.* (1994). PCR amplification of the 16S rDNA preparations was carried out using the methods described by Karuppiah *et al.* (2011). The resultant PCR products were purified and the purified fragment was directly sequenced using an Ampli Tag FS DNA sequencing Kit (Applied Biosystem). The data were analyzed using applied biosystem DNA editing and assembly software and sequence comparisons were obtained using the Micro Seq Software.

### Analysis of sequence data

Sequence similarity search was made for the 16S rDNA sequence of all the isolates by applying their sequence to BLAST search of the NCBI (National Centre for Biotechnological Information, USA). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura *et al.,* 2007) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou and Nei (1987) from $K_{nuc}$ values (Kimura, 1980). The topology of the phylogenetic tree was evaluated using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

**Table 1.** Phenotypic and biochemical characteristics of four actinobacterial strains isolated from the subtropical front.

| Characteristics | SSTF1 | SSTF2 | SSTF3 | SSTF4 |
|---|---|---|---|---|
| **Spore arrangement** | Hooks and spiral | RA-S | Hooks and flexous | S |
| **Aerial mycelium** | White | Grey | White | White |
| **Reverside pigment** | Whitish yellow | - | - | - |
| **Soluble pigment** | - | - | - | - |
| **Melanoid pigment** | - | - | - | - |
| **Utilization of carbohydrates as solecarbon sources** | | | | |
| **L-arabinose** | - | + | - | + |
| **D-galactose** | - | + | + | - |
| **D-lactose** | - | + | - | - |
| **D-maltose** | + | + | + | - |
| **D-raffinose** | + | - | + | - |
| **L-rhamnose** | - | + | - | - |
| **Sucrose** | - | + | + | + |
| **D-xylose** | + | - | + | + |

RA-Retinaculiaperti; S-Spiral; (+) Positive utilization; (-) Negative utilization

## Results

### Isolation of actinobacteria

Bimodal distribution of actinobacteria in the nearshore tropical marine environments has been reported by Jensen *et al.* (1991) and Takizawa *et al.* (1993). Existence of indigenous marine actinobacteria from oceanic sediments has been suggested from the earlier studies (Weyland and Helmke, 1988; Takizawa *et al.*, 1993; Ravel *et al.*, 1998). Though, there are more evidences for the presence of actinobacteria in the marine sediments, studies on the marine actinobacterial distribution in sea water is less. Ghanem *et al.* (2000) proved the existence of uneven distribution of marine actinobacteria, in accordance with the occurrence of microenvironments, providing with sites of activity, governed by intermediate factors by recording higher population density in marine sediments rather than the sea water. Actinobacterial population density recorded from the sub-tropical sea water was $1.2x10^{-2}$ CFU/ml. Based on the distinct colony morphology, four isolates (SSTF1, SSTF2, SSTF3 and SSTF4) were selected for further investigation.

### Phenotypic and biochemical characteristics

Cultural, microscopic and biochemical characterisitics of the four strains are shown in Table 1.

### Chemotaxonomy

Chemotaxonomic studies were performed to check the cell wall aminoacid type and to ensure the characteristic sugar patterns. The study revealed that the strains SSTF1 and SSTF3 possessed mesodiaminopimelic acid as the cell wall aminoacid with the characterisitic sugar pattern of galactose and arabinose, indicating cell wall type IV. Whereas, the strains SSTF2 and SSTF4 possessed L-Diaminopimelic acid and glycine as the cell wall amino acids with no characteristic sugar patterns indicating cell wall type I.

### Analysis of sequence data

Results of the 16S rRNA gene sequence comparison clearly demonstrated that the strains SSTF1 and SSTF3 are members of the genus *Saccharopolyspora* and the strains, SSTF2 and SSTF4 are the members of the genus *Streptomyces*. In the phylogentic tree based on the neighbor-joining algorithm, strain SSTF1 formed a distinct subclade with *Saccharopolyspora gregorii* and strain SSTF3 formed a distinct subclade with *Saccharopolyspora gloriosa* (Fig.1). In the same way, strain SSTF2 formed a distinct subclade with *Streptomyces flavoviridis* and strain SSTF4 formed a distinct subclade with *Streptomyces variabilis* (Fig.1). The 16S rRNA gene sequence similarities between the strains SSTF1 and SSTF3 were 89.8% and 92.8%, respectively. Similarly, the 16S rRNA gene sequence similarities between the strains SSTF2 and SSTF4 were 91.7% and 93.4%, respectively. Though the strains SSTF1 and SSTF3 formed a subclade with the respective *Saccharopolyspora* sp., the sequence similarity difference suggested that these strains belong to the genus *Saccharoployspora*. Though the strains SSTF2 and SSTF4 formed a subclade with the respective *Streptomyces* sp., the sequence similarity difference suggested that they belong to the genus *Streptomyces*.

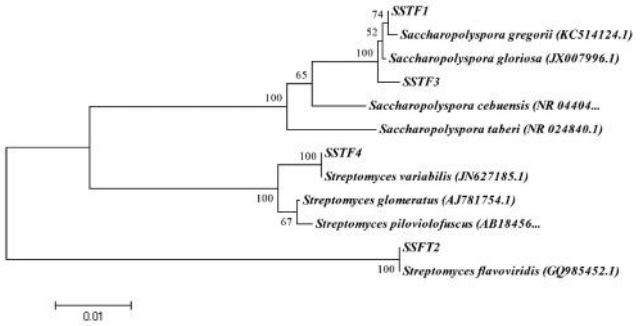


**Fig. 1.** Neighbour-joining phylogenetic tree, based on almost-complete 16S r RNA gene sequences, showing the relationships between the isolated strains and the type strains of recognized species of the genus *Saccharopolyspora* and *Streptomyces*. Numbers at the nodes indicate bootstrap percentages (based on a neighbor-joining analysis of 1000 resampled data sets). Bar, 0.01 substitutions per nucleotide position.

## Discussion

In general, it is assumed that the occurrence of actinobacteria is largely associated with the marine sediments or they occur as symbionts in the invertebrates but their planktonic lifestyle is rare (Bull and Stach, 2007). Hence, cultivation efforts were

made to get considerable actinobacterial diversity from marine samples, either from sediments or from marine organisms (Magarvey *et al*., 2004; Jensen *et al*., 2005; Moldonado *et al*., 2005; Gontang *et al*., 2007), rather than the sea water. Today, actinobacteria are consistently observed in sea water when culture-independent techniques are applied to marine samples. Application of molecular techniques has also provided with a new perspective to the diversity of marine actinobacteria (Ward and Bora, 2006), which are omnipresent and even a small portion of them occurs as bacterioplankton in sea water (Giovannoni and Stingl, 2005). In open ocean regions, proportion of actinobacteria can rise from zero to 35% of the bacterioplankton (Bull and Stach, 2007). Further, the actinobacterial diversity from sea water can be elucidated easily using the culture independent techniques.

Present study reporting two genera of *Streptomyces* and *Saccharopolyspora* from the subtropical sea water, signifies that there is no dearth of the microbes in the sea water and it would be worth attempting to explore their diversity, using metagenomics. Such culture independent studies may reveal and include rare and novel forms of planktonic actinobacteria from the marine realm.

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