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Full Length Research Paper

Isolation and identification of *Streptomyces* ST1 and ST2 strains from Tsunami affected soils: Morphological and biochemical studies

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Tsunamis are one of the natural disasters that are a series of long oceanic waves. A large volume of water gets displaced from the ocean because of undersea earthquakes or land slides or volcanic eruptions or extra terrestrial collisions. Our current research focuses on screening and characterization of microbes in water, soil and other extraneous material from the Tsunami affected areas along with substrates. Our present research work rests with *Actinomycetes* spp. possessing wide diversity of Genera. The morphological and biochemical characteristics of two *Streptomyces* spp. were identified from collected samples at different places of Pondicherry and Chennai. The screening methods, biochemical characterization and tentative identification methods will be discussed.

Key words: Tsunami, *Actinomycetes* spp., substrates, *Streptomyces*.

INTRODUCTION

The genus *Streptomyces* is represented in nature by the largest number of species and varieties among the family Actinomycetaceae. They differ greatly in their morphology, physiology and biochemical activities, producing the majority of known enzymes (Suneetha and Zaved, 2011). Among the genera of Actinomycetes, the genus streptomyces is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities. Interestingly, the majority of the antibiotic-producing Actinomycetes are found among those species, which led to a growth in economic importance for this group of organisms. Enrichment procedures always gives wonderful organisms which have potential applications in present world. In the 2004 Tsunami crisis blown off the south east coast of India. States of Tamil Nadu and the Union Territory of Puducherry were predominantly affected. Sea water entered into the cultivable lands increasing the soil pH notably from 6.5 to 7.5 to 8.5 inducing huge marked damage to the Indian agriculture (Dietz, 1986; Bentley et al., 2002) (Figure 1). In this study we screened various soil samples for screening of Actinomycetes from various Tsunamis affected areas.

The *Actinomycetes* are a morphologically, physiologically and ecologically diverse group of bacteria. Absolute majority of antibiotic-producers are encountered among these species making them economically potent. Keeping in view the significant contribution of *Actinomycetes* in the areas of soil ecology and industrial exploitation, an attempt has been made to isolate and identify potential *Actinomycetes* from Chennai and Kalapet (Puducherry). Two most dominant *Streptomyces* strains from Tsunami soil samples were screened and further characterized for micro morphology, cultural characterization followed by physiological and biochemical characterization. Based on these characteristics, the two dominant *Actinomycetes* strains were identified as *Streptomyces* ST1 and ST2 which could be further utilized for Biodegradation of Industrial effluents and microbial biodiversity studies. *Streptomyces*, the wonderful pigmented organisms bearing fungal characteristics but still are deliberated to be prokaryotic by their cellular status (Gulyas, 1978). Few species are pathogenic to both humans and plants. This group is an excellent reservoir for maximum number of antibiotics with commercial application (Bibb, 1996). The classification of the Prokaryotes is a complex issue and takes into consideration different characters.

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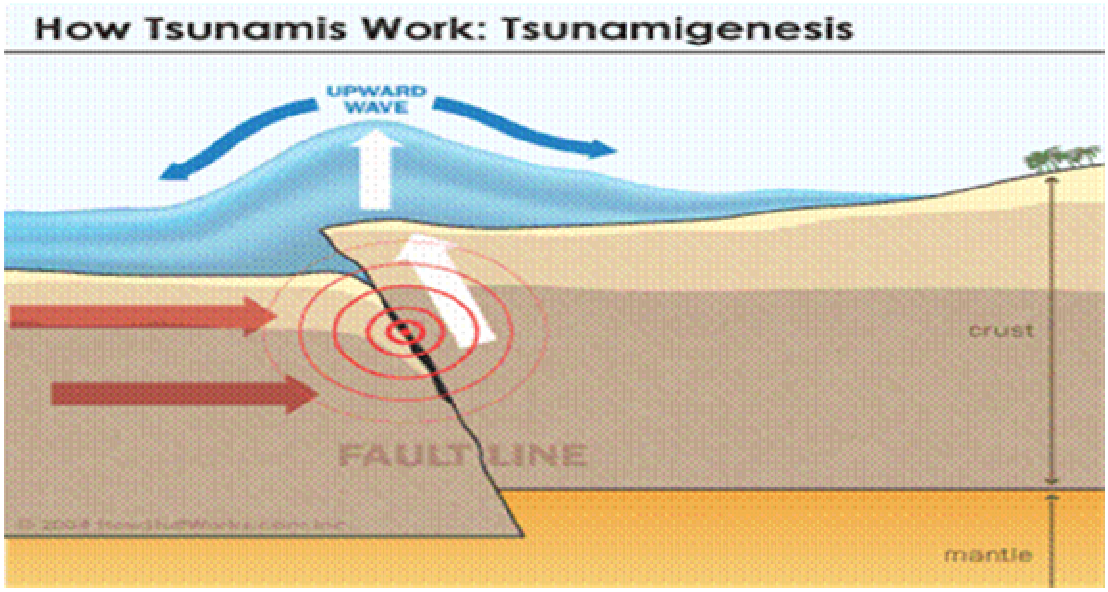

Figure 1. Tsunamigenesis.

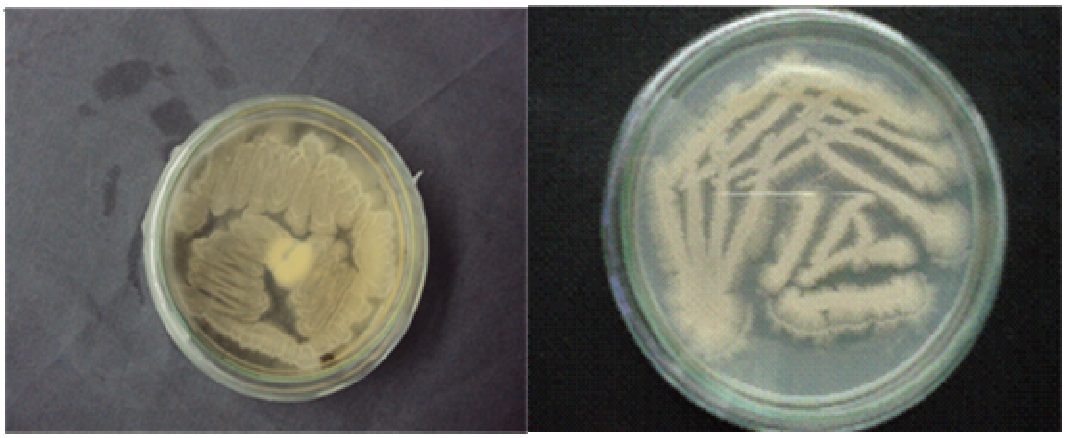

Figure 2. Screened *Actinomycetes* on Petri plates.

Major subdivisions account to bacteria (Schizophyta), the blue green algae (Cyanophyta) and the Actino-mycetales (Teddi et al., 2006, 1999; Suneetha and Zaved, 2011).

MATERIALS AND METHODS

Media/Buffers and Reagents Employed

Basal medium

KH_2PO_4	1.5
$ZnSO_4$	0.5
$MgSO_4$ .7 H_2 O	0.5
Glycerol	10 mM
$CaCl_2$	0.25
$(NH_4)_2SO_4$	10.0
$FeSO_4$	0.015
Agar	20.0

pH of the medium was adjusted to 8.3 before sterilization. Cyclohexamide (50 µg/ml) was added to the media to facilitate selective isolation of Actinomycetes (Malviya et al., 1992) (g/L).

Basal media without glycerol

Same as aforementioned without glycerol.

Starch casein agar medium (g/L)

Starch	10.0
Casein (vitamin free)	0.3
KNO_3	2.0

Table 1. Identification of the cultures.

Characters	Characteristics of ST1	Characteristics of ST2
Spore chain morphology	Rectiflexibilis	Straight to flexuous
Substrate myclia	Yellow to Green in colour	White in colour
Colour of spore mass	Dark green colour	White in colour
Diffusible pigment	No pigments	No pigments
Melanin pigment formation	+	+

NaCl	2.0
K_2HPO_4	2.0
$MgSO_4$ 7H $_2O$	0.05
$CaSO_4$	0.02
$FeSO_4$	0.01
Agar	20.0

pH of the medium was adjusted to 8.2 before sterilization.

Production medium

Galactose	4.0
K_2HPO_4	1.5
$FeSO_4$ $7H_2O$	0.015
$MgSO_4 7H_2O$	0.05
$(NH_4)_2SO_4$	5.0
$ZnSO_4.7H_2O$	0.005
$CaC1_2$	0.25

pH was adjusted to 7.8 before sterilization (Mukhopadhyay and Chandra, 1990) (g/L).

CSPY-ME medium (g/L)

K_2HPO_4	0.5
Casein	3.0
Maize starch	10.0
Peptone	1.0
Yeast extract	1.0
Malt extract	10.0
Agar	20.0

pH of the media was adjusted to 7.5.

***Yeast extract Malt extract medium* (g/100 ml)**

Yeast extract	0.5
Malt extract	1.0
Glucose	1.0
Agar	2.0

pH was adjusted to 7.8 before sterilization. This media was used to maintain Actinomycetes cultures.

***Ringers solution* (g/litre)**

NaCl	2.15
$CaCl_2$ (anhy)	0.12
$Na_2S_2O_3$ 5 H_2O	0.5
KCl	0.075

pH was adjusted to 6.6 before sterilization.

Screening of Actinomycetes from Tsunami soil samples

Soil is a natural source for the isolation of microbes. For the isolation of Actinomycetes, soil samples were collected from different places of Tsunami affected areas. The superficial loose soil layers were removed and the underlying soil sample was dug. The samples were collected in polythene bags, labeled and the collected soil samples were transported to the laboratory and were screened for Actinomycetes (Suneetha and Lakshmi, 2006).

Cultural and biochemical screening

Cultural characterization followed for Actinomycetes isolates were identified up to the generic level (tentatively) by studying morphological, physiological and ecological characters. Morphological characters were studied by observing the growth on cover slip under (10X and 45X lens). Bio-chemical characterization was done by using different tests like spore chain morphology, substrate mycelia, glucose, mannitol, xylose, lactose, starch, saccharose and l-Ramose (Mahr et al., 2000) (Tables 1 - 2 and Figure 5).

Genus identification and morphological characteristics

Morphological studies were carried out by microscopic observation and studies on growth characteristics in Petri dishes. Genus identification and morphological characteristics were observed. Visual observation of both morphological and microscopic characteristics using light microscopy, acid fastness and gram-staining properties were performed. All morphological characters were observed on CSPY-ME and were used for classification and differentiation as follows:

Aerial mass color: The mass color of the mature sporulating aerial mycelium was observed following growth on the CSPY-ME plates. The aerial mass was classified according to the Bergey's manual of systemic bacteriology (Locci, 1989) in the following color series: grey (Gy), white (W).

Substrate mycelium: Distinctive colors of the substrate mycelium were recorded. The observed colors were: yellow (Y), green (G) and white (W).

Melanoid pigments: The production of melanoid pigments was also considered. It was reported as a positive result and the production of melanoid pigments was clear.

Spore chain morphology: According to the shape of the spore chains observed under light microscopy, the isolates were grouped as follows: Rectus-Flexibilis (RF) and spores in straight flexous chains (Table 2 and Figure 5).

Table 2. Physiological characteristics of growth temperature.

Characters	Characteristics of ST1	Characteristics of ST2
4℃	-	-
37℃	+	+
45℃	+	-
Use of carbon source (1% w/v)	+	+
Glucose	+	+
Mannitol	+	+
Xylose	+	+
Lactose	+	+
Starch	+	+
Saccharose	-	-
L-Rhamnose	-	-

Figure 3. *Actinomycetes* on slants.

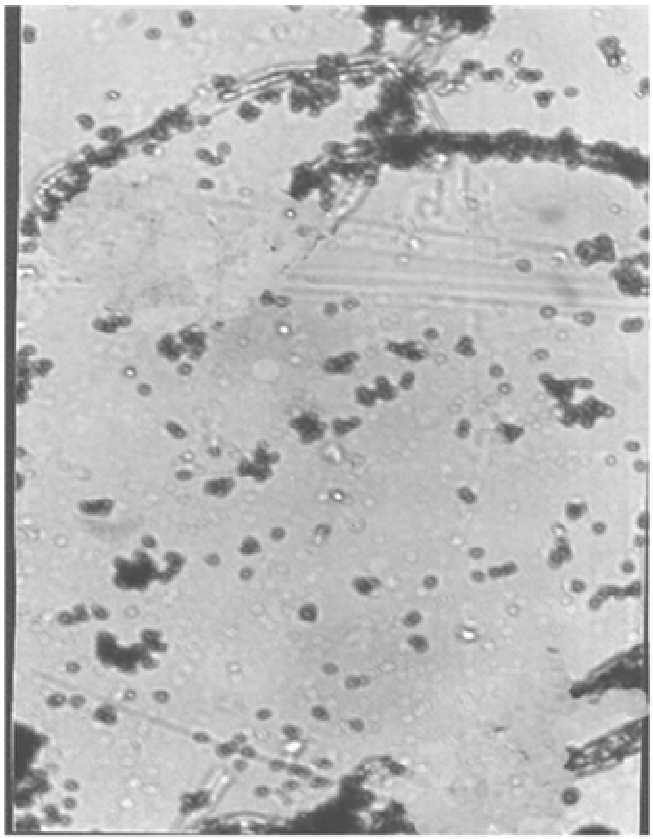

Figure 4. Micromorphology of *Actinomycetes*.

Biochemical screening: Physiological criteria such as ability to degrade casein, tyrosine and xanthine as substrates by the various *Streptomyces* strains were used for genus confirmation. The utilization of different carbon sources and degradation of amino acids as well as the production of melanine and utilization of urea were studied in order to reach a possible classification to species. (Shirling and Gottileb, 1966; Lechevalier, 1968; Korn-Wendisch and kutzner, 1991). Figure 4 and 5.

RESULTS AND DISCUSSION

Most of the colonies that grew on CSPY-ME plates belong to the genus S*treptomyces* since the colonies were slow growing, aerobic, glabrous (Figure 1-3) or chalky, heaped, folded and with aerial and substrate mycelia of different colors. In addition, all colonies possessed an earthy

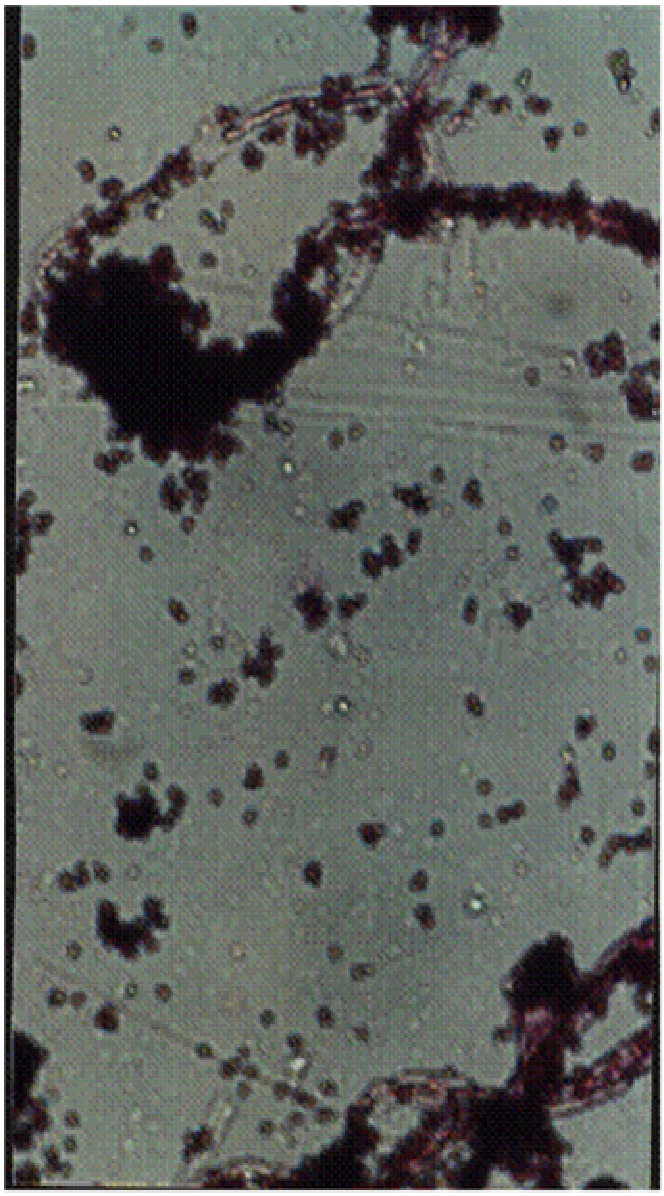

Figure 5. Micromorphology of *Actinomycetes*.

odour. Some of them also produced antibiotics and enzymes (Suneetha, 2011) as reflected by zones of growth inhibition among other inhabitants of soil samples. A confirmatory identification to genus was based on acid-fastness, gram-stain and degradation of casein, tyrosine and xanthine. All *streptomyces* strains were acid-fast negative and gram-stain positive. The degradation of substrates casein, tyrosine and xanthine was variable according to each isolate. Microscopically, it was observed that the morphology of the sore chains varied depending on the species, showing the exact straight and flexuous forms, hooks, open loops and coils, which were used, among other features, to establish differences between them (Table 1 and Figure 5). It was also observed that some of the strains produced diffusible melanoid pigments in the surrounding medium. In accordance with the aerial mycelium color series established in the Bergey's manual of determinatives bacteriology (Buchanan and Gibbons, 1974) and in the category 4 of the Bergeys manual of systemic bacteriology, the isolates could be grouped in the following series: grey and white.

Various colors of mycelia were also observed, the shades beige, yellow and ivory being the most pre dominant. Few of them had substrate mycelia violet-purple or red-violet. As per the utilization of the several carbohydrates, it was noted that strain ST1 were able to use all of the tested carbon sources, while the ST2 were able to use cellulose as carbon source.

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