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Full Length Research Paper

Isolation and characterization of actinomycetes in Vellar Estuary, Annagkoil, Tamil Nadu

D. Dhanasekaran[1]*, S. Selvamani [2], A. Panneerselvam[3] and N. Thajuddin[4]

[1]School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Vellore-632 014, India.
[2]Department of Biotechnology, Arulmugu Kalasalingam College of Engineering, Krishnan koil, India.
[3]P.G. and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, 613 503, Thanjavur District, Tamil Nadu, India.
[4]Department of Microbiology, Bharathidasan University, Trichirapalli, 620 024, India.

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Vellar Estuary was investigated as a source of actinomycetes to screen for production of novel bioactive compounds. The presence of relatively large populations of *Streptomyces* in Vellar Estuary soil samples indicates that it is an eminently suitable ecosystem for actinomycetes. Actinomycetes counts ranged 12 x 10^4 cfu/g of soil. The actinomycetes isolated from these ecosystems are capable of producing antibiotics that strongly inhibit the growth of Gram positive and Gram-negative bacteria and yeast like fungi. Further cultural and physiological characterization and DNA homology suggest that strain DPTD-5 is identical to *Streptomyces bikiniensis.*

Key words: Actinomycetes, estuary, characterization, antimicrobial activity.

INTRODUCTION

Actinomycetes have provided important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes (Okami and Hotta, 1988). Actinomycetes are abundant in terrestrial soils, a source of the majority of isolates shown to produce a number of bioactive compounds. The result of intensive screening program carried out over the past several decades is that there is a growing problem of rediscovery of already known bioactive compounds (Nolan and Cross, 1988). An approach to address this problem is to expand the source of actinomycetes by carrying out ecological assessment of environments other than terrestrial soils. There is growing interest in the Streptomycete actinomycetes from Vellar Estuary soil and it was found that majority of isolates were *Streptomycetes*, indicating that the Vellar Estuary soil is a suitable source of actinomycetes to screen for production of novel bioactive compounds.

Jensen et al. (1991) reported a bimodal distribution in the actinomycete population in relation to depth, with *Streptomycetes* predominating at shallow depths and an increase in *Actinoplanetes* with increasing depth. Ghanem et al. (2000) reported that variation in total nitrogen and organic matters were significant for total actinomycetes population in soil. However, actinomycetes from marine samples have rarely undergone screening for novel metabolites and there is evidence that actinomycetes usually make up only a small proportion of the bacteria flora of marine habitats, with absolute numbers of actinomycetes much lower than in terrestrial habitats (Ghanem et al., 2000). The consequence is that it should be more difficult to obtain large number of isolates from marine samples for screening purposes (Goodfellow, 1983; Goodfellow and Haynes, 1984). In view of the potential importance of marine actinomycetes as a source of novel bioactive compounds, we present the identification of strain DPTD-5 through a study of biological properties.

MATERIALS AND METHODS

Soils

The soil samples used in these studies were obtained from the Ve-

*Corresponding author. E-mail: dhansdd@yahoo.co.in.

Table 1. Physiochemical characteristics of estuarine soil.

Soil characteristics	Quantity (ppm)*
P^H	7.66 - 7.94
EC (MS)	1.869 - 2.080
Organic Matter	0.25 - 0.60
Nitrogen	1.7 - 1.8
Available phosphorus	BDL - 5.3
Reserved Phosphorus	73 - 78
Potassium	49 - 54
Calcium	208 - 215
Magnesium	129 - 135
Sodium	626 - 850
Zinc	0.17 - 0.18
Ferrous	6.83 - 7.12
Manganese	1.48 - 1.81
Copper	0.08 - 0.14
Boron	0.3 - 0.4
Chloride	10.50 - 12.20

BDL = Below the detectable level.
*Values given in ppm (parts per million) except pH and EC.

llar Estuary, Tamil Nadu (India). Vellar River originating from Servarayan hills in Salem district, opens into the Bay of Bengal near Port Novo (Lat. 11 °30' N, long. 79°46' E) after flowing zigzag over a distance of 480 km. The estuary is an open type as it has connection with the adjoining sea throughout the year and is subjected to semidiurnal tides. The estuary is about 600 m in width at its junction with sea.

Isolation of actinomycetes

Tenfold serial dilution of soil samples were made using sterile water. The soil suspensions were plated using starch casein agar supplemented with 40 mg/mL actidione, which inhibits the development of eukaryotic microorganisms. The plates were incubated at 28°C for 7-10 days.

Determination of colour of the actinomycete isolates

Colour of aerial mycelium was determined from mature, sporulating aerial mycelia of the actinomycetes colonies on different media such as starch casein agar, glycerol aspargine agar, yeast extract malt extract agar, and oat meal agar. Colour was determined using the colour names lists (Pridham, 1964).

Colour of the soluble pigments was determined visually by observing the colour changes in the medium due to the diffusing pigments produced by actinomycete isolates (Shirling and Gottlieb, 1966).

Morphological characterization

The morphology of the spores was determined by light microscopy. A small peripheral portion of well-grown mature parts of the colony was picked using a sterile inoculation loop. This was then transferred to a glass slide and observed under Nikon photo micrographic unit at the magnification 1000 X.

Physiological characterization

Utilization of carbohydrates was investigated with a basal carbon nutrient medium (Pridham and Gottlieb, 1948; Waksman, 1961). Methods and media used for physiological test were as described by Luedemann and Brodsky (1964) and Luedemann (1971). All the cultures were incubated at 28°C for 10 days except for the gelatin liquefaction (15°C for 21 days). The assay for enzymatic activity was performed according to Hopwood (1957).

Chemotaxonomic characterization

The actinomycetes isolate was grown in bennett's broth at 28°C for 7 days. Biomass was harvested by centrifugation and the pellet was washed twice with aqueous KCl solution (0.85%, wt/vol). The cell wall was partially purified with a Bead Beater and the resultant suspension was collected by centrifugation. Analysis of diaminopimelic acid and sugar were performed by the method of Lechevalier and Lechevalier (1970).

Antimicrobial characterization

Using inoculation loop, each actinomycete isolate was transferred from starch casein agar plate to nutrient agar plate. This is done by streaking a straight line of the actinomycetes inoculum across the surface of nutrient agar medium in the plate and incubated at 25-28°C for 3 days. Inoculum of the test microorganisms were then streaked at right angles to the actinomycetes straight-line colony as described by Prescott and Dunn (1959). Alexander (1977) or Brock et al. (1994).

DNA-DNA Homology study

DNA-DNA relatedness was determined by the method of Ezaki et al. (1989).

RESULTS AND DISCUSSION

Physiochemical characteristics

The physiochemical characteristics of soils samples collected from Vellar Estuary showed insignificant variation in temperature, pH and dissolved phosphate, but that variation in total nitrogen and organic matter were significant (Table 1).

Isolation of actinomycetes

Of the 20 actinomycetes isolate, only 4 isolates showed antimicrobial activity and among them only one showed broad-spectrum activity, hence the strain DPTD-5 was further characterized for its identification. Earlier studies carried out by Joe D' Souza and Nelson De Souza (2000) revealed that estuarine soils are rich in antibiotic producing actinomycetes. The soil samples from estuary are reported as rich habitat for microbial diversity. The presence of pectinolytic, oil degrading yeast, and photosynthetic bacteria reported by Da Costa and D'

Table 2. Cultural characteristics of *Streptomyces* strain DPTD-5 on different culture medium.

Name of the medium	Growth	Aerial mycelium	Substrate mycelium	Pigments
Starch casein agar	Good	Grey	Brownish grey	None
Glycerol asparagines agar	Good	Grey	Dark grey	None
Glucose asparagines agar	Moderate	Pale grey	Grey	None
Yeast malt extract agar	Moderate	Grey	Grey	None
Tyrosine agar	Good	Grey	Dark grey	None
Maltose extract agar	Good	Grey	Grey	None

Table 3. Physiological characteristics of strain DPTD-5 and *S. bikiniensis.*

Name of the test	DPTD-5	*S. bikiniensis*
Lecithin hydrolysis	+	+
Lipid hydrolysis	+	+
Starch hydrolysis	+	+
Protein hydrolysis	+	+
Pectin hydrolysis	+	+
Nitrate reduction		
H_2S Production	+	+
Blood haemolysis		
Resistance of rifampicin, penicillin	+	+
Growth at 45°C	+	+
Growth with 7% Nacl	+	+
Utilization of Carbon and Nitrogen		
L-Cysteine		
L-Valine		
L-Phenylalanine	+	+
L-Histidine	+	+
Sucrose	+	+
Inositol		
Mannitol		
L-Rhamnose		
Fructose	+	+
Lactose	+	+

+ = Positive; - = negative.

Souza (1979), D' Souza and Fritas (1976), Aguiar and D' Souza (1978). However, in the present investigation it was found that estuarine actinomycetes, which remained largely ignored showed promising antibacterial activities. The promising antibiotic producing isolate identified as *Streptomyces* sp.

Cultural characteristics

The strain DPTD-5 grew well on most of the synthetic and organic media tested. Typically the colonies were elevated, coloured with gray, the reverse side is yellow-brown, diffusible pigments are not produced (Table 2).

Physiological characteristics

It is capable of growth in the presence of penicillin and sodium chloride. It utilizes phenylalanine, histidine as a sole nitrogen source and fructose, lactose as carbon source; test for lecithin hydrolysis, lipid hydrolysis, pectin hydrolysis, starch hydrolysis and H_2S production showed positive results but nitrate reduction and haemolysis showed negative results.

Cell wall characterization

The cell wall contains L-diaminopimelic acid, and hexose diagnostic sugar is present in the cell wall fraction (Chemotype I).

Table 4. Antimicrobial activity of *Streptomyces* strain DPTD-5 and *S. bikiniensis.*

Organism	Zone of inhibition (mm)	
	DPTD5	*S. bikiniensis*
Staphylococcus aureus	30.2	29.0
Escherichia coli	26.4	25.0
Klebsiella pneumoniae	00.0	00.0
Candida albicans	24.8	20.0
Candida tropicalis	31.4	27.2
Saccharomyces cerevisiae	32.0	29.6
Micrococcus sp.	00.0	00.0
Pseudomonas sp.	04.0	03.5
Bacillus sp.	02.0	02.0

Antimicrobial activity

The ability of actinomycetes isolate DPTD-5 to inhibit growth of some pathogenic bacteria and fungi showed strong (> 30 mm inhibition zone) moderate (20 – 30 mm) and week (< 20 mm) antibiosis against some of the test organisms (Table 4). This isolate did not show any antibiosis activity against *Klebsiella* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. These results were observed within 3 days after streaking of the test organisms.

The strain DPTD-5 showed a broad-spectrum antimicrobial activity against gram positive, gram-negative bacteria and yeast (Korzybski et al., 1967). Earlier studies carried out by Kokare et al. (2004), Jensen et al. (1991), Ghanem et al. (2000) revealed that marine soil actinomycetes are rich in bioactive compound producing antibiotics. The properties of *Streptomyces bikiniensis* were tested for comparison. *S. bikiniensis* was distinct from strain DPTD-5 in having more spirals of spore chains and spore mass. Both strains showed similar pattern of carbohydrate utilization and enzyme production (Table 3), but *S. bikiniensis* demonstrated a narrow antimicrobial spectrum that strain DPTAD-5. The DNA-DNA homology experiment indicated a near 98% DNA similarity between strain DPTD-5 and *S. bikiniensis,* suggesting a significant genomic relatedness. Consequently, strain DPTD-5 belongs to the genus *Streptomyces* sp., and is identical to *Streptomyces bikiniensis.*

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