Screening Of Salt Pans Actinomycetes For Antibacterial Agents

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Abstract

The actinomycetes exist in various habitats in nature. The terrestrial ones from the soil have been extensively used for the production of secondary metabolites useful to human. However the aquatic counterparts have reminded relatively unknown and unexploited. The soil from the saltpan regions of Cuddalore and Parangipettai (Porto-novo) were screened for the isolation of actinomycetes. In this investigation 17 actinomycete isolates were obtained and were screened for primary antibacterial activity. Three actinomycetes namely two species of Streptomyces and one species of Saccharomonospora collected and showed promising antimicrobial activity against eight test organisms.
Secondary screening of the actinomycetes isolates also indicated positive antibacterial activity against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Vibrio cholerae, salmonella typhi, Staphylococcus aureus, and Shigella dysenteriae the present study indicate that the saltpan soil harbors diverse species of actinomycetes possessing potent antibacterial activity, which could be later scaled up for large scale commercial production of antibiotics if found suitable. The extraction of antibacterial activity in various solvents indicated that the active organic moiety responsible for antibacterial activity was soluble in aniline and ethyl acetate was found active against the test microorganisms.

INTRODUCTION

Actinomycetes are numerous and widely distributed microorganisms in nature. They are the best common source of novel antibiotics (Okami and Hotta, 1988). Since the remarkable discovery of streptomycin from streptomyces gresius in 1942 by Waksman (1969), there has been a worldwide search for antibiotics from various terrestrial substrates and geographic regions, (Hocken Hull, 1963, Podogi, 1984). However the saltpan and the marine Streptomyces counterparts reminds largely unexploited for useful metabolites.

In the present investigation soil samples from various saltpans of Cuddalore, Tamil Nadu, India were examined for the presence of Streptomyces and the 17 isolates thus obtained were tested for the production of antibacterial metabolite.

MATERIALS AND METHODS

Sample collection. The soil samples (100-500g) were collected during field trip in sterile petridishes and polythene bags to avoid external contamination, from the saltpan regions of Cuddalore (Lat 11° 45' N and Long 79° 47' E) and Parangipettai, (Lat 11° 32' and Long 79° 45' E) Tamil Nadu, India. The samples were collected from 6 inches from the soil surface, inorder to avoid the contamination.

Physico-chemical analysis of soil. The physical and chemical analyses of the soil samples were carried out by using standard methods (APHA, 1975).

Isolation of actinomycetes. Starch casein agar was used for isolation of actinomycetes. The media components were sterilized in an autoclave at 121°C at 15lbs pressure for 15 minutes. The antifungal (50µg/ml cyclohexamide) and antibacterial (20µg/ml of tetracycline) were added to medium after sterilization and prior to the pouring of the agar medium. The saltpan soils were diluted up to 10^{-6}, poured on agar plates of the media stated above and incubated at 28°C for 7 to 10 days.

Primary screening (agar overlay method). The promising isolates identified in the present study were subjected to primary screening procedures to assess the ability of the actinomycetes to produce secondary metabolites. Soft nutrient agar containing test pathogens was poured on to the

starch casein agar plates containing 40-50 actinomycetes colonies. The plates were incubated at 37°C for 24 hours.

Secondary screening. The primary isolates possessing antibacterial activity were subjected to the secondary screening procedures. Promising cultures in starch casein broth was incubated at 28°C for 7 days on a rotary shaker. After filtering off the biomass, through the membrane filter, the crude filtrate obtained was tested against pathogens. Using agar diffusion method. The crude filtrate broth was also subjected to solvent extraction using chloroform, acetone, pyridine, aniline and ethyl acetate. The solvent extract was vacuum dried and the active principle was tested for antibacterial activity. Among 17 isolates, the culture filtrates of 4 isolates exhibited antibacterial activity against different pathogens except B. subtilis and P. mirabilis (Table-3).

RESULTS AND DISCUSSION

The physico-chemical analysis of the soil samples from two different saltpans, indicated in table 1. Except P^H, salinity, copper, and zinc, other elements showed much variation in their physico-chemical properties. This variation is shows the biodiversity of actinomycetes in different saltpans. Cuddalore and Parangipettai soil had 9 and 8 actinomycetes isolates respectively.

Figure 1

Table: 1 Physico-chemical analysis of soil.

S. No	Name of Test	Location of saltpan	
		Parangipettai	Cuddalore Port novo
1	Soil texture	Sandy loam	Sandy loam
2	P^H	8.03	8.06
3	Ec (ms)	39.700	11.240
4	Organic matter (%)	75	0.49
5	Nitrogen	BDL	1.3
6	Phosphorous	BDL	46.3
7	Potassium	2408	430
8	Calcium	11032	1929
9	Magnesium	5267	1229
10	Sodium	4516	7120
11	Zinc	0.49	0.77
12	Ferrous	2.08	16.00
13	Manganese	7.07	4.11
14	Copper	1.50	1.48
15	Boron	17.6	6.3
16	Salinity	385.35	370

- Values given in ppm-parts million except P^H & Ec
- BDL- Below the detectable level.

The cultural and microscopic characterizations of actinomycetes were shown in Table-2. These studies indicate that species of Streptomyces and Saccharomonospora are dominant in the saltpan of Cuddalore, Tamil Nadu. The four promising isolates of actinomycetes (3 species of Streptomyces and one species of Saccharomonospora) were used in further studies.Earlier studies carried out by Pathiranana, et.al (1991),Jensen et.al (1991)Joe D.Souza,(2000),Kokare et.al (2000) revealed that saltpans are rich in antibiotic producing actinomycetes.

The bacteria free culture filtrates were extracted using organic solvents also showed antibacterial activity similar to that of culture filtrates (Table 4). It is interesting to note that in the broth as well as solvent extracted substance retained the antibacterial activity. The results show that antibiotic principles extracted in solvent or present in the crude culture filtrate are active against Escherichia coli and Klebsiella pneumoniae.

Figure 2

Table 2: Identification of actinomycete isolates based on morphological & cultural characteristics.

Isolate code	Colony characteristics on starch casein agar after 7 days	Microscopic characteristics on the 5 days	Identification of actinomycetes
DPTD 21	Size – 7mm AM – Dark green SM – Pale brown PG – Nil	Aerial mycelium with long chains of spores	*Streptomyces sp*
DPTD 12	Size – 3mm AM – White SM – Grey green PG – Nil	Single spores formed mainly on the aerial hyphae	*Saccharomonospora*
DPTD 14	Size – 8mm AM – Grey SM – Yellowish orange PG – Nil	Long unfragmented hyphae with long chains of spores	*Streptomyces* sp
DPTD 11	Size – 6mm AM – White SM – Pale brown PG – Nil	Long unfragmented hyphae with long chains of spores	*Streptomyces sp*

AM – Aerial mycelium, SM – Substrate mycelium, PG – Pigment.

Figure 3

Table 3: Inhibition spectrum (mm) of the actinomycetes isolates against the test bacteria.

Code name of actinomycete isolates	Zone of inhibition (mm)	
	Staphylococcus aureus MTCC 96	*Escherichia coli* MTCC 739
DPTD 11	-	-
DPTD 12	12	8
DPTD 13	-	-
DPTD 14	3	-
DPTD 15	17	14
DPTD 16	-	4
DPTD 17	-	-
DPTD 18	-	-
DPTD 19	-	-
DPTD 20	-	-
DPTD 21	18	18
DPTD 22	3	-
DPTD 23	-	-
DPTD 24	-	8
DPTD 25	12	16
DPTD 26	-	-
DPTD 27	-	-
DPTD 28	-	-

Figure 4

Table 4: Antibacterial activity of actinomycetes starch casein broth by agar diffusion method.

S. No	Name of the bacteria	Zone of inhibition (mm) Actinomycetes culture code			
		DPTD 12	DPTD 15	DPTD 21	DPTD 25
1	*Staphylococcus aureus*	13	9	18	12
2	*Escherichia coli*	10	6	12	17
3	*Klebsiella pneumoniae*	12	5	17	4
4	*Pseudomonas aeruginosa*	8	7	14	7
5	*Vibrio cholerae*	11	8	7	12
6	*Shigella dysentriae*	7	11	10	11
7	*Salmonella typhi*	10	9	15	0
8	*Proteus mirabilis*	0	0	0	0
9	*Bacillus subtilis*	0	0	0	0

Figure 5

Table 5: Antibacterial activity of selected isolate DPTD 21 extracted in different organic solvents.

S. No	Name of the bacteria	Name of organic solvents			
		Aniline	Chloroform	Pyridine	Ethylacetate
1	*Escherichia coli*	21.5	-	19.5	17
2	*Klebsiella pneumoniae*	8.5	-	21	14
3	*Pseudomonas aeruginosa*	0.9	-	10	0
4	*Vibrio cholerae*	13	-	-	12
5	*Salmonella typhi*	8.5	-	12	9
6	*Shigella dysentriae*	10	-	-	11
7	*Staphylococcus aureus*	8.5	-	0	0
8	*Proteus mirabilis*	-	-	-	-
9	*Bacillus subtilis*	-	-	-	-

Among the different organic solvents tested, Aniline, Pyridine and Ethyl acetate showed prominent antibacterial activity against different pathogens. The chloroform extraction doesn't showed antibacterial activity against different pathogens tested (Table 5).

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