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A comparative study on selected marine actinomycetes from Pulicat, Muttukadu, and Ennore estuaries

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ABSTRACT

Objective: To isolate and make a comparative study of marine sediments actinomycetes from Pulicat estuary, Muttukadu estuary and Ennore estuary, TamilNadu, India. **Methods:** A unique selective enrichment procedure has resulted in the isolation and identification a total of 304 actinomycetes colonies which were isolated from different stations of marine soil sediments in Pulicat estuary, Muttukadu estuary and Ennore estuary, TamilNadu, India. **Results:** Among them, 277 isolates were morphologically distinct on the basis of spore mass colour, aerial and substrate mycelium formation and production of diffusible pigment. The majority (60%; 162 isolates) were assigned to the genus *Streptomyces*. (35%; 104 isolates) were assigned to the genus *Actinopolyspora*, (5%; 11 isolates) were assigned to the genus *Nocardiodes*. **Conclusions:** The present study concluded that the physiological characteristics of actinomycetes *Streptomyces, Actinopolyspora* and *Nocardiodes varied* by available nutrients in the medium and the physical conditions.

1. Introduction

The marine ecosystem has been widely recognized as a source that nurtures abundant compounds that contain novel composition and organic characteristics. These compounds that are extracted from the ocean are applied as antimicrobial and enzymes [1]. The process of identifying and exploring the previously undiscovered natural products from microorganisms are rate limiting while isolating and screening. It starts with Actinomycetes belong to in the order of actinomycetales that are categorized under the class actinomycobacteria which possesses numerous readily developed genera, including mycobacterium, Streptomycetaceae and Rhodococus [2]. Actinomycetes are the dominant group of soil population together with bacteria and fungi. They are Gram-positive bacteria having high G+C (>55%) content in their DNA and they are originally considered as an intermediate group between bacteria and fungi. They are free living, saprophytic bacteria, and a major source for production of antibiotics. They play a major role in recycling of organic matter [3]. When the soil population is considered, actinomycetes are the prevailing faction along with bacteria and fungi. A little is known about the actinomycetes diversity in marine sediments, which is an inexhaustible resource that has not been properly exploited. Many reports described Indian coastal area as a major source of actinomycetes [4-7]. In the present study an attempt is made to estimate the actinomycetes populations in different soil stations viz. from Pulicat estuary, Muttukadu estuary and Ennore estuary, TamilNadu, India, and to screen their antimicrobial properties. Further, the identified actinomycetes were characterized based on morphological, biochemical, and physiological characteristics.

2. Materials and Methods

2.1. Soil Sampling

A total of 175 soil sediment samples were collected during (2009-2010) from different soil stations. The samples were collected from Pulicat estuary between 13° 33′ to 13° 66′ North latitude and 80° 23′ to 80° 25′ East longitude, in Muttukadu estuary between 13° 59′ to 13° 16′ North latitude and 80° 15′ to 80° 04′ East longitude, and Ennore estuary 13° 14′ N, 80° 22′ E, TamilNadu, India. The Samples were collected from 6-10 cm depth and were stored in sterile polythene bags. They were stored at 4℃ in the laboratory for further study.

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2.2. Enrichment and Isolation of slowly growing marine actinomycetes

The process of enrichment and isolation of the slowly growing marine actinomycetes was carried out by employing starch casein nitrate (SCN) agar medium (Himedia, Mumbai, India) to which 10 μg/ml amphotericin and 25 μg/ml streptomycin (Himedia, Mumbai, India) were added in order to restrain fungal and bacterial contamination respectively [8]. The conservative procedure termed, Dilution Plate Technique was followed in suspending 10g of marine soil samples in 100 ml of sterile sea water. From the obtained suspension 0.5 ml was smeared over 50% sea water starch casein agar medium [9]. This was subject to incubation for a period of 7-9 days maintained at 28℃.

2.3 Characterization of actinomycetes isolates

The studies on the physiological morphological and biochemical characteristics of the effective actinomycetes were conducted [10]. Microscopic description was established by cover slip culture method and development of aerial and substrate mycelium, and the array of spores on mycelium were examined under high power objective of light microscope [11]. The investigation further extended to analyze the cell wall sugars [12] and cell wall amino acid that makes up the chemotaxonomical features [13]. Physiological characterization such as, the effect of pH (5-9), temperature (10-50℃) and salinity (NaCl concentrations 1-4%) and antibiotic sensitivity against ten different antibiotics (Himedia, Mumbai, India) [ampicillin (A10), cloxacillin (Clo), streptomycin (S10), chloramphenicol (C30), tetracycline (T30) trimethoprin (Tr), bacitracin (B10), erythromycin (Er10) and nalidixic acid] were also examined. The advantages and benefits of carbon sources such as starch, dextrose, fructose, maltose and mannitol, and nitrogen sources namely D-alanine, L-arginine, potassium nitrate, L-phenylalanine and L-tyrosine were tested on starch casein agar medium.

3. Results

Figure 1 shows, starts with the different actinomycetes isolates were identified in Pulicat estuary, Muttukadu estuary and Ennore estuary [Streptomyces-162 isolates (60%), Actinopolyspora-104 isolates (35%), Nocardiodes-11 isolates (5%)]. Actinomycetes colonies were isolated from different stations of marine soil sediments of the three areas. Among them, 277 isolates were morphologically distinct on the basis of spore mass colour, aerial and substrate mycelia formation and production of diffusible pigment. The majority (60%; 162 isolates) were assigned to the genus Streptomyces.(35%; 104 isolates) were assigned to the genus Actinopolyspora,(5%;11 isolates) were assigned to the genus Nocardiodes. As presented in Table 1, the sediments taken from Pulicat estuary 118 actinomycetes isolates were identified. These isolates comprised of various actinomycetes such as Streptomyces RM 17-45, Streptomyces RM 4 -32, Actinopolyspora sp.Halophila-19, Actinopolyspora sp.Mortivallis-14 and Actinopolyspora sp.Iraqiensis-8. In Muttukadu estuary the actinomycetes that were identified are Streptomyces RM 17-34, Streptomyces RM 42-20, Actinopolyspora sp.Halophila -16, Actinopolyspora sp.Mortivallis-12 and Actinopolyspora sp.Iraqiensis-9, which together amount to 91 actinomycetes isolates. The estuary of Ennore contained 68 actinomycetes. Isolates showing Streptomyces RM 17- 14, Streptomyces RM 42-17, Actinopolyspora sp.Halophila-11, Actinopolyspora sp.Mortivallis -7, Actinopolyspora sp.Iraqiensis- 8 and in addition Nocardiodes -11, that was not identified in Pulicat and Muttukadu estuaries.

Table 1.
Identification of Actinomycetes

Sediments	No. of Actinomycetes Isolates	Actinomycetes
Pulicat Estuary	118	Streptomyces RM 17-45
		Streptomyces RM 42 -32
		Actinopolyspora sp.Halophila - 19
		Actinopolyspora sp.Mortivallis - 14
		Actinopolyspora sp.Iraqiensis- 8
Muttukadu Estuary	91	Streptomyces RM 17-34
		Streptomyces RM 42-20
		Actinopolyspora sp.Halophila - 16
		Actinopolyspora sp.Mortivallis - 12
		Actinopolyspora sp.Iraqiensis - 9
Ennore	68	Streptomyces RM 17-14
		Streptomyces RM 42-17
		Actinopolyspora sp.Halophila - 11
		Actinopolyspora sp.Mortivallis - 7
		Actinopolyspora sp.Iraqiensis - 8
		Nocardiodes - 11

3.1. Phenotypic Characteristics of Selected Actinomycetes

The selected actinomycetes exhibited certain morphological characteristics as follows

Morphological characterization of Pulicat Estuary isolates Streptomyces RM17 and Streptomyces RM42 developed dark grey coloured aerial mycelia, the strain Streptomyces RM17 developed coffee brown coloured substrate mycelium, and Streptomyces RM42 developed brick red coloured substrate mycelium and spore mass was of dark grey. Streptomyces RM17 developed spiral nature spore chain and Streptomyces RM42 developed hooked spore chain. However, the strains of Actinopolyspora sp.Halophila developed white coloured aerial mycelium, yellow ocher substrate mycelium, its spore mass was white in colour and developed a long elongated

Table 2.
Morphological Characteristics

Properties	Sterptomycessp. RM17	Sterptomycessp. RM42	Actinopolysporasp. Halophila	Actinopolysporasp. Mortivallis	Actinopolysporasp. Iraqiensis	Nocardiodes
Pulicat Estuary						
Sporophore morphology	Spiral Dark	Hook like	Long elongated	Cylindrical	Spherical	Not found
Colour of aerial mycelium	Grey	Dark grey	White	Pale yellow	Pale brown	Not found
Colour of substrate mycelium	Coffee brown	Brick red	Yellow ocher White	Yellow	Brown	Not found
Spore mass	White	Dark white	White	White	Brown	Not found
Muttukadu Estuary						
Sporophore morphology	Spiral	Hook like	Long elongated	Cylindrical	Spherical	Not found
Colour of aerial mycelium	Dark grey	Dark grey	White	Pale yellow	Pale brown	Not found
Colour of substrate mycelium	Coffee brown	Brick red	Yellow ocher White	Yellow	Brown	Not found
Spore mass	White	Dark white	White	White	Brown	Not found
Ennore Estuary						
Sporophore morphology	Spiral	Hook like	Long elongated	Cylindrical	Spherical	Spherical
Colour of aerial mycelium	Dark grey	Dark grey	White	Yellow	Pale brown	Brown
Colour of substrate mycelium	Coffee brown	Brick red	Yellow	Yellow	Brown	Brown
Spore mass	Dark grey	Dark grey	White	White	Brown	Brown

Table 3.
Biochemical Characteristics

Properties	Sterptomyces sp.RM17	Sterptomycessp. RM42	Actinopolysporasp. Halophila	Actinopolyspora sp.Mortivallis	Actinopolysporasp. Iraqiensis	Nocardiodes
Pulicat EstuaryIndole Production	−	−	−	−	−	
Not found						
Voges Proskauer	−	−	−	−	−	Not found
Methyl red	−	−	−	−	−	Not found
Catalase	+	+	+	+	+	Not found
Oxidase	+	+	+	+	+	Not found
Citrate utilization	+	+	+	+	+	Not found
Melanin production	−	−	−	−	−	Not found
Casein hydrolysis	+	+	+	+	−	Not found
Lipid hydrolysis	+	+	−	−	−	Not found
Starch hydrolysis	+	+	+	+	−	Not found
Gelatin hydrolysis	+	+	+	+	+	Not found
Muttukadu Estuary						
Indole Production	−	−	−	−	−	Not found
Voges Proskauer	−	−	−	−	−	Not found
Methyl red	−	−	−	−	−	Not found
Catalase	+	+	+	+	+	Not found
Oxidase	+	+	+	+	+	Not found
Citrate utilization	+	+	+	+	+	Not found
Melanin production	−	−	−	−	−	Not found
Casein hydrolysis	+	+	+	−	−	Not found
Lipid hydrolysis	−	−	−	−	−	Not found
starch hydrolysis	+	+	+	+	−	Not found
Gelatin hydrolysis	+	+	+	+	+	Not found
Ennore Estuary						
Indole production	−	−	−	−	−	−
Voges Proskauer	−	−	−	−	−	−
Mythyl red	−	−	−	−	−	+
Catalase	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+
Citrate Utilization	+	+	+	+	+	+
Melanin production	−	−	−	−	−	−
Casein hydrolysis	+	+	+	+	−	−
Lipid hydrolysis	+	+	−	−	−	−
Starch hydrolysis	+	+	+	+	−	−
Gelatin hydrolysis	+	+	+	+	+	+

+ Positive ; − Negative

Table 4.
Carbon Utilization

Properties	Streptomyces sp .RM 17	Streptomycessp .RM 42	Actinopolyspora sp . Halophila	ActinoPolyspora sp .Mortivallis	Actinopolyspora sp .Iraqiensis	Nocardiodes
Pulicat Estuary						
Starch	++++	+++++	+++	+++	++	Not found
Dextrose	++	+++	+	++	++	Not found
Fructose	+	+	+	+	+	Not found
Maltose	+++	+	+	+	+	Not found
Muttukadu Estuary						
Starch	+++	+++	+++	+++	+++	Not found
Dextrose	++	+++	++	++	++	Not found
Fructose	+	+	++	++	++	Not found
Maltose	+++	++	++	++	++	Not found
Ennore Estuary						
Starch	++++	+++++	+++	+++	++	+
Dextrose	++	+++	+	++	++	−
Fructose	+	+	+	+	+	−
Maltose	+	+	+	+	+	+

++++Excellent; +++Good; ++Moderate; +Poor; −Nil

Table 5.
Chemotaxonomic Characters

Properties	Streptomyces sp.RM 17	Streptomyces sp.RM 42	Actinopolyspora sp. Halophila	Actinopolyspora sp.Mortivallis	Actinopolyspora sp.Iraqiensis	Nocardiodes
Pulicat Estuary						
Whole cell sugar analysis	−	−	Galactosearabinose & ribose	Arabinose & ribose	Arabinose& galactose	Not found
Cell Wall amino acid analysis	L-DAP	L-DAP	meso-DAP	Meso-DAP	meso-DAP DD-DAP	Not found
Muttukadu Estuary						
Whole cell sugar analysis	−	−	Galactose, arabinose& ribose	Arabinose & ribose	Arabinose & galactose	Not found
Cell Wall amino acid analysis	L-DAP	L-DAP	meso-DAP	Meso-DAP	meso-DAP DD-DAP	Not found
Ennore Estuary						
Whole cell Sugar analysis	−	−	Galactose arabinose & ribose	Arabinose & ribose	Arabinose & galactose	rabinose & galactose
Cell Wall amino acid analysis	L-DAP	L-DAP	Meso-DAP	Meso-DAP	Meso-DAP	Meso-DAPDD-DAP

−Nil

spore chain. Actinopolyspora sp.Mortivallis developed pale yellow coloured aerial mycelium, yellow coloured substrate mycelium,its spore mass was white in colour and developed a cylindrical spore chain. Actinopolyspora sp.Iraqiensis had a pale brown coloured aerial mycelium, substrate mycelium and spore mass was brown in colour and developed a spherical spore chain. The details of morphological and biochemical characteristics, utilization of carbon and nitrogen sources, and chemotaxonomical properties of the test isolates are given in the Table 2 to Table 6.

The Muttukadu Estuary isolates Streptomyces RM17 developed grey coloured aerial mycelia and grey coloured for Streptomyces RM42, the strain Streptomyces RM17 developed coffee brown coloured substrate mycelium, and Streptomyces RM42 developed brick red coloured substrate mycelium and spore mass was dark grey for Streptomyces RM17 and grey for Streptomyces RM42. RM17 developed spiral nature spore chain and Streptomyces RM42 developed hooked like spore chain. However, the strains of Actinopolyspora sp.Halophila developed white coloured aerial mycelium, yellow ocher substrate mycelium, its spore mass was white in colour and developed a long elongated spore chain. Actinopolyspora sp.Mortivallis developed pale yellow coloured aerial mycelium, yellow coloured substrate mycelium,its spore mass was white in colour and developed a cylindrical spore chain. Actinopolyspora sp.Iraqiensis had a dark brown coloured aerial mycelium, pale brown substrate mycelium and spore mass was brown in colour and it developed a spherical spore chain.

The details of morphological and biochemical characteristics, utilization of carbon and nitrogen sources, and chemotaxonomical properties of the test isolates are given in the Table 2 to Table 6

Morphological characterization of the Ennore isolates of Streptomyces RM17 developed grey coloured aerial mycelia and dark grey colour for Streptomyces RM42, the strain Streptomyces RM17 developed coffee brown coloured substrate mycelium, and Streptomyces RM42 developed brick red coloured substrate mycelium and spore mass was dark grey for both Streptomyces RM17 and Streptomyces

Table 6.
Nitrogen Source Utilization

Properties	Streptomyces sp.RM 17	Streptomyces sp.RM 42	Actinopolyspora sp. Halophila	Actinopolyspora sp.Mortivallis	Actinopolysporasp. Iraqiensis	Nocardiodes
Pulicat Estuary						
D-alanine	+++	+++	+++	+++	+++	Not found
L-arginine	++	++	+	+	+	Not found
Potassium nitrate	++	++	+	+	+	Not found
L-Phenylalanine	++++	++++	+++	+++	++	Not found
L-tyrosine	++++	++++	++++	++++	+++	Not found
Muttukadu Estuary						
D-alanine	+++	+++	+++	+++	+++	Not found
L-arginine	+++	+++	++	+	+	Not found
Potassium nitrate	++	++	+	+	+	Not found
L-Phenylalanine	++++	++++	++++	++++	++++	Not found
L-tyrosine	++++	++++	+++	+++	+++	Not found
Ennore Estuary						
D-alanine	+++	++++	+++	+++	+++	+++
L-arginine	++	++	+	+	+	+
Potassium nitrate	++	++	+	+	+	+
L- Phenylalanine	++++	+++++	+++	+++	++	+++
L-tyrosine	++++	+++++	+++	+++	+++	+++

++++Excellent; +++Good; ++Moderate; +Poor

Table 7.
Temperature

Test	Streptomyces sp.RM 17	Streptomyces sp.RM 42	Actinopolyspora sp. Halophila	Actinopolyspora sp.MortivalLIS	Actinopolysporasp. Iraqiensis	Nocardiodes
Pulicat Estuary						
10℃	+	+	+	+	+	Not found
20℃	++	++	++	++	++	Not found
30℃	++++	++++	+++	+++	+++	Not found
40℃	++++	++++	++++	++++	++++	Not found
50℃	+++	+++	+	+	++	Not found
Muttukadu Estuary						
10℃	+	+	+	+	+	Not found
20℃	++	++	++	++	++	Not found
30℃	+++	+++	+++	+++	+++	Not found
40℃	++++	++++	++++	++++	++++	Not found
50℃	+++	+++	+	+	++	Not found
Ennore Estuary						
10℃	+	+	+	+	+	+
20℃	++	++	++	++	++	++
30℃	++++	++++	++++	++++	++++	++++
40℃	++++	++++	++++	++++	++++	++++
50℃	+++	+++	+	+	++	+

++++ Excellent; +++Good; +Moderate; +Poor

RM42. Streptomyces RM17 developed spiral nature spore chain and Streptomyces RM42 developed hook like spore chain. Actinopolyspora sp.Halophila developed white coloured aerial mycelium, yellow substrate mycelium,its spore mass was white in colour and developed a long elongated spore chain. Actinopolyspora sp.Mortivallis developed yellow coloured aerial mycelium and substrate mycelium, its spore mass was white in colour and developed a cylindrical spore chain. Actinopolyspora sp.Iraqiensis had a pale brown coloured aerial mycelium, brown substrate mycelium, spore mass in brown and it developed a spherical spore chain.

The details of morphological and biochemical characteristics, utilization of carbon and nitrogen sources, and chemotaxonomic properties of the test isolates from all the three areas are shown in the Table 2 to Table 6.

3.2 Physiological Characteristics of Selected Actinomycetes

It was evident that different physiological characteristics are influencing the growth rate of the actinomycetes [14-16]. In the present study, the assessment of physiological characteristics of Pulicate Estuary strains Streptomyces RM17 and Streptomyces RM42 revealed that they could grow excellent at 30℃ and 40℃, but Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis and

Table 8.
pH

Properties	Streptomyces sp.RM 17	Streptomyces sp.RM 42	Actinopolyspora sp. Halophila	Actinopolyspora sp.Mortivallis	Actinopolysporasp. Iraqiensis	Nocardiodes
Pulicat Estuary						
5	++	++	+	+	+	Not found
6	+++	+++	++	++	++	Not found
7	++++	++++	+++	+++	+++	Not found
8	++++	++++	+++	+++	+++	Not found
9	++++	++++	+++	+++	+++	Not found
Muttukadu Estuary						
5	+	+	+	+	+	Not found
6	+++	+++	+++	+++	+++	Not found
7	++++	++++	++++	++++	++++	Not found
8	+++	+++	++	++	++	Not found
9	+++	+++	+++	+++	+++	Not found
Ennore Estuary						
5	++	++	+	+	+	+
6	++	++	++	++	++	++
7	+++	+++	+++	+++	+++	+++
8	+++	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++	+++

++++Excellent; +++Good; ++Moderate; +Poor

Table 9.
NaCl concentrations

Test	Streptomyces sp.RM 17	Streptomyces sp.RM 42	Actinopolyspora sp. Halophila	Actinopolyspora sp.Mortivallis	Actinopolyspora sp.Iraqiensis	Nocardiodes
Pulicat Estuary						
Without NaCl	+	+	+	+	+	Not found
1g/l	++++	++++	++++	++++	++++	Not found
2g/l	+++	+++	+++	+++	+++	Not found
3g/l	++	++	++	++	++	Not found
4g/l	+	+	+	+	+	Not found
Muttukadu Estuary						
Without NaCl	+	+	+	+	+	Not found
1g/l	++++	++++	++++	++++	++++	Not found
2g/l	+++	+++	+++	+++	+++	Not found
3g/l	++	++	++	++	++	Not found
4g/l	+	+	+	+	+	Not found
Ennore Estuary						
Without NaCl	+	+	+	+	+	+
1g/l	++++	++++	++++	++++	++++	++
2g/l	+++	+++	+++	+++	+++	+
3g/l	++	++	++	++	++	+
4g/l	+	+	+	+	+	+

++++Excellent;+++Good;++Moderate ;+Poor

Actinopolyspora sp.Iraqiensis had an excellent growth only at 40℃ . In pH Streptomyces RM17 and Streptomyces RM42 had excellent growth level between pH 7.0 and 9.0. Whereas Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis had a good growth level between pH 7.0 and 9.0. The strains of Streptomyces RM17, Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis showed an excellent growth rate at a NaCl concentration of 1g/l. The physiological characteristics of the isolates are shown in Table 7 to Table 9

Physiological characteristics of Muttukadu Estuary strains Streptomyces RM17 and Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis revealed that they could grow excellent only at 40℃. In pH Streptomyces RM17 and Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis had an excellent growth level only at pH 7.0 respectively. However, the strains of Streptomyces RM17, Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis showed an excellent growth rate at a NaCl concentration of 1g/l. The physiological characteristics of the isolates are shown in Table 7 to Table 9

The Physiological characteristics of Ennore Estuary strains Streptomyces RM17 and Streptomyces RM42, Actinopolyspora

Table 10.
Antibiotic Sensitivity

Test	Streptomyces sp .RM 17	Streptomyces sp .RM 42	Actinopolyspora sp . Halophila	Actinopolyspora sp .Mortivallis	Actinopolyspora	Nocardiodes sp.Iraqiensis
Pulicat Estuary						
Ampicillin (Ak30)	R	R	S	R	S	Not found
Coloxacillin (Clo)	R	S	S	S	R	Not found
Streptomycin (S10)	S	S	R	R	R	Not found
Chloramphenicol(C30)	S	S	S	S	S	Not found
Tetracycline(T30)	S	S	S	S	S	Not found
Trimethoprin (Tr)	R	R	R	R	R	Not found
Bactracin (B10)	S	S	S	S	S	Not found
Erythromycin(Er10)	S	S	S	S	S	Not found
Nalidixic acid (Na30)	R	R	R	R	R	Not found
Muttukadu Estuary						
Ampicillin (Ak30)	R	R	S	S	S	Not found
Coloxacillin (Clo)	S	S	S	S	S	Not found
Streptomycin (S10)	S	S	R	R	R	Not found
Chloramphenicol(C30)	S	S	S	S	S	Not found
Tetracycline(T30)	S	S	S	S	S	Not found
Trimethoprin (Tr)	R	R	R	R	R	Not found
Bactracin (B10)	S	S	S	S	S	Not found
Erythromycin(Er10)	S	S	S	S	S	Not found
Nalidixic acid (Na30)	R	R	R	R	R	Not found
Ennore Estuary						
Ampicillin (Ak30)	R	R	S	R	S	S
Coloxacillin (Clo)	R	S	S	S	R	R
Streptomycin (S10)	S	S	R	R	R	R
Chloramphenicol(C30)	S	S	S	S	S	S
Tetracycline(T30)	S	S	S	S	S	R
Trimethoprin (Tr)	R	R	R	R	R	S
Bactracin (B10)	S	S	S	S	S	S
Erythromycin(Er10)	S	S	S	S	S	S
Nalidixic acid (Na30)	R	R	R	R	R	S

R Resistant
S Sensitive

sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis and Nocardiodes revealed that they could grow well at 30 ° and 40℃. In pH Streptomyces RM17 and Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis had a good growth level only at pH 7.0 to 9.0. However, the strains of Streptomyces RM17, Streptomyces RM42, Actinopolyspora sp.Halophila, Actinopolyspora sp.Mortivallis , Actinopolyspora sp.Iraqiensis had excellent growth rate at a NaCl concentration of 1g/l .Whereas Nocardiodes had moderate growth rate at a NaCl concentration of 1g/l . The physiological characteristics of the isolates are shown in Table 7 to Table 9

In general, biochemical and physiological characteristics and antimicrobial susceptibility patterns of the actinomycetes vary from isolate to isolate depending on the growth conditions. The antibiotic sensitivity patterns against the test isolates from all the three areas are shown in the Table 10.

4. Discussion

The marine soil sediments in Pulicat Estuary, Muttukadu estuary and from the estuary of Ennore , TamilNadu, India had a wide range of salinities and were selected as an ecosystem for studying the diversity of actinomycetes and their antimicrobial properties. Identification of strains by morphological and phenotypic characteristics revealed that the isolates Streptomyces, Actinopolyspora showed dominant genera in Pulicat estuary, Muttukadu estuary and Ennore estuary. Nocardiodes were isolated only from the soil sediments of Ennore. All the isolated actionmycetes were identified at a generic level which was based on colony morphology and microscopic morphology. Chemotaxonomy plays a major role in the identification of actinomycetes in the generic level. The present study concluded that the physiological characteristics of actinomycetes varied by available nutrients in the medium and the physical conditions.

The present study revealed that among the isolates, Streptomyces and Actinopolyspora were the dominant genera. Frequency and dominance of Streptomyces among actinomycetes in various soil types were reported by several workers [17-20]. Whereas actinomycetes isolated from marine sediments of Visakapatnam, exhibited only 18% of

antimicrobial activity as stated by Chandra Sekhar et al.[21]. Whereas the isolation and characterization of actinomycetes from west cost of India prevailed that major (47%) of the isolates belonged to the genera Streptomyces sp RM 17 and Streptomyces sp RM 42 stated by Remya and vijayakumar [22], Similarly, the present study also determined that the isolates showed both antibacterial and antifungal activity. The isolates namely Streptomyces sp. RM17 and Streptomyces sp. RM42, Actinopolyspora sp.Halophila, Actinopolyspora. mortivallis and Actinopolyspora sp.Iraqiensis sp had broad spectral antimicrobial activity, but Nocardiodes showed a low profile and was isolated only on the Ennore estuary.

This investigation concludes that the biochemical and physiological characteristics of the six actinomycetes varied from isolate to isolate depending on the growth and physical conditions.

Conflict of interest statement

We declare that we have no conflict of interest.

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