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BIOACTIVITY OF ACTINOMYCETES ISOLATED FROM COSTAL SEDIMENTS

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ABSTRACT

Marine soil sample from different depths at two different sites were collected and all the samples were subjected for the analysis of the actinomycetes diversity. This reseach revealed that costal region of Cuddalore to Rameswaram is a potential source for wide spectrum of antimicrobial and anticancer metabolite producing actinomycetes. Findings of the present study conclude that marine environment actinobacteria are the potential ecosystem for antagonistic actinobacteria which deserves for bioprospecting. Isolation of actinomycetes from marine sediment of Cuddalore costal region to Rameswaram was successfully achieved. Screening of the actinomycete strains for antimicrobial activity shows positive results and one of the most promising potential isolate was selected and partially Characterized by followed International streptomyces project. The isolate NKC2 was aerobic, Gram-positive and grew well on PDA. Aerial mycelium was grey to black; basal mycelium yellowish orange; reverse side of the colony yellow. Light yellow diffusible pigment was produced; no melanin was produced. Tolerated 0-10% NaCl level but grew well at 2% NaCl concentration. It can grow in a wide range of pH 4-12, with optimum growth at pH 7 and 9. Growth in different temperatures showed that growth occurred between 20-35ºC, with optimum at 27ºC. The isolate also hydrolysed lipid, casein and starch and catslase positive. Utilised wide range of sugars as sole source of carbon.

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INTRODUCTION

Actinomycetes comprise a group of microorganisms producing of radiating colonies of branching mycelia. The mycelium may be of only one type called substrate or vegetative or of two types, substrate and aerial. They reproduce by fission or by means of special spores or conidia. They resemble true bacteria in their size, chemical composition and biochemical activities. Hence, they commonly are considered as higher, filamentous bacteria.

Chemical diversity of Actinomycetes: Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. 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 one abundant in terrestrial soils, and majority of the isolates shown to produce bioactive compounds. Actinmycetes produce till today the highest chemical diversity regarding novel structure of secondary metabolites; 61% in total of all bioactive microbial metabolites were isolated from actimomycetes till now (48% from the genus Streptomyces and 13% from the rare Actinomycetes). The number of bioactive compounds, which were usually discovered by special pharmaceutical and aerobiological screens, is exponentially increasing more rapidly than the antibiotics do. In the last couple of years, up to 40-45% of discovered microbial metabolites exhibit some of kind of this or other non-antibiotic activities.

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The percentage of actinomycetes derived metabolites in this group of compounds is about 40% less than their rate among antibiotics.

Bioactive compounds produced by common Actinomycetes: The number of bioactive secondary metabolites isolated from various genera and species of actinomycetes during 1974-1996. In the recent years numerous selective techniques were developed for isolation of these morphologically and physiologically unusual species, not only from soil samples but from the most diverse other source such as muds, various sediments and from the soil sediments. It is clear that Streptomyces and non-streptomyces actinomycetes are still equally frequent and important sources of new bioactive secondary metabolites. The shift of interest from Streptomyces species to rare actinomycetes does not mean that the Streptomyces genus is no longer important.

Rare Actinomycetes: It is a fact that different groups of microbes one not equally potential producers of secondary metabolites. In the late sixties, especially after the discovery and success of the Micromonospora derived gentamicin, the study of actinomycetes-received increasing attention. Theses relatively rare species, which are difficult to isolate from nature by common methods, soon proved to be rich sources of new antibiotics, growth factors and other secondary metabolites. Extensive screening of the soil actinomycetes has led to the discovery of many novel strains that produce useful secondary metabolites (Tanaka and Omura, 1990). Presently the course of screening for new antibiotics, several studies are oriented towards isolation of new actinomycetes from different habitats. One of the strategies for enhancing the likelihood of obtaining novel secondary metabolites is to analyze uncommon ecosystems. Uncommon environment or extreme environments have been found by many investigators to be inhabited by microorganisms, which are especially adapted to these ecological niches. In the last few years, the discovery of new actinomycetes and new metabolites found in microbiologically poorly explored areas in the world suggests that a careful exploration of new habitats might continue to be useful for the these purposes (Ouhdouch et al., 2001). With the above facts in mind in the present investigation an attempt was made to evaluate marine sediments, as a source of actinomycetes for the screening program. Marine sediments, either man-made or natural represent a unique aquatic habitat. So far no work has been carried out to isolate and study their antimicrobial potential.

The present study aims at isolation and screening of actinomycetes for antimicrobial activity from marine sediments from the cuddalore costal region to Rameswaram. The objectives of the present investigation are the Isolation of actinomycetes from marine sediments,Screening broad spectrum bioactivity and Selection of a potential isolate for antagonism .

MATERIALS AND METHODS

Marine sample collection: Soil sample was collected from Cuddalore costal region near college and Rameswaram region. mud samples were collected from various points (periphery and the middle section of the sea shore) within the marine sediment using a mud sampler. All the sub-samples collected from the marine sediment were immediately transferred to a sterile polythene bottle sealed, labeled and transported to laboratory. In the laboratory the composite mud sample was spread in a clean aluminium tray and dried in a hot air oven for seven days at 40°C. The dry mud sample after removing large debris was transferred to a fresh clean polythene bag, secured, labeled and stored at 4°C until examined.

Serial dilution: Ten gram soil sample was aseptically transferred to a 250 ml conical flask containing 100 ml sterilized distilled water and shaken vigorously for 10 minutes. This was designated as 10^{-1} dilution. This suspension was serially diluted by transferring 10 ml aliquots successively to four 250 ml conical flasks containing 90 ml sterilized water. Up to seven dilutions were made viz.10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} The last two dilutions were used for inoculation.

Isolation of actinomycetes using pour plate method: One-milliliter aliquots of each of the two dilutions (10^{-6},10^{-7}) were aseptically transferred to sterilized Petri plates. Two sets of five plates for each dilution were prepared. To one set of plates starch casein agar (45°C) (Kuster and Williams, 1964) was added at the rate of 20ml/plate and swirled gently for uniform distribution of the inoculum with the medium. To another set of plates molten (45°C) starch ammonium sulphate agar was added at the rate of 20ml/plate and mixed thoroughly. All the plates were incubated under laboratory conditions (25±2°C). Plates were examined for the appearance of actinomycete colonies from 2nd day onwards and up to 30-40 days. Total number of colonies in each plate and in each medium was recorded.

This procedure was repeated for the other marine sediment sample too. Selected colonies from the plates were sub-cultured on PDA (Riker and Riker, 1936)slopes and incubated for 7-10 days. When sufficient growth has occurred in the tubes the tubes were stored at 4°C in a refrigerator.

Test organisms: The test organisms, six filamentous fungi *Rhizoctonia solani*, *Fusarium solani*, *Drechslera oryzae*, *Colletotrichum gloeosporioides*, and *Curvularia lunata* used in this screening program was procured from the Institute of Microbial Technique and Culture Collections, Chandigarh, Pune, India.

Dual culture screening: Four different isolates were point inoculated around the periphery of the plate at equidistance. All the plates were then incubated for ten days at 25±2°C. After ten days the plates were inoculated with a mycelial plug (5 mm dia.) of actively growing *Rhizoctoniasolani*, *Colletotrichum gloeospoioides*, *Drechslera oryzae* and *Fusarium oxysporium* and *Curvularia lunata* plate cultures. Separate plates were inoculated for each fungus. Plates were observed for growth inhibition after 3-8 eight days depending on the fungus. Reduction in radial growth was measured and recorded.

Preparation of culture filtrate: Culture filtrate (CF) containing inhibitory metabolites of the isolate NKC2 was prepared by growing the isolate in Potato-Dextrose Broth (PDB). Fifty-milliliter portions of PDB (pH 7.2 before sterilization) was poured into six 250 ml conical flasks and sterilized in an autoclave for 20 minutes at 15psi. The broth was then inoculated with a 100µl of five days old broth culture

and mixed well. All the inoculated flasks were incubated as static culture at room temperature (25±2°C) for twenty days.
At the end of the incubation period two observations were made ie. type of growth – submerged growth or surface growth or both and colour of the culture broth. Then the broth was filtered through a handmade filter paper to separate the mycelial biomass and the liquid containing metabolites. The broth was then centrifuged at 3000 rpm for 10 minutes to remove spores and mycelial bits. The clear supernatant was used for bioassay. At this stage pH of the culture filtrate was also recorded in a digital pH meter.

Colony characterization: Characterization of the selected isolates was carried out according to the methods recommended by International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966). Spore chain and sporophore morphology of a mature colony were determined under light microscope. Colour of aerial surface growth, colour of substrate mycelium and diffusible pigments, other than melanin were also determined. Whenever diffusible colours other than brown or black were produced on any medium, the colour was also recorded. Production of melanin pigments on tyrosine agar (ISP-7) and tryptone-yeast extract agar (ISP-1) was also observed. The various media used and their compositions are given below:

Growth in different pH: Ability of the isolates to tolerate a wide range of pH was tested in PD broth. pH of the PD broth was adjusted with 0.1N NaOH or 0.1N HCl to get 4, 5, 6, 7, 8, 9, 10, 11 and 12 and sterilized following standard procedures. The liquid medium (15ml) in test tubes was inoculated with 100µl of spore suspension and incubated at 25±2°C for 15 days. After 15 days the tubes were scored for growth and recorded.

Growth in different temperatures: Growth in different temperatures was tested by incubating PDA slants inoculated with spore suspension of the test isolate at the following temperatures 20, 25, 28, 35, 40 and 50°C in an incubator for 15 days.

Sodium chloride tolerance: Salt tolerance studies were carried out on Glucose-yeast extract-malt extract medium as the basic medium. The NaCl concentrations (w/v) used were: 0%, 2%, 4%, 6%, 8%, 10%, 12%, 15%, 18% and 20%. The slants were inoculated by streaking the agar surface with a loopful of spore suspension of the isolate. The inoculated tubes were incubated at 25±2°C for 20 days. After 20 days presence or absence of growth in different salt concentrations was recorded.

Utilisation of different carbon sources: Ability of the test strain, to use 20 different sugars as sole carbon sources for energy and growth was examined in carbon utilization medium suggested by Shirling and Gottlieb (1966). The various compounds were added to the liquid medium to get a final concentration of 1%. The inoculated tubes were incubated at 25±2°C for 20 days. The test strain was also inoculated on the basal medium (without any carbon source) which constituted negative control and a positive control with glucose (1%) was also included. A positive result was recorded when growth was greater than that in the negative control and that equal to or less than that in the negative control as negative.

The basal medium used was as follows:

RESULTS AND DISCUSSION

Successfully fifty six actinomycetes with diverse morphology and colour series were isolated from the cuddalore costal sediments. Among the different colour series grey colour colonies were dominant and represented by 29 colonies followed by yellow (8), sandal colour (5), light yellow (7), dirty sandal colour (6), and rose, brown, black, white and cream colour colonies were represented by one each. Out of the 56 isolates 36 did not survive on PDA slants after transfer from isolation plates. From the remaining isolates 20 isolates were selected based on colony morphology for bioactivity screening. All the isolates (100%) displayed growth suppressive effect on one or more of the test organisms. Interestingly all the isolates inhibited one or more fungi. However, only one isolate inhibited the unicellular fungus *Candida albicans*. Eight isolates (40%) arrested growth of Gram-positive bacteria used in this screening and only one isolate exhibited activity towards Gram-negative as well as Gram-positive bacteria (Fig. 1). Among the six filamentous fungi *Drechslera oryzae* was the most sensitive and was inhibited by 17 isolates (85%), with inhibition percentage ranging from 17 % - 88 %. Closely followed by *Colletotrichum gloeoporioides* which was inhibited by 14 isolates (70%), *Curvularia lunata* by 12 isolates (60%), *Fusarium oxysporum* and *Rhizoctonia solani* by 8 isolates (40%) each and *Pythium aphanidermatum* by 7 isolates (35%). Growth of *Candida albicans* an unicellular target fungus of this investigation was reduced by 55 % by only one isolate (table 1). In the case of bacteria eight isolates (40%) exhibited activity towards bacteria. Out of the eight active isolates only one isolate (NK/C2) inhibited growth of both Gram-negative bacteria and Gram-positive bacteria, whereas the remaining seven isolates exhibited activity towards Gram- positive bacteria only.

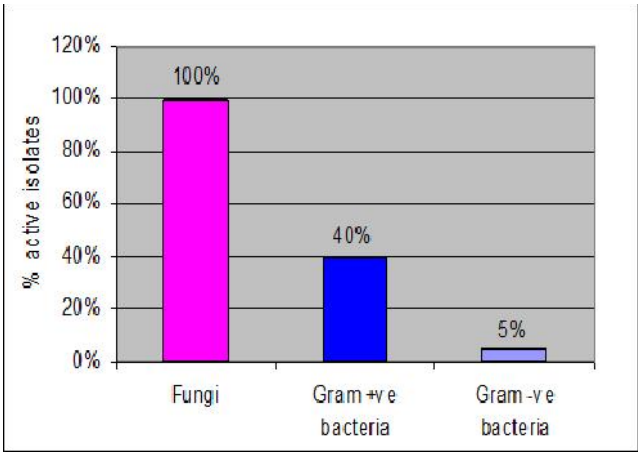


Figure 1. Antifungal and antibacterial activity

From this results (Table 1) it is clearly shows that the isolate NKC2 is the most promising strain with strong and broad-spectrum activity. The bioactivity spectrum of the isolate NKC2 to a range of bacteria and fungi is presented in Table 2. Since NKC2 was the only isolate to inhibit *Candida albicans* and the only organism to inhibit all the test organisms it was selected for further study.

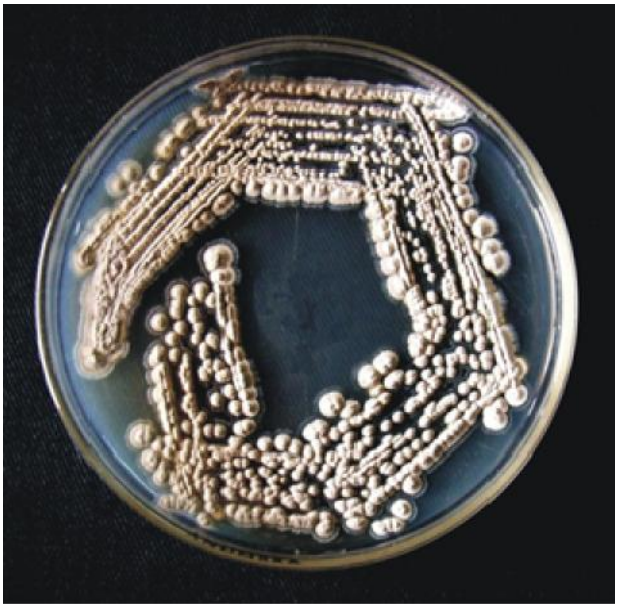
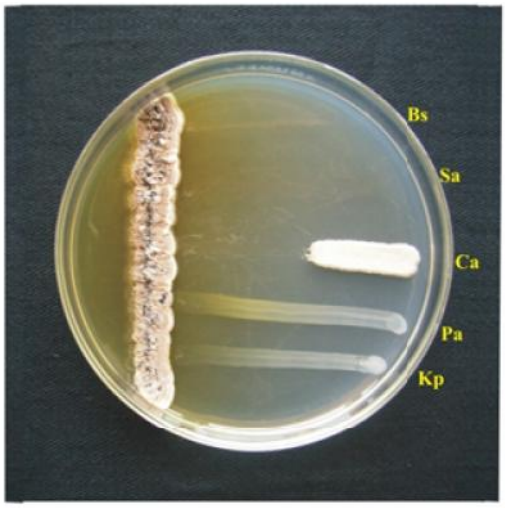


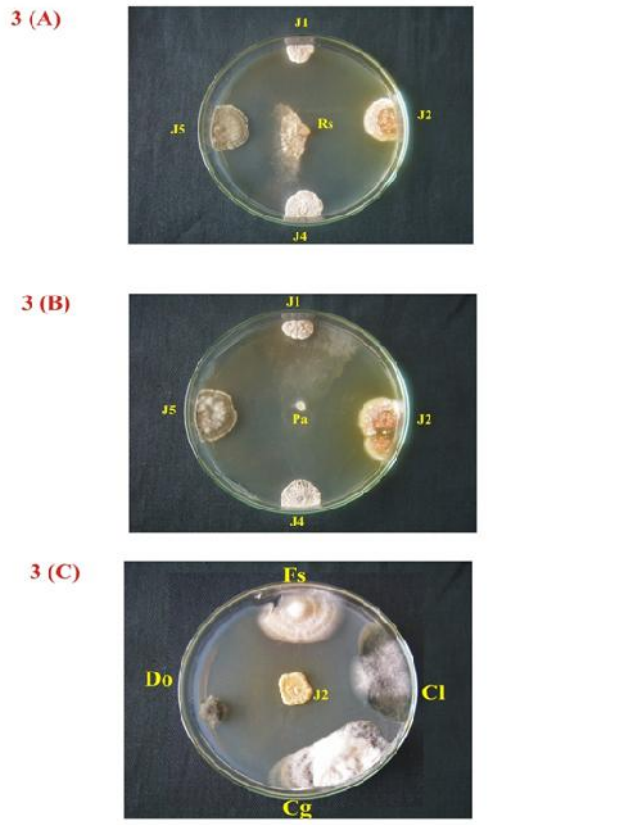
Plate 2. Atibacterial and and anticandidal activity



Test Organisms : *Bs = Bacillus subtilis; Sa = Staphylococcus aureus; Ca = Candida albicans; Pa = Pseudomonas aeruginosa ; Kp = Klebsiella pneumoniae*

Pure culture of the isolate is shown in Plate 1. The isolate NKC2 exhibited strong to moderate toxicity towards the various test organisms used in this study. Among the bacteria tested the two Gram - positive bacteria i.e. *Bacillus subtilis* and *Stphylococcus aureus* were highly susceptible and were completely inhibited (100%). The two Gram – negative bacteria were not much effected (Plate 2). Among the six filamentous fungi growth of *R. solani* was completely inhibited (100%). *D. oryzae* and *P. aphanidermatum* were strongly inhibited by 84% and 83% respectively. Closely followed by *C. gloeosporioides* (68%), *F. solani* (58%) and *C. lunata* (50%) (Plate 3).

Cultural characteristics of the antagonistic actinomycete strain NKC2 was determined on eleven different types of media recommended by International *Streptomyces* Project (ISP) and others. The data are summarized in table 3. tagonist grew well on ISP-4, ISP-5, ISP-7, potato dextrose agar (PDA) and Bennet's agar (BA). Moderate growth was noticed on ISP-2, ISP-3 and starch casein agar (SCA).



3(A). *Rhizoctonia solani*
3(B). *Pythium aphanidermatum*
3(C). Fs - *Fusarium solani;* Cl - *Curvularia lunata;* Cg - *Colletotrichum gloeosporioides;* Do - *Drechslera oryzae*

Antifungal activity of some actinomycetes isolates plate -3

No growth occurred on ISP-1 nutrient agar (NA) and Czapek's agar (CZ). Aerial mycelium was grey or grey mixed with rose or black in almost all the media tested. Basal mycelium was yellow to light yellow in all media except in ISP-2. Similarly reverse side of the colony was light yellow to dark yellow in all media tested. Yellow coloured diffusible pigment was observed in all media except ISP-2 and SCA. No melanin pigment was produced in ISP-1, but brown colour was noticed in ISP-7. Physiological and biochemical properties of the isolates NKC2 are presented in Table 4. The colony grew copiously on the medium without NaCl (0%); moderate growth occurred at 2% and 4% NaCl concentrations. Less growth at 6% NaCl and no growth beyond this concentration. The antagonist grew between 20ºC to 35ºC with optimum at 28ºC. pH tolerance studies revealed that the isolate could tolerate and grow between pH 6 to pH 10, with optimum at pH 7 and 8 . The isolate was catalase positive and could hydrolyse starch, casein and lipid. The results on the ability of the isolate NKC2 to utilize 20 different sugars as sole source of carbon is presented in table 5. The actinomycete grew better in sorbitol and trehalose. Moderate growth was observed in cellobiose, dextrose, fructose, inositol, maltose, melibiose, rhamnose, sucrose, adonitol dulcitol, inulin and salicin. No growth in galactose and lactose. Only a weak response was noticed in mannitol, mannose, raffinose and xylose. Out of the screened isolates only three isolates exhibited strong antagonism towards the pathogen with 10-17 mm inhibition zone. Five isolates showed moderate activity (3-6mm) and 11 isolates showed weak antagonism (less than 3mm), and 13 isolates exhibited no inhibitory effect on mycelial growth of tested fungi.

Table 1. Antimicrobial properties of actinomycetes

S. No	Iso. No	*Antifungal activity* % Inhibition							Antibacterial activity	
		Cg	Cl	Fs	Do	Rs	Pa	Ca	Gram -+ve	Gram --ve
1	NK/C1	20	20	-	83	86	-	-	+	-
2	NK/C2	68	50	58	84	100	83	34	+	+
3	NK/C3	-	-	-	-	14	17	-	+	-
4	NK/C5	77	40	54	73	46	68	-	-	-
5	NK/J6	33	36	36	78	-	-	-	-	-
6	NK/C7	55	41	17	83	-	20	-	-	-
7	NK/J8	-	-	-	17	-	-	-		-
8	NK/C12	33	06	16	64	-	11	-	-	-
9	NK/C14	-	-	-	17	-	-	-	+	-
10	NK/C15	43	35	35	73	-	-	-	-	-
11	NK/C16	11	-	-	31	-	-	-	-	-
12	NK/C17	-	-	-	88	-	-	-	+	-
13	NK/C18	07	27	20	55	09	05	-	-	-
14	NK/C19	14	12	-	50	06	-	-	-	-
15	NK/C20	13	09	-	83	06	-	-	+	-
16	NK/C21	19	-	-	50	05	-	-	+	-
17	NK/C23	-	25	-	-	-	05	-	-	-
18	NK/C26	-	-	15	18	-	-	-	-	-
19	NK/C29	15	21	-	-	-	06	-	+	-
20	NK/J30	08	00	-	20	-	-	-	-	-

Colletotrichum gloeosporioides; Cl- *Curvularia lunata F.s -Fusarium solani; Do- Drechslera oryzae; Rs- Rhizoctonia solani Pa-Pythium aphanidermatum; Ca- Candia albicans (-) = no activity; (+) = Activity present*

Tabble 2. Antimicrobial effects of the isolate NKC2

Test Organisms	% Inhibition
I. *Bacteria*	
a. Gram-positive	
Bacillus subtilis	100
Stphylococcus aureus	100
b. Gram-negative	
Klebsiella pneumoniae	21
Pseudomonas aeruginosa	24
II. Fungi	
a. Unicellular	
Candida albicans	55
b. Filamentous	
Colletotrichum gloeosporioides	68
Curvularia lunata	50
Drechslera oryzae	84
Fusarium solani	58
Pythium aphanidermatum	83
Rhizoctonia solani	100

Table 3. Colony characteristics of the strain NKC2

Medium	Colony characters					
	Growth	Aerial mycelium	Basal mycelium	Colour on reverse side	Diffusible pigment	Melanin Production
ISP-1	±	±	-	Colourless	-	-
ISP-2	++	Grey	Colourless	Dull yellow	-	-
ISP-3	++	Grey-black	Yellowish black	Yellowish-black	Light yellow	-
ISP-4	+++	Sandal-black	Light yellow	Yellow-black	Light yellow	-
ISP-5	+++	Grey-rose	Yellow	Dark yellow	Yellow	-
ISP-7	+++	Rose-grey-black	Yellow	Dark yellow	Light yellow	Yes
CZ	±	-	-	-	-	-
SCA	++	Greyish	Light yellow	Yellow	-	-
NA	-	-	-	-	-	-
PDA	++++	Light white-grey-black	Yellow	Yellow	Light yellow	-
BA	+++	Grey-sandal-black	Light yellow	Dark yellow	Yellow	-

(++++) = Good growth, (+++) = Moderate growth, (++) = Less growth (+) = Poor growth, (±) = Doubtful, (-) = No growth

However, six isolates of the last category strongly inhibited sclerotial production, though had no effect on radial growth. The results indicate that 9.4% of the isolates exhibited strong antagonism to the test pathogen. Even among the three isolates only 2 isolates produced clear well defined inhibition zone, suggesting strong antagonism. There are several reports on antagonism of the actinomycetes against various pathogens (Broad bent *et al.,* 1971; Ghaffar, 1971; Reddi and Rao, 1971; Knauss, 1976; Janaki, 2002). Since only 3 out of 32 isolates were found antagonistic, to all fungi are considered to be highly resistant to the antibiotic effect of the actinomycetes used in this investigation.The above observations confirm the earlier report by Turhan and Grossman (1986), who observed that among the six soil borne fungi *R. solani* and *A. alternata* were relatively resistant against the antibiotic effects of the actinomycetes.

Table 4. Physiological and Biochemical characteristics of the strain NKC2

Reaction	Response	Result
1. Melanin Reaction		
Medium ISP-1.	No browning of medium	Negative
Medium ISP-7	Blackish	Negative
2. Tyrosine Reaction		
Medium ISP-7	Browning of Medium	Positive
3. Diffusible pigment		
Bennet's agar	Yellow	Positive
PDA	Light brown	Positive
ISP-7	Blackish	Positive
4. Starch Hydrolysis	Zone appeared	Positive
5. Casein hydrolysis	Zone appeared	Positive
6. Urease	Phenol red to deep pink	Positive
7. Lipase	Opaque zone	Positive
8. Catalase	Brisk effervescence	Positive
9. Growth at (°C)		
20°C	+	Poor growth
25°C	++	Moderate growth
28°C	+++	Good growth
35°C	++	Moderate growth
40°C	-	No growth
50°C	-	No growth
10. NaCl Tolerance (%)		
0%	+++	Good growth
2%	++	Moderate growth
4%	++	Moderate growth
6%	+	Less growth
8%	-	No growth
10%	-	No growth
12%	-	No growth
15%	-	No growth
11. Growth in pH		
4	-	No growth
5	-	No growth
6	+	Poor growth
7	+++	Good growth
8	+++	Good growth
9	++	Moderate growth
10	+	Poor growth
11	-	No growth
12	-	No growth

Table 5. Carbon utilization pattern of the strain NKC2

Utilization	Carbon source
Positive	Sorbitol, Trehalose (+++)
Moderate	Cellobiose, Dextrose, Fructose, Inositol, Maltose, Melibiose Rhamnose, Sucrose (+++), Adonitol, Dulcitol, Inulin, Salicin (++)
Weak positive	Mannitol, Mannose, Raffinose, Xylose (+)
Doubtful	Galactose, Lactose

+++ = Good growth, ++ = Moderate growth, + = Poor growth

Again in 1994, the same authors in another study observed that *R. solani* and *Pythium debaryanum* were least sensitive to *Myrothecium* isolates. In anticancer aspect, it appears that there have been only a few studies focusing on finding bioactive compounds derived from marine actinobacteria to be used as anticancer agents, as well as agents against infectious organisms. Pure active compounds extracted from the marine actinobacterium, Salinispora tropica have shown inhibitory effects in manymalignant cell types (Prudhomme et al. 2008). In particular, Salinosporamide A is a novel rare bicyclic beta-lactonegamma lactam isolated from marine actinobacterium, S. tropica (Feling et al. 2003; Jensen et al. 2007). Salinosporamide A is an orally active proteasome inhibitor that induces apoptosis in multiple myeloma cells with mechanisms distinct from the commercial proteasome inhibitor anticancer drug. The isolate NKC2 displayed wide spectrum activity towards a range of fungi. Among the test organisms, only *F. solani* was insensitive to the antagonist.

Within the fungi maximum growth inhibition was recorded for *R. solani* (68%) followed by *D. oryzae* (45%). Such broad range of activity has been reported earlier for several species of *Streptomyces* and *Streptoverticillium* (Haque *et al.*, 1996; Chattopadhyay and Sen, 1998; Sujatha *et al.*, 2005). A gradual rise in the antifungal spectrum was observed with increasing age of the culture up to 7 days. Thereafter, a subsequent decline in its antimicrobial and anitcancer activity was noticed. These results are in similar with the earlier work done by Muharram *et al.* (2013). They proved the metabolites collected from 24 hr old culture of the *Streptomyces* sp. exhibited antifungal activity against *C. albicans* and *F. oxysporum*. The maximum activity and growth was observed after five to seven days of incubation. The activity of the isolate was observed from the second day of incubation and reached maximum after eight days. Previous studies reported different incubation periods including, nine days (Vijayakumar *et al.*, 2009).

The few recent studies that focused on the chemical characterization of bioactive compounds produced by actinobacteria support the potential isolation of novel molecules with biological activity (Carr et al., 2012). Thus, an exploration program of isolation of bioactive molecules from actinobacteria from marine sediments certainly will result in the discovery of novel compounds with activity against cancer cell lines and microorganisms that are potentially pathogenic to humans. The data discussed in the research work is primary attempt to identify potential marine actinomycetes isolate from south east costal region. It reveals promising antimicrobial activity. Examination of the characteristics of the antagonist on different media recommended by International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966) revealed certain characteristics of the isolate NKC2. These results demonstrate that cultivable actinobacteria from mangrove soil are potentially rich sources for discovery of new antibacterial metabolites and new actinobacterial taxa.

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CONCLUSION

It could be concluded that marine region south east coast of India is rich with Actinomyces. The isolates NKC2 from the investigated actinomycetes exhibited high antagonistic activity against the tested pathogens and cell lines particularly lung cancer cell lines. The phylogeny of the active isolate revels the Streptomyces sp are rich source for bioactive metabolites. Further investigations are needed in order to determine the active metabolites of the isolates. These results demonstrate that cultivable actinobacteria from mangrove soil are potentially rich sources for discovery of new antibacterial metabolites and new actinobacterial taxa. The research work is a primary attempt to identify potential marine actinomycetes isolate from cuddalore costal region to rameswaram. It reveals promising antimicrobial activity.

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