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Studies on the Antagonistic Activity of Actinomycetes Isolated from Soil Samples

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ABSTRACT

The need for the research on antimicrobial agents is a worldwide alarm due to the emergence of multiple drug resistant pathogens. Soil is a diverse medium in which one can find an immense microbial diversity. A good number of isolates were obtained from the different soil samples collected. 37 isolates were obtained from sample collected from in and around Madurai city. Of the 37 isolates screened so far, 30 of them showed antimicrobial activity against one or more of the test pathogens. 26 isolates showed good antimicrobial activity during secondary screening. Among those strains five actinomycetes SA1, SA4, SA12, SA13 and SA14 showed antagonistic activity only against gram-negative organism of *E.coli*. Four strains of SA3, SA10, SA24 and SA30 revealed antibacterial activity against gram positive organism of *Streptococcus mutans*. Actinomycete samples of SA7 and SA26 exhibited antibacterial activity against one tested organism of *Vibrio parahaemolyticus* and *Bacillus subtilis* respectively. Only 2 isolates of SA18 and SA28 showed antifungal activity against *Aspergillus niger*. Among these bioactive isolates, SA5, SA17, SA18, SA21 and SA28 show inhibition zone of 20 mm and above. Comparing with all the results obtained, the study revealed that SA28 is a promising strain and identified as *Streptomyces sp*. The strain seems to be potential not only by their broad-spectrum activity against most of the target organisms, it also revealed more than 14mm zone of inhibition. The FTIR spectrum of SA28 fermentation broth exhibited absorption at 3431.13 cm^{-1}, 2088.76 cm^{-1}, 1639.38 cm^{-1}, 1311.5 cm^{-1}, 1085.85$cm^{-1}$and 989.41cm^{-1}. These bands correspond to the amide and it is revealed that this actinomycetes colony SA28 is a hopeful strain for antibiotic production.

KEYWORDS: actinomycetes, agar well diffusion method, antimicrobial activity, antagonism, pathogenic microorganisms

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INTRODUCTION

The discovery of novel antimicrobial agents is a worldwide concern due to the emergence of multiple drug resistant strains. A large number of pathogenic bacteria and fungi have developed resistant to antibiotics in frequent use. These resistance mechanisms by pathogens are presently an urgent focus of research and in search for new novel antimicrobial products[1-3]. Actinomycetes form a large and important segment of the microflora of most natural environments. Soils, freshwater, lake and river bottoms, manures and compost contain an abundance of these organisms. They are of worldwide occurrence in nature, living and multiplying in both cold and tropical zones, and have been reported to occur even under the most extreme conditions of the desert [4-7].

Actinomycetes, filamentous soil bacteria, are widely documented as significant microorganisms because of their capability to produce many kinds of secondary metabolites like antibiotics, enzymes, pesticides, herbicides, immunomodulators, anti-infective and anticancer agents with diverse chemical structures and biological activities[8-9]. In the midst of all actinomycetes, the streptomycetes are the foremost one comprising large number of species. The non-streptomycetes are called rare actinomycetes, comprising approximately 100 genera. The discovery of new antibiotics reached a peak in the 1970s then decline in the late 1980s and 1990s due to a decrease in screening efforts rather than an exhaustion of compounds.

Soil is haunted with immense microbial diversity. The majority of the commercially available antibiotics have been obtained from soil microbial inhabitants. Investigations can possibly reveal actinomycetes species that produce novel antibiotics. It is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics and novel species of actinomycetes. The present study focused on the isolation of soil actinomycetes from poorly explored areas where the ecological habitat has terrestrial and it is a preliminary search for actinomycetes having both antibacterial activities and antifungal activities.

2. EXPERIMENTAL SECTION

2.1. Collection of samples

Five soil samples were selected randomly from Madurai city (9° 56' 20.7348" N & 78° 7' 18.1884"E) and the selected area was Balarengapuram (BR), Thiagarajar College (TC), Thiruppavanam (TPM), Theppakulam (TP) and Thirupparankundram (TPK) in and around Madurai city. Sample collection was done during the rainy season. The soil samples were collected in pre–sterilized glass bottles. The collected soil sample were divided into two portions, one portion of the sample was subjected to physico-chemical parameter analysis which was performed at soil testing laboratory, Dindigul District. The other portion was used for the isolation of actinomycetes.

2.2. Isolation of actinomycetes

The actinomycetes used in this study were isolated from the soil by spread plate technique on media after serial dilution using sea water. One mL of tested sea water was mixed with 9 mL of sterile sea water, followed by serial dilution with sterilized sea water in the range of 10^{-1} – 10^{-10}. The diluted samples of 100µL were spread with different isolation agar media of actinomycetes isolation agar and starch casein agar for actinomycetes. The plates were incubated and monitored for growth[10]. According to morphology, colonies were streaked on fresh agar plates for purification. This procedure was repeated several times till pure culture plates were obtained. For actinomycetes colonies starch casein agar and actinomycetes isolation agar were supplemented with cyclohexamide and nystatin (50 mg/L) to inhibit the growth of bacteria, yeast and fungi.

2.3. General storage of bacteria

All the isolated actinomycetes colonies were maintained on agar slants and stored at 4ºC. These strains were subcultured every month in order to keep them alive.

2.4. Organisms tested for biological characterization

Bacterial and fungal specimens were obtained from Microbial Type Culture Collections (MTCC), IMTECH, Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Pune, Maharastra, India. The cultures were kept on slants and stored at 4ºC. These strains were subcultured every month in order to keep them alive.

Table-1. List of microorganisms tested in antimicrobial studies

Test organisms	Strain Number
Gram-positive bacteria	
Bacillus subtilis	NCIM – 2063
Streptococcus mutans	MTCC –890
Gram-negative bacteria	
Escherichia coli	MTCC – 46
Vibrio cholerae	MTCC – 1738
Vibrio parahaemolyticus	MTCC – 451
Fungal Culture	
Aspergillus niger	NCIM – 586

2.5. Screening for biological characterization

2.5.1. Primary screening

For detection of antagonistic activity, an agar spot test and a well diffusion assays were performed. The agar spot test was performed with isolated actinomycetes[11]. Overnight cultures of the strains to be tested for production of an antimicrobial compound were spotted onto the surface of Mueller Hinton agar plates and incubated for 24 hours at 35ºC to allow the colonies to develop. An indicator organism (usually at a turbidity of 0.5 McFarland standards) is used to inoculate into 7mL of soft agar (MHB + 0.7% agar) and poured over the plate upon which the producer was grown. After incubation at 35ºC the plates were checked for zone of inhibition. Clear zone around the colonies of producer strain with a width of 0.5 mm or larger was scored positive.

2.5.2. Extraction of antimicrobial compounds

The cultures were incubated at 35ºC with 121 rpm agitation for 30 days. 10-20mL of each sample was harvested every 2 days and centrifuged for 15 minutes at 5,000 rpm for the separation of supernatant. Then the supernatant was filtered through bacterial filter (Millipore filter 0.45 µm) to get cell free samples. Antimicrobial activity of each cell free supernatant was determined using well diffusion assay, 100 to 200 µL of samples was loaded and tested to test the antagonistic activity [12].

2.5.3. Secondary screening

Secondary screening was performed using well diffusion assay[12]. Mueller Hinton Agar with a pH of 7.2 ± 0.2 medium was poured into the plates to a uniform depth of 7mm and refrigerated for solidification. Prior to use, the plates were transferred to the incubator at 37ºC for 30 minutes to clear the moisture content that develops on the agar surface. The plates were then heavily inoculated with standardized inoculums (usually at a turbidity of 0.5 McFarland standards) by means of cotton swab to ensure the confluent growth of the organism. Wells of 7 mm in diameter were cut into these agar plates and loaded with 100 -150 µL of filtered culture supernatant of selected strain samples from primary screening. The plates were incubated for 24 hours at 37ºC and were subsequently examined for the clear zones surrounding each well. Zone of inhibition was measured by the diameter of the zone in millimetres (mm) and the results were recorded. All the experimental procedures were done in triplicates.

2.6. Identification of actinomycete isolates

The actinomycete isolate selected by the above screening procedure was subjected for identification procedure. To identify the isolated actinomycetes colonies the following studies were performed.

2.6.1. Morphological characterization

Morphological characterization was performed with a magnified lens on actinomycetes strains grown for 3 days on starch casein agar plates. Several characteristic features were examined such as surface characteristics, consistency, type of margins and elevation, obverse and reverse view. Whole colony form and colour were determined with naked eye. Gram – Staining was examined under a light compound microscope. Gram – reaction of a smeared colony was done by using with crystal violet, gram's iodine, decolourisation with 95% alcohol and counter staining with saffranin.

2.6.2. Biochemical characterization

Unless otherwise indicated, all physiological and biochemical characteristics were studied using standard procedures. Experiments were performed in sterile test tubes filled with 5 ml of test media. An incubation temperature of 30ºC and test media containing equivalent salts content to half strength sea water was chosen for the following tests: Catalase activity was determined using 3% H_2O_2; presence of cytochrome oxidase was tested using standard oxidase discs. Production of H_2S gas and indole were tested using SIM broth medium. Indole production was detected with Kovac's reagent. The methyl red reaction and the Voges - Proskauer test assay were performed by using a culture grown in MR-VP medium at 37ºC for 48 hours. Production of acid from different carbohydrates was also performed[13 – 14].

2.7. Chemical characterization

2.7.1. Fourier Transform Infra Red Spectroscopy (FTIR)

NEXUS – 672 and 8400S Shimadzu MODEL Fourier Transform Infra Red Spectroscopy was used for the analysis of culture supernatants. The spectrum was taken in the mid IR region of 400 – 4000 cm^{-1}. The spectrum was recorded using ATR (Attenuated Total Reflectance) technique. The sample was directly placed in the sodium chloride crystal and the spectrum was recorded in the transmittance mode.

3. RESULTS AND DISCUSSION

Microbial natural products are a key source of both existing and new drugs. An important reason for discovering novel secondary metabolites is to circumvent the problem of resistant pathogens, which are no longer susceptible to the currently used drugs. Amongst the producers of commercially essential metabolites, bacteria have proven to be a productive source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered till date. Among these, Actinomycetes are the most economically and biotechnologically priceless prokaryotes. Actinomycetes have been recognized as the potential producers of metabolites such as antibiotics, growth promoting substances for plants and animals, immune modifiers, enzyme inhibitors and many other compounds of use to man. They have provided about two-thirds (more than 4000) of the naturally occurring antibiotics discovered, together with many of those important in medicine, such as aminoglycosides, anthracyclines, chloramphenicol, *b*-lactams, macrolides, tetracyclines etc.[15-16]. Actinomycetes have been intensively surveyed in several un- and underexplored environments, in various parts of the world in the last few decades [17].

The present work was conducted to study antimicrobial activity of actinomycetes isolated from soil samples taken from various places in and around Madurai. Five soil samples were collected from various locations in and around Madurai. 37 isolates were obtained from sample collected from in and around Madurai. 11 (BR), 7 (TC), 6(TK), 7(TP), and 6 (TPM) isolates were obtained from the soil samples of Balarengapuram, Thiagarajar College, Thiruppankundram, Teppakulam and Thirupavanam respectively (Table-2). The physico-chemical parameters for the collected soil samples were analysed in Soil Testing Laboratory, Dindigul District. The physic-chemical parameter values are tabulated (Table-3).

Table-2. Collection sites and number of strains isolated

S.No	Collection sites	Number of strains isolated
1	Balarengapuram (BR)	11
2	Thiagarajar College (TC)	7
3	Thiruppankundram (TK)	6
4	Theppakulam (TP)	7
5	Thirupavanam (TPM)	6
Total isolates		**37**

Table: 3 Physico – chemical parameters of the collected soil samples

S.No	Lab Code	Sample Code	EC ds/m	pH	Texture	Total Nitrogen kg/ha	P kg/ha	K kg/ha	OC %	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Bo (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
1.	Dgl	BR	0.27	7.2	Sandy Clay Loam	175	161	252.875	0.27	5.608	1.272	1.67	0.596	0.12	1300	300	9.8
2.	Dgl	TC	0.23	7.4	Sandy Clay Loam	177.5	107.63	265	0.30	8.192	1.234	1.83	0.678	0.19	1360	312	6.1
3.	Dgl	TK	0.26	7.1	Sandy Clay Loam	180.25	172.9	293.5	0.27	7.520	1.072	1.076	0.94	0.22	1320	348	9.8
4.	Dgl	TP	0.25	7.3	Sandy Clay Loam	183.5	114.4	277.25	0.37	7.440	1.264	1.93	1.07	0.31	1400	360	13.6
5.	Dgl	TPM	0.29	7.2	Sandy Clay Loam	181.25	121.16	257.25	0.30	7.187	1.286	1.312	0.86	0.34	1440	324	9.8

Of the 37 isolates screened so far, 30 of them showed antimicrobial activity against one or more of the test pathogens (Table-4). 26 isolates showed good antimicrobial activity during secondary screening. Of these, 13 isolates showed antimicrobial activity against the Gram-positive bacteria, mostly against *B. subtilis*, 8 against Gram-positive bacteria *Streptococcus mutans*, 16 isolates revealed good antimicrobial activity against Gram-negative organism of *E.coli*, 10 isolates exhibited good antimicrobial activity against *Vibrio parahaemolyticus*, only 8 isolates showed antagonistic activity against *Vibrio cholerae* and only 2 isolates of SA18 and SA28 showed antifungal activity against *Aspergillus niger*. The antimicrobial profiles of selected actinomycete isolates are shown in Table-5; Figure -1.

Among these bioactive isolates, SA5, SA17, SA18, SA21 and SA28 showed inhibition zone of 20 mm or above. The isolate exhibiting broad spectrum of activity are SA18 and SA28. Five actinomycetes strain of SA1, SA4, SA12, SA13 and SA14 showed antagonistic activity only against Gram-negative organism of *E.coli*. Four strains of SA3, SA10, SA24 and SA30 revealed antibacterial activity against Gram positive organism of *Streptococcus mutans*. Actinomycete samples of SA7 and SA26 exhibited antibacterial activity against one tested organism of *Vibrio parahaemolyticus* and *Bacillus subtilis* respectively. SA17 and SA21 actinomycetes samples showed broad spectrum antibacterial activity against all tested bacterial organisms but not against *Aspergillus niger*. So, these two samples did not show antifungal activity. SA9 actinomycetes supernatant showed antibacterial activity only against Gram negative organisms tested.

Table-4. Primary Screening of Actinomycetes for antimicrobial activity

Test organisms	Actinomycetes
Gram-positive bacteria	
Bacillus subtilis	2,,5,6,11,16,17,18,19,21,22,23,26,28,30
Streptococcus mutans	3,10,17,18,21,24,28,30,33
Gram-negative bacteria	
Escherichia coli	1,2,3,4,6,8,9,12,13,14,16,17,18,21,22,28
Vibrio cholerae	5,9,11,17,18,21,22,28
Vibrio parahaemolyticus	2,5,6,7,8,9,17,18,21,28
Fungal Culture	
Aspergillus niger	18,28,34

*All the actinomycetes colonies are numbered as SA1- SA37

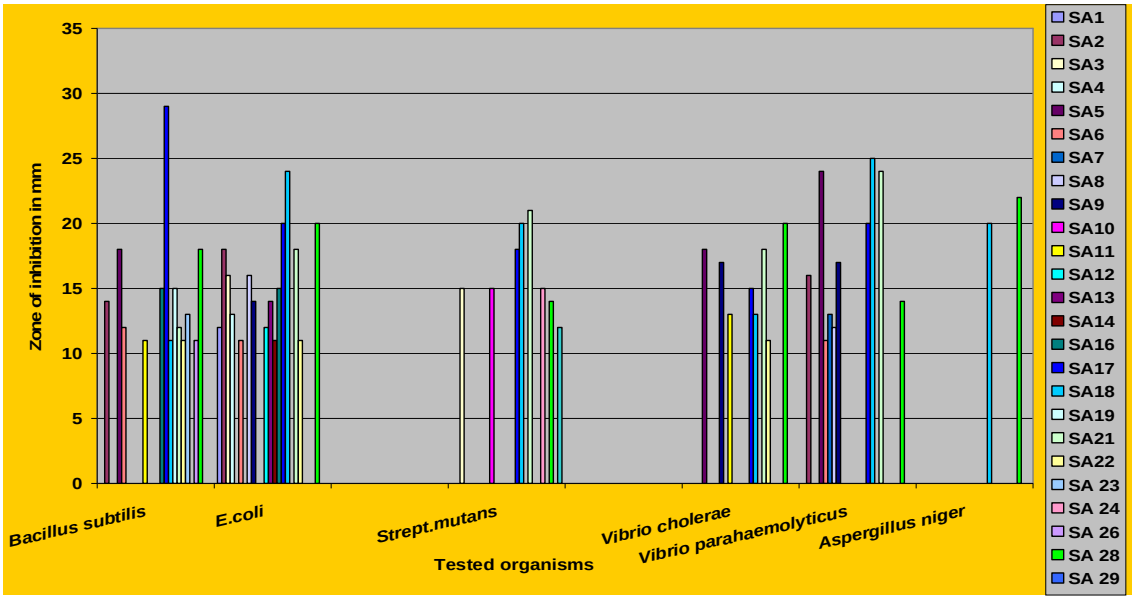
Table-5. Secondary screening for antimicrobial activities by soil actinomycetes

S. No	Target organisms	Zone of inhibition in mm																									
		SA1	SA2	SA3	SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	SA12	SA13	SA14	SA16	SA17	SA18	SA19	SA21	SA22	SA 23	SA 24	SA 26	SA 28	SA 29	SA30
1	*Bacillus subtilis*	-	14 ±0.5774	-	-	18 ±0.5774	12 ±0.5774	-	-	-	-	11 ±0.5774	-	-	-	15 ±1,0000	29 ±1.0000	11 ±0.5774	15 ±0.5774	12 ±0.5774	11 ±0.5774	13 ±0.5774	-	11 ±0.5774	18 ±1.1547	-	-
2	*Escherichia coli*	12 ±1.5275	18 ±0.5774	16 ±1.1547	13 ±0.5774	-	11 ±0.5774	-	16 ±0.5774	14 ±1.0000	-	-	12 ±1.5275	14 ±0.5774	11 ±0.5774	15 ±1.0000	20 ±1.1547	24 ±1.1547		18 ±1.1547	11 ±0.5774	-	-	-	20 ±0.5774	-	-
3	*Streptococcus mutans*	-	-	15 ±0.5774	-	-	-	-	-	-	15 ±0.5774	-	-	-	-	-	18 ±0.5774	20 ±1.1547	-	21 ±1.000	-	-	15 ±0.5774	-	14 ±0.5774	-	12 ±0.5774
4	*Vibrio cholerae*	-	-	-	-	18±0.5774	-	-	-	17 ±1.0000	-	13 ±1.0000	-	-	-	-	15 ±1.0000	13 ±0.5774	-	18 ±1.1547	11 ±0.5774	-	-	-	20 ±0.5774	-	-
5	*Vibrio parahaemolyticus*	-	16 ±1.0000	-	-	24±1.1547	11 ±0.5774	13 ±0.5774	12 ±1.0000	17 ±1.0000	-	-	-	-	-	-	20 ±1.1547	25 ±1.000		24±1.0000	-	-	-	-	14 ±1.0000	-	-
6	*Aspergillus niger*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20 ±1.1547	-	-	-	-	-	-	22 ±1.0000	-	-

- Indicates negative; SA- Soil Actinomycetes

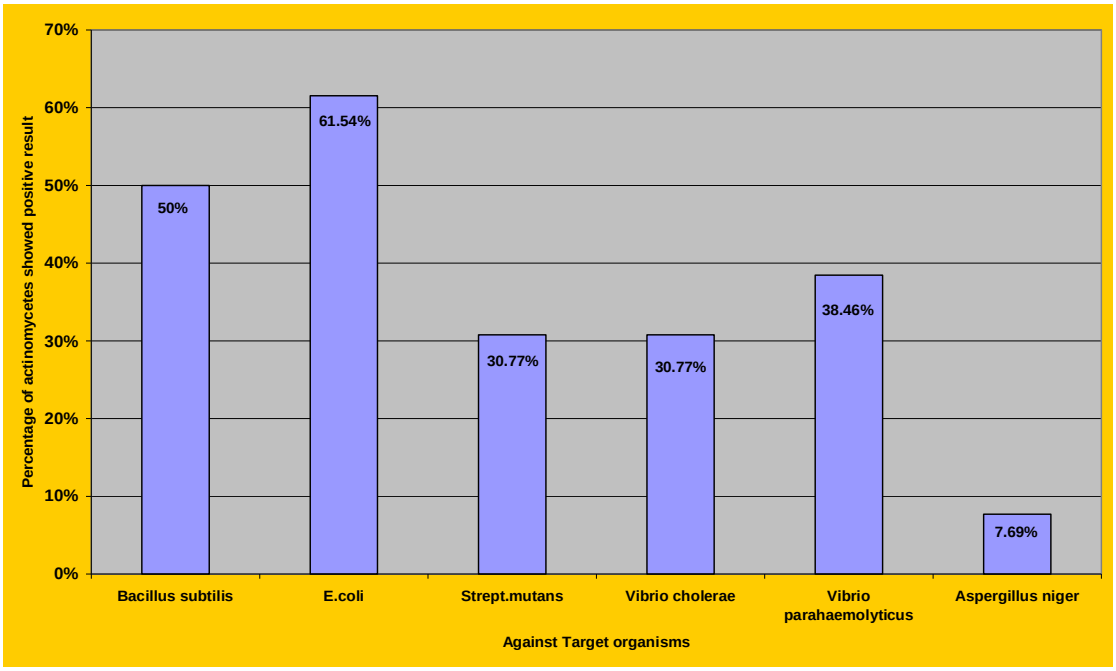
Note: All experiments were done in triplicates

Figure-1. Secondary screening for antimicrobial activities by soil actinomycetes



Five actinomycetes supernatants of SA2, SA5, SA6, SA9 and SA22 showed 50% antibacterial activity against three tested organisms. 50% (13) of the tested actinomycetes samples showed positive result against *Bacillus subtilis*, 61.54%(16) of the tested actinomycetes samples revealed positive result against *E.coli*, 30.77% (8) against *Streptococcus mutans* and *Vibrio cholerae* , *38.46%* (10) of the actinomycetes supernatants showed antibacterial activity against *Vibrio parahaemolyticus* and only 7.69% (2)actinomycetes exhibited antifungal activity against *Aspergillus niger* (Figure-2).

Figure-2. Percentage of actinomycetes supernatants showed positive antagonistic activity



The study revealed that SA28 is a promising strain for antibiotic production not only by showing broad spectrum activity, it also revealed more than 14 mm zone of inhibitionin diameter. According to antibacterial activity and spectrum broadness, SA28 was selected and identified. Morphological characterization of the SA28 potential isolate revealed as dry powdery white colonies with long chains arranged in bead like structures. Biochemical characterizations of the SA28 isolate was showed in Table-6. The overall biochemical characteristics were matched to the genus of *Streptomyces sp.* (SA28).The small percentage of active strains might not specify that the source is not a good one, but rather that our method of screening was such that all isolates of actinomycetes could not grow well at the same incubation time. Moreover, there are so many factors which affect actinomycetes growth and antimicrobial compounds, including the chemical and biological environment. Hence, different specific antimicrobial-producing strains of actinomycetes need different kinds of media for producing substances and optimization is also required.

The culture supernatant of SA28 antibiotic potent sample was active against a number of test organisms like *A. niger, B. subtilis, E.coli, Vibrio cholerae, Vibrio parahaemolyticus* and *Streptococcus mutans.* A broad spectrum of antifungal and antibacterial activity was observed in 13.7% of the strains and antifungical activity only in 7.69%. The broad spectrum of activity shown in these cases is possibly due to the production of different compounds. Antibiotics belonging to both families are produced by the same biosynthetic pathway. These results confirm that the actinomycetes are able to produce a wide variety of antibiotics with antifungal activity [18-19].

In general, the active isolates showed a wide spectrum of activity against bacteria and fungi. Present work data may be skewed by the fact that the Gram-positive strains were highly resistant to many antibiotics and were consistent with the known susceptibility differences among similar target organisms [20]. Although most of the actinomycetes isolates could inhibit only Gram-positive bacteria, some of them were rare actinomycetes from which novel antimicrobial substances might be expected to be found. However, there were 4 strains in the strain SA17, SA18, SA21 and SA28, which produced antimicrobial substances inhibiting both Gram-positive and Gram-negative bacteria are in agreement with the high percent of fresh isolates reported to be producers of these compounds [21].

TABLE: 6 Identification of selected actinomycetes isolate (SA28) based on morphological & biochemical tests

Character	SA28 (*Streptomyces sp.)*
Colony	Dry Powdery, white
Spores	Long Chains with bead like structures
Gram Staining	+
Catalase Test	+
H_2S Production	+
Glucose Utilization	+
Citrate Utilization	+
Lactose Utilization	+
Starch Hydrolysis	+
Gelatin Hydrolysis	-
Casein Hydrolysis	+
Reduction of Nitrate	-
Urea Hydrolysis	-
Growth at 27°C	+++,good
at 35°C	+++, good
at 40°C	++ moderate
at 45°C	+
at 50°C	-
Nacl 1%	++ moderate
3%	-
5%	-
7%	-

Note : '+' - Indicate Positive Result

'-' - Indicate Negative Result

It is also found a high percentage of activities against Gram positive cocci and bacilli and a lower number of activities against *Mycobacterium vaccae*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. Such differences in susceptibility reflect our previous experiences in the screening for antimicrobial products [22]. These data clearly show that soils from the lands would be one of the valuable resources of novel antibiotic compounds, especially from rare actinomycetes apart from the sediments or in the oceans. Preliminary tests for antimicrobial activity of the isolated bacterial strains clearly demonstrated the ability of many genera to produce antimicrobial compounds. In this work, we have shown that a total of 26 different actino*mycetes* isolates associated with soil have the ability to produce antimicrobial compounds against microorganisms,

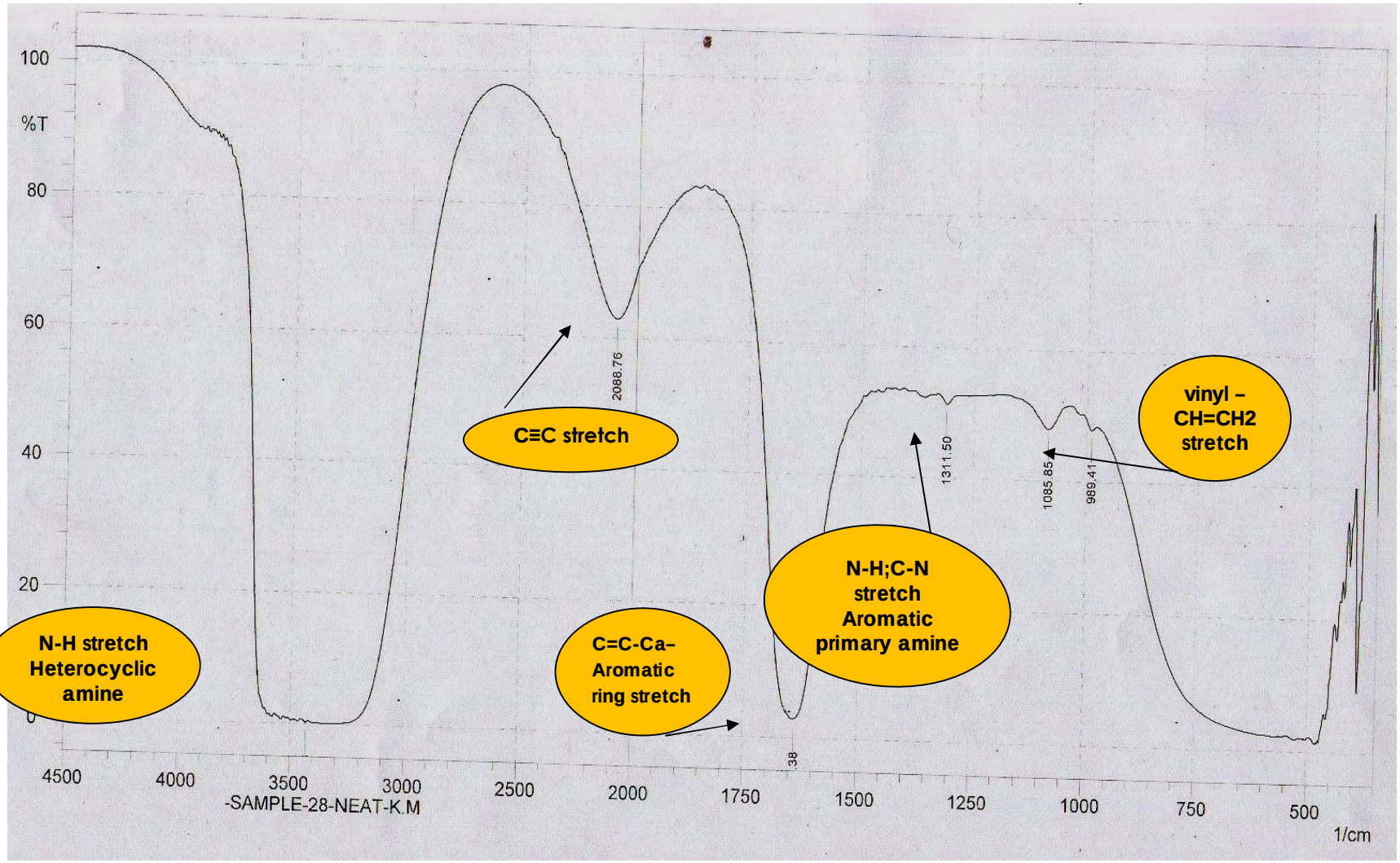
especially Gram positive and Gram negative bacteria. Further investigations are needed in order to further determine the active metabolites of these isolates.

Figure-3 showed the FTIR spectrum of the antbiotic compound from the strain SA28. The FTIR spectrum of SA28 fermentation broth exhibited absorption at 3431.13 cm^{-1}, which indicate hetrocyclic amine N-H stretch, and the absorption at 2088.76 cm^{-1} indicating a C≡C stretch, alkyne group and double bond of polyenic compound. The FTIR spectrum of extracts of SA28 fermentation broth exhibited absorption at 1639.38 and 1311.5 cm^{-1}, which indicate N-H; C-N stretch, aromatic primary amine and 1085.85 $cm^{-1}$and 989.41 cm^{-1} vinyl –CH=CH2 stretch. These bands may be assigned to the amide I and II bands of proteins, respectively. With the IR spectral data, the antibiotic probably is a straight chain polyhydroxy polyether compound with a single double bond, indicating a non-polyene (lacking conjugated double bonds) antifungal antibiotic. The data also indicate that the antibiotic is a non-polyene, antifungal antibiotic [23].

CONCLUSION

Actinomycetes from soils rich in minerals are promising producers of antibacterial and antifungal compounds. The high number of polyene macrolide producer strains demonstrates the effective and powerful antifungal activity of the compounds belonging to this family and their important protective effect to the actinomycetes' life. The need for new, safe and more effective antibacterials and antifungals are a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immune-compromised host. On this background, the biotechnological potential of SA28 in terms of production of antibiotic inhibiting pathogenic bacteria, and mold is noteworthy. In summary, the potential utility of these actinomycetes in screening programmes for bioactive natural products is confirmed. This ability is not restricted to one family or genus within actinomycetes, but rather, all of them offer opportunities to obtain bioactive compounds.

Figure-3. IR spectra of the compounds from selected actinomycetes SA28 supernatant sample



REFERENCES

1. Arifuzzaman M, Khatun MR, Rahman H. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity, Afr. J. Biotechnol. 2010; 9 (29): 4615-4619).
2. Patel JD, Parmar M, Patel P, Rohit P, Ramesh T, Ansari P, Bhattacharya B, Vyas D, Kumar V, Sahay NS, Singh PK.) Dynamism of Antimicrobial Activity of Actinomycetes- A Case Study from Undisturbed Microbial Niche. Adv. Microbiol. 2014; 4:324-334.
3. Velayudham S, Murugan K. Diversity and Antibacterial Screening of Actinomycetes from Javadi Hill Forest Soil, Tamilnadu, India. J. Microbiol. Res. 2012; 2: 41-46.
4. Bakheit SEA, Saadabi AM. Antagonistic affects of Actinomycetes isolated from Tuti island farms (Central Sudan) against *Fusarium oxysporum f.sp.vasinfectum* a phytopathogenic fungus. Int. J. Adv. Res. 2014; 2:114-120.
5. Rakshanya UJ, Shenpagam HN, Devi KD. Antagonistic activity of actinomycetes isolates against human Pathogen. J. Microbiol. Biotechnol. Res. *V*2011; 1: 74-79..
6. Luzhetskyy A, Pelzer S, Bechthold A. The future of natural products as a source of new antibiotics. Curr. Opin. Invest. Dru. 2007; 8: 608-613.
7. Elamvazhuthi P, Subramanian M. Antagonistic Activity of Actinomycetes from Jeypore Paddy Soils against Selective Phytopathogenic Fungi. J. Mod. Biotechnol. 2013; 2: 66–72.
8. Newman DJ, Cragg MG. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 2007; 70: 461-477.
9. Takahashi YO, Omura S. Isolation of new actinomycete strains for the screening of new bioactive compounds. J. Gen. Applied. Microbiol. 2003; 49: 141-154.
10. Cappuccino JG, Sherman N. Microbiology – A laboratory manual (Harlow: Benjamin). 1999; 263-264.
11. Lemos ML, Toranzo AE, Barja JL. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microbiat. Ecol. 1985; 11:149-163.
12. Schillinger U, Lucke FK. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 1989; 55: 1901-1906.
13. Bowman JP, McCammon SA, Brown JL, Nichols PD, Mc Meekin TA. *Psychroserpensburtonensis* gen. nov., sp..nov., and *Gelidibacteralgens*gen. nov., sp. nov., psychrophilic bacteria isolated from antarctic lacustrine and sea ice habitats. Int. J. Syst. Bacteriol. 1997; 47(3): 670-677.
14. Cappuccino JG, Sherman N. Microbiology – A laboratory manual (Harlow: Benjamin). 1999; 263-264.

15. Solanki R, Khanna M, Lal R. Bioactive compounds from marine actinomycetes. Ind. J. Microbiol. 2008; 48:410–43.
16. Gupte M, Kulkarni P, Ganguli BN. Antifungal Antibiotics. Appl. Microbiol. Biotechnol. 2002; 58: 46–57.
17. Ningthoujam DS, Sanasam S, Nimaichand S, Sanjenbam P. Screening and optimization studies of native anticandidal actinomycetes from Manipur (Indo-Burma Biodiversity Hotspot), India. Proceeding of the 15th International Symposium on the Biology of Actinomycetes Aug. Shanghai Jiaotong University, Shanghai. http://www.isba15.org/pdf/ISBA15_Handbook_09. 2009; 20 -25.
18. Asolkar RN, Maskey RP, Helmke E, Laatsch H. Chalcomycin B, a new macrolide antibiotic from the marine isolate Streptomyces sp. B7064. J Antibiot. 2002; 55: 893–898.
19. Usuki Y, Goto K, Kiso T. UK-2A, B, C and D, novel antifungal antibiotics from *Streptomyces sp*. 517–02 VI (2). Structure-activity relationships of of UK-2A. J. Antibiot**.** 2002; 55: 607–610.
20. Suay I, Arenal F, Asensio FJ, Basilio A, Cabello A, Diez MT, Garcia JB, Gonza´lez del Val A. Screening of basidiomycetes for antimicrobial activities. Antonie van Leeuwenhoek. 2000; 78: 129–139.
21. Etienne G. A screening method to identify antibiotics of the aminoglycoside family. J. Antibiot. 1991; 44 : 1357–1366.
22. Gonza´lez del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, Vicente F, Portillo E. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Inter. Microbiol. 2001; 4: 35–40.
23. Augustine SK, Bhavsar SP, Kapadnis BP. A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23. J. Biosci. 2005; 30(2): 201–21.