African Journal of Microbiology Research Vol. 6(10), pp. 2265-2271, 16 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.856
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Distribution and bioactive potential of soil actinomycetes from different ecological habitats

M. George[1], A. Anjumol[1], G. George[1] and A. A. Mohamed Hatha[2]*

[1]School of Environmental Sciences, Mahatma Gandhi University, Kottayam- 686560, India.
[2]Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Cochin – 682 016, India.

Accepted 18 November, 2011

Emergence of drug resistance among pathogenic bacteria to currently available antibiotics has intensified the search for novel bioactive compounds from unexplored habitats. In the present study actinomycetes were isolated from two relatively unexplored and widely differing habitats such as mountain and wetlands and their ability to produce antibacterial substances were analyzed. Pure cultures of actinomycetes were identified by morphological and biochemical tests. Various genera of actinomycetes encountered included *Nocardia, Pseudonocardia, Streptomyces, Nocardiopsis, Streptosporangium, Micromonospora, Rhodococcus, Actinosynnema, Nocardiodes, Kitasatosporia, Gordona, Intrasporangium* and *Streptoalloteichus.* The frequency of occurrence of each genus was found to vary with sample. About 47% of wetland isolates and 33% of mountain isolates were identified as various species of *Nocardia.* The isolated strains differed among themselves in their ability to decompose proteins and amino acids and also in enzyme production potential. Antibiotic activities of these actinomycetes were evaluated against 12 test pathogenic bacteria by well diffusion method using agar wells in glycerol-yeast extract agar. About 95% of actinomycete isolates from wetland ecosystem and 75% of highland isolates suppressed in different degrees the growth of test pathogens. Relatively high antibacterial activity among these isolates underlined their potential as a source of novel antibiotics.

Key words: Actinomycetes, antibacterial activity, wetland, mountain ecosystem.

INTRODUCTION

Actinomycetes are attractive, bodacious and charming filamentous Gram-positive bacteria with true aerial hyphae, belonging to the phylum *Actinobateria* (order actinomycetales) that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria (Olano et al., 2009). Bergey's manual divides actinomycetes in eight diverse groups and comprise 63 genera. The majority of actinomycetes are free living, spore forming, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomycetes have been recognized primarily on their morphological criteria.

*Corresponding author. E-mail: mohamedhatha@gmail.com. Tel: +919446866050.

Although actinomycete populations have been identified as one of the major group of soil organisms, they may vary with the soil type. They are important in soil biodegradation and humus formation by the recycling of nutrients associated with recalcitrant polymers such as keratin, lignocelluloses and chitin (Stach and Bull, 2005), and produce several volatile substances like geosmin responsible for the characteristic "wet earth odor" (Wilkins, 1996). They also exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes (Schrempf, 2001), antibiotics and other bioactive molecules, which are of considerable importance in medicine, industry and agriculture.

The secondary metabolites obtained from the class actinobacteria are of special interest because of their diverse biological activities such as antibacterial, antifungal, antioxidant, antitumor and antiviral. Species of

Streptomyces, account for more than 70% of the total antibiotic production and *Micromonospora* is the runner up with less than one-tenth as many as *Streptomyces* (Lam, 2006). However, the survey of *Streptomyces* and other common terrestrial actinomycetes is nearly exhausted. This and the rise of multiple drug resistant pathogens imply the need to search for novel antimicrobials in actinomycetes from under explored ecosystems (Wise, 2008; Demain and Sanchez, 2009). The approaches considered in these research programmes include the isolation of new antibiotics from actinomycetes other than the genus *Streptomyces* and the exploration of new and particular ecological systems. Species richness has been linked to ecosystem type and soil pH, indicating that certain parameters, such as soil chemistry and ecological context, can affect the distribution of bacteria in the environment (Wawrik et al., 2007).

The actinomycetes exist in various habits in nature. Actinomycetes from exotic locations such as Antarctic soils were shown to have good antibacterial activity against Gram-positive and Gram-negative bacteria (Moncheva et al., 2002). The unusual antibiotic profile of these isolates underlined their potential as a source of novel antibiotics. Kharat et al. (2009) isolated actinomycetes from marine environment. They found that, out of the 24 actinomycete isolates subjected to secondary screening, 12 isolates were active against *Bacillus subtilis,* 13 against *Staphylococcus aureus*, 7 against *Escherichia coli*, 3 against *Proteus vulgaris* and 4 against *Salmonella typhi*.

In the present study two widely differing and relatively unexplored ecological habitats such as mountain ecosystem and wetland ecosystem were explored to determine the distribution of actinomycetes and potential of these strains in the development of new and novel antibiotics.

MATERIALS AND METHODS

Study area

Soil samples were collected from various mountain plantation sites in Kerala, which spreads along Manimala River basin (Mundakkayam – Peruvanthanam region) (75-450 m above MSL, 9°28'-9°32' N, 76°54'-76°57' E, with an average temperature of 27°C) and Pamba river basin (Ranni Forest division) (440 m above MSL, 9°25' N, 76° 99' E, with an average temperature of about 25°C). Diverse habitats were selected in this mountain area such as Rubber plantations (R), Teak plantations (T), disturbed forest region of Peruvanthanam (F_1), undisturbed forest of Pamba (F_2) and barren land (BL), which is devoid of plantations. Soil samples were also collected from Kumarakom region (9°37'52"-9°37'40" N, 76°25'08"-76°25'45" E, with an average temperature of 29°C) of Vembanadu - Kol wetland of Kerala, which spreads along the southwest coast of India. Three different habitats in the wetland area were selected for the study such as: mangrove forest (M), bird sanctuary (BS) and an area affected by anthropogenic activity (A). Figure 1 showing various sampling sites in highland and wetland ecosystems.

Collection of soil samples

Soil samples were collected during pre-monsoon and monsoon seasons of the year 2008. Composite soil samples (2-5 cm depth) were taken from each area in sterile plastic bags and transported to the laboratory under ambient conditions and was air dried at room temperature. Later the soil samples were subjected to dry-heat treatment at 50°C for 1hr to depress the number of other bacteria and for preferential isolation of actinomycetes.

Isolation and maintenance of isolates

Isolation and quantification of actinomycetes were done by applying standard serial dilution plate technique. Different aqueous dilutions (10^{-1} to 10^{-3}) of samples were prepared and spread plated on Kusters Agar (Glycerol- 10 g, Casein- 0.3 g, KNO_3- 2 g, K_2HPO_4-2 g, $MgSO_4$- 0.05 g, $CaCO_3$- 0.02 g, $FeSO_4$- 0.01 g, Agar- 18 g, Distillled water- 1 L, pH 7± 0.1) (Lakshmanaperumalsamy et al., 1986). After incubation of the plates at room temperature for 2-3 weeks typical actinomycete colonies were selected on morphological basis (Shirling and Gottlieb, 1966) and purified on Kusters Agar plate by restreaking and incubating at room temperature.

Identification of isolated strains

Pure cultures of actinomycete strains were characterized by morphological tests as per Bergey's Manual of Determinative Bacteriology (Holt et al., 2000) and by physiological tests (Gordon, 1967).

Morphological characterization

Gross morphology was observed after 2-3 days of growth on Kusters agar plates by cover slip culture technique. The mycelium structure, arrangement of conidiospore and arthrospore on the mycelium was observed through the oil immersion (1000×). The observed structure was compared with the Manual and the organism was identified.

Physiological characterization

These tests were performed as described by Gordon (1966, 1967). Physiological tests included decomposition of Casein, Tyrosine, Xanthine, Hypoxanthine, Urea and Esculin, evaluation of lysozyme resistance and the ability to produce acid from various carbohydrates such as arabinose, fructose, galactose, inositol, lactose, mannitol, mannose, rhamnose, sorbitol and xylose.

Evaluation of antibacterial activity

Antibacterial activity of the strains was determined by well diffusion method using agar wells in Glycerol- Yeast Extract Agar (Waksman, 1961). Lawn cultures of the test microorganisms (*Salmonella bovis, Salmonella typhimurium, Salmonella senftenberg, Salmonella typhi*, *Salmonella mgulani, Salmonella enteritidis, Salmonella welteverden, Salmonella bareilly, Vibrio cholerae, Bacillus subtilis*, enterotoxigenic *Escherichia coli* and enteropathogenic *Escherichia coli* which were isolated from environmental samples) were prepared by swabbing young culture over the agar medium and 3mm diameter wells were punched. About 30 µl of four day old broth culture suspension of actinomycetes were pipetted into the wells and plates were incubated for 24 h at room temperature. Zone of inhibition around the wells were recorded in mm.

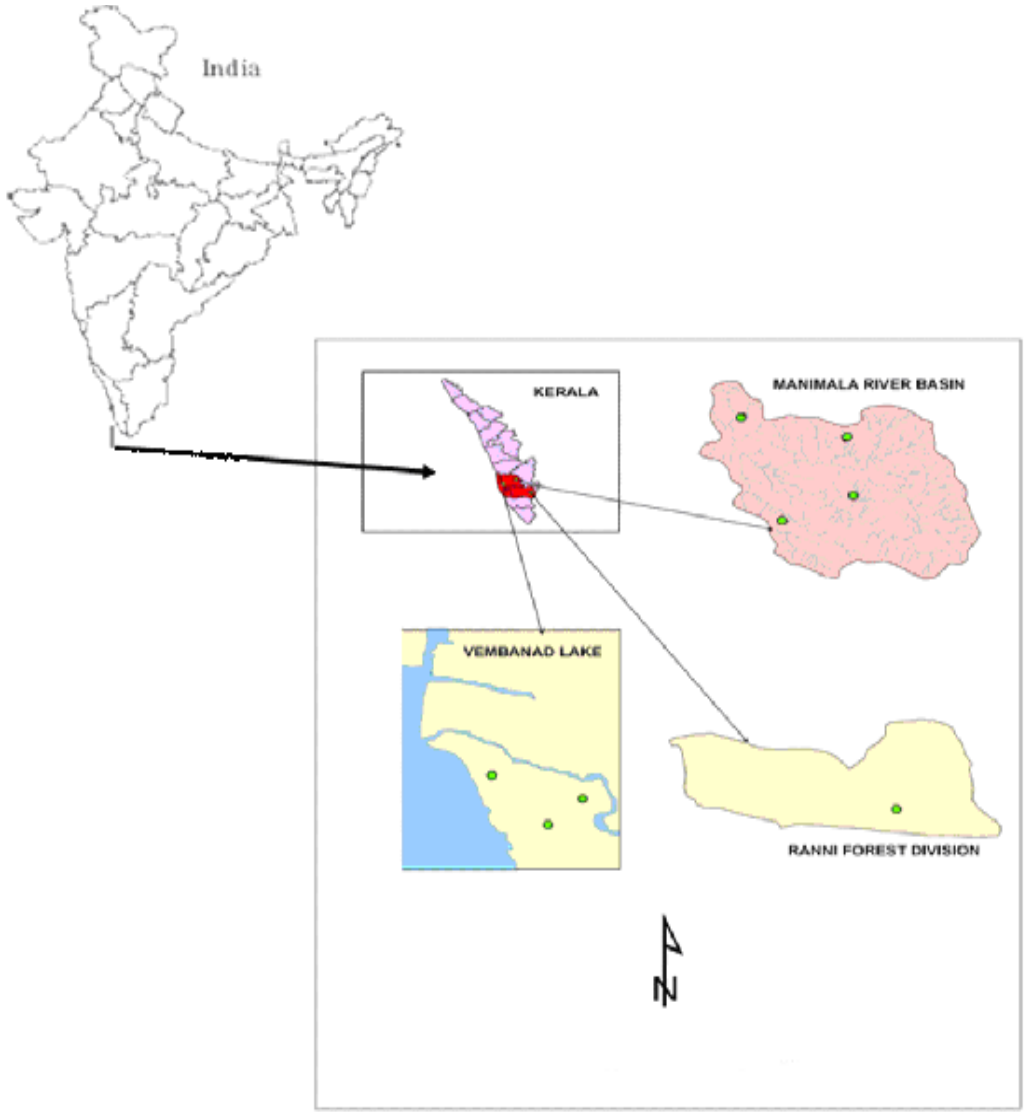

Figure 1. Map showing various sampling sites in the highland and wetland ecosystems.

Soil analysis

The soil samples collected for the isolation of actinomycetes were analysed for various physico-chemical parameters. pH of soil samples determined potentiometrically using pH meter (Systronics-model 361). Total Nitrogen in the soil samples was determined by microkjeldahl method, the available phosphorus was estimated by Bray and Kurtz method and organic carbon content was determined by Walkey and Black method (Maiti, 2003). The available potassium in the soil samples were extracted using neutral ammonium acetate as extractant and determined by using flame photometer (Systronics–model 128) (Maiti, 2003).

Statistical analysis

Average load of actinomycetes between the study areas were compared by using Students't' test. Pearson Product Moment Correlation Coefficient, usually known as Pearson's correlation coefficient (PCC) was used to test the correlation between physico-chemical properties of the soil and actinomycete load, by using statistical package SPSS version 11 (SPSS for windows 2001).

RESULTS

Microbial and pedological analysis of soil samples

Good numbers of isolates were obtained from different soil samples collected. A total of 74 actinomycete cultures were isolated from all sites. Pedological characteristics and load of actinomycetes in soils from different sampling sites is given in Table 1. The soil pH of the various sampling sites in wetland and highland were acidic in nature. While N_2 content of the highland soil was high, available phosphorous of wetland soil was higher than that of highland soils. Actinomycete population of highland region ranged from 41×10^2 to 12×10^2 cfu/g,

Table 1. Mean paedological characteristics and load of actinomycetes in different ecosystems.

Source of soil	Temp. (°C)	pH	N_2 (%)*	P (ppm)**	K (ppm)**	Organic carbon (%)	Actinomycetes (cfu/g)
Highland region							
Rubber Plantation	28	5.23	2.52	0.102	31.1	2.36	93.66×10^2
Teak plantation	28	5.80	2.8	0.052	29.13	3.04	79×10^2
Forest-1	26	5.06	3.26	0.036	15	5.88	68.66×10^2
Forest -2	25	5.47	1.86	0.053	23	3.41	73×10^2
Barren land	28	5.14	2.24	0.029	26.2	3.32	53.66×10^2
Wetland region							
Mangrove	29	4.83	1.91	0.3	22.4	1.99	460×10^2
Bird sanctuary	29	4.85	0.99	0.41	14.3	2.04	273.3×10^2
Area affected by anthropogenic activity	30	5.74	0.76	0.45	16.63	1.90	150×10^2

*= Total Nitrogen, ** = Available phosphorus and potassium.

while that of wetland region ranged from 90×10^2 to 750×10^2 cfu/gm. Analysis of the data by Students't' test reveals that there is significant difference ($t(22) = 4.026$, $p< 0.05$) in the average load of actinomycetes between the study areas. Mean load of actinomycetes in wetlandsoil (mean=294.44×10^2, Standard deviation=15607) was higher than that of highland soil (mean=73.6×10^2, Standard deviation=1461). In the present investigation it was found that there was correlation co-efficient between physico-chemical properties of the soil and total actinomycete population. Positive correlation was observed between soil organic carbon content and load of actinomycetes in highland region ($r= 0.4$) and wetland ($r= 0.1$). But in the wetland there is a significant positive correlation between total nitrogen and actinomycete load ($r =0.867$, $p< 0.01$).

During the study period 22 actinomycete strains were recovered from the three wetland soil sampling sites and 52 strains from the five sites in highland soil. Isolates from the mountain plantation sites were identified as *Streptomyces, Nocardia, Pseudonocardia, Nocardiopsis, Micromonospora, Rhodococcus* and *Streptosporangium*. While 11 genera were identified in the wetland soil samples. They are: *Nocardia, Pseudonocardia, Streptomyces, Micromonospora, Rhodococcus, Actinosynnema, Nocardiodes, Kitasatosporia, Gordona, Intrasporangium* and *Streptoalloteichus.* The percentage frequency of occurrence and distribution of actinomycetes in different ecosystems is given in Table 4. Nocardioform actinomycetes were present in all the sampling sites. They have well-formed, branched filaments with aerial mycelium which sometimes visible grossly. These isolates have fragmented hypha (both in substrate and aerial) which is the morphological feature of *Nocardia*.

Though the number of actinomycete isolates from wetland soils was low more diverse forms encountered among them. There was an equal distribution (5%) of the genera such as: *Actinosynnema, Gordona, Kitasatosporia, Intrasporangium* and *Streptoalloteichus* in the wetland ecosystem.

Physiological properties of the Isolates of actinomycetes

Results of the physiological capabilities of the actinomycetes isolates are given in Table 2. The utilization of various carbohydrates (such as lactose, galactose, xylose, inositol, sorbitol, mannitol, mannose, rhamnose and arabinose) by the isolated actinomycetes suggests a wide pattern of carbon source assimilation. The most favoured carbon source for actinomycetes from both of the ecosystems was found to be xylose while least preferred one was found to be inositol. More than 90% of the isolates from mountain ecosystems and 77% of wetland isolates utilized xylose.

Antibacterial activity of the actinomycete isolates against pathogenic bacteria

The antibacterial activity of actinomycetes to test pathogens is presented in Table 3. About 95% of the isolates from wetland ecosystem and 75% of mountain plantation isolates suppressed in different degrees the test organisms. Nearly 43% of isolates from various highland soils showed antagonism towards *Salmonella bovis*, but it is only 36% in the case of wetland isolates. Most of the wetland isolates showed antagonism towards *B. subtilis* (41%). But only 30% of isolates from mountain area showed antibiotic potential against *B. subtilis.* Isolates from wetland soils showed excellent activity against *S. bareilly.* They were identified as *Actinodassonvillei* and *Streptoalloteichus*. The least antagonistic activity was found among the isolates from Rubber plantation.

Table 2. Physiological and biochemical characteristics of actinomycete isolates from various ecosystems.

Substrates	Percentage of isolates capable of utilizing various substrates							
	Rubber plantation	Teak plantation	Forest 1	Forest 2	Barren land	Mangrove	Bird sanctuary	Area affected by anthropogenic activity
Casein	56	55	56	44	83	63.6	50	20
Xanthine	33	27	22	56	16	36.3	16.6	40
Hypoxanthine	44	55	89	33	50	72.7	50	40
Tyrosine	44	45	44	44	50	45.4	16.6	40
Esculin	33	73	44	78	100	54.5	66.6	40
Urea	11	55	67	56	83	45.5	50	20
Lysozyme	44	73	22	67	83	81.8	66.6	100
Lactose	89	73	56	89	100	72.7	50	60
Inositol	67	64	56	56	33	54.5	50	60
Galactose	89	45	67	89	83	72.7	66.6	80
Mannitol	89	73	56	100	100	72.7	83	60
Mannose	100	64	67	67	100	82	83	40
Rhamnose	89	100	22	89	67	36.3	83	80
Arabinose	89	64	67	100	100	72.7	66.6	80
Sorbitol	89	73	67	100	67	72.7	66.6	80
Xylose	89	73	78	100	100	72.7	83	80

DISCUSSION

The actinomycetes have wide distribution and they show variation in their population dynamics. We have analysed various soil parameters such as pH, temperature, total nitrogen, organic carbon, available phosphorous and potassium, and actinomycetes load. It was found that there was positive correlation between organic carbon content of soil samples and actinomycete load in highland and wetland soil samples. In the wetland soil samples there is significant positive correlation between total nitrogen and actinomycete load. Saadoun and Al-Momoni (1996) and Mansour (2003) reported similar findings. Mansour (2003) reported that higher count of actinomycetes was highly correlated ($r = 0.892$, $p<0.01$) with per cent of organic matter of the soil studies. Ghanem et al. (2000) reported that the variation in temperature, pH and dissolved phosphate showed non-significant values, but variation in total nitrogen and organic matter was significant in the population of actinomycetes isolated from marine sediment samples in Alexandria. However there are conflictory reports. Jensen et al. (1994) reported that there was no correlation between the percentage of organic content of marine sediment and actinomycetes population.

All the soil samples of this present study had an acidic pH ranging from 4.28–6.76. Though soil actinomycetes for the most part show their optimum growth in neutral and slightly alkaline conditions, existence of large diversity of acidophilic actinomycetes that differed morphologically and physiologically from neutrophilic species have been reported by Khan and Williams (1975) and Williams et al. (1977). The isolated strains were identified based on their colony morphology and microscopic morphology. The mycelium structure, colour, and arrangement of spore were observed by cover slip technique and they were identified by using the Manual along with the help of biochemical characters. Similar method has been followed by Berd (1973) and Mansour (2003).

About 47% of wetland isolates and 33% of mountain isolates were identified as various species of *Nocardia.* The dominance of *Nocardia* isolates in these ecosystems may be because of their ability to adapt to particular microhabitats which satisfy their ecological requirements. Some among them was having totally sporulated aerial mycelium, which is found in genus *Nocardiopsis*. Species of *Nocardiopsis* are xerotolerant and are able to utilize a wide range of substrates, which suggests good survival in different environments (Holt et al., 2000). It is also reported that like other actinomycetes *Nocardiopsis* spp. are capable of producing metabolites with biological activities (Tsujibo et al., 1990; Kroppenstedt, 1992).

Actinomycetes are producers of potent metabolic compounds having agricultural and medicinal importance (Shiomi and Takeuchi, 1990; Kharat et al., 2009; Kavitha et al., 2010). Our work highlights the broad host range exhibited by the metabolites of the actinomycetes isolated from various ecosystems. About 95% of the isolates from wetland ecosystem and 75% from mountain plantation isolates have an inhibitory effect on test organisms used. This prevalence of antimicrobial activity is higher than those available in the data from surveys of sediment derived actinomycetes (Pisano et al., 1985;

Table 3. Antimicrobial activity of actinomycete isolates from different ecosystems.

Sampling sites	Percentage activity of actinomycetes against pathogenic strains											
	S. b	Ec.78	S. t	S. s	*S. typhi*	S. m	V. c	Ec.12	S. e	B. s	S. w	*S. bareilly*
Rubber Plantation	33	0	55	22	0	0	0	0	0	22	22	22
Teak Plantation	45	36	27	27	27	18	9	18	27	18	18	27
Barren land	33	33	33	16	16	66	16	50	33	50	50	16
Forest 1	66	11	11	0	11	0	22	11	22	11	22	44
Forest 2	33	33	22	33	0	22	0	11	11	55	33	22
Mangrove	27	0	9	9	36	9	36	36	0	46	0	9
Bird sanctuary	33	0	17	0	0	33	0	33	17	33	17	17
Area affected by anthropogenic activity	60	20	40	0	0	20	0	20	20	40	40	20

S.b = *Salmonella bovis*, E.c = *Escherichia coli,* S.t = *Salmonella typhimurium*, S.s = *Salmonella senftenberg,* S.m = *Salmonella mgulani,* V.c = *Vibrio cholerae,* S.e = *Salmonella entritidis*, B.s = *Bacillus subtilis*, S.w = *Salmonella weltsverden*.

Table 4. Taxonomic diversity of actinomycetes in different ecosystems.

Genera of actinomycetes	Percentage distribution in different ecosystems							
	Highland					Wetland		
	R	T	F1	F2	BL	M	BS	A
Nocardia	4	8	4	2	2	8	8	2
Micromonospora	2	0	2	0	4	0	2	0
Streptomyces	6	2	0	2	2	4	0	0
Rhodococcus	0	2	2	2	0	0	0	2
Streptosporangium	0	2	0	0	0	0	0	0
Pseudonocardia	0	0	0	0	2	2	0	0
Actinosynnema	0	0	0	0	0	2	0	0
Gordona	0	0	0	0	0	2	0	0
Kitasatosporia	0	0	0	0	0	2	0	0
Actinodassonvillei	0	0	0	0	0	0	2	0
Intrasporangium	0	0	0	0	0	0	0	2
Streptoalloteichus	0	0	0	0	0	0	0	2

R = Rubber plantation, T = Teak plantation, F1 = disturbed forest region of Peruvanthanam, F2 = undisturbed forest of Pamba, BL = barren land, M = Mangrove, BS = Bird Sanctury, A = Wetland soil affected by anthropogenic activity.

Ellaiah et al., 1996). Both these researchers reported antibiotic activity (< 20%) among their actinomycete isolates.

The results also revealed that the actinomycetes strains had both broad spectrum and narrow spectrum of activity against the pathogenic isolates. Strains from wetland ecosystem have broad spectrum activity against pathogens used in this study. The isolates, *Streptoalloteichus* (H5) and *Actinodassonvillei* (B2) from wetland soil samples showed the maximum inhibition zone of 35 mm against *S. bareilly.* The isolate *Streptoalloteichus* showed antagonism towards *S. mgulani, S. bareilly*, enteropathogenic *E. coli*, *B. subtilis* and *S. weltevreden*. This strain (H5) was able to decompose urea, esculin, casein and hypoxanthine and is able to resist lysozyme (0.001 %). They were able to utilize lactose, galactose, xylose, arabinose, rhamnose and sorbitol. But, *Actinodassonvillei* showed antibiotic potential against 8 out of 12 test pathogenic strains used in this study, such as: *B. subtilis*, *S. enteritidis*, *S. mgulani*, enteropathogenic *E. coli*, *S. weltevreden*, *S. bareilly*, *S. mgulani* and *S. bovis*. This isolate (B2) was able to decompose urea, esculin, casein, xanthine, hypoxanthine and sodium hippurate. But are unable to decompose tyrosine or to utilize any of the carbohydrates used in this study.

This study concludes that the search for novel metabolites especially from actinomycetes requires a large number of isolates (over thousands) in order to discover a novel compound of pharmaceutical interest. The search will be more promising if diverse actinomycetes are sampled and screened (Oskay, 2004).

Results of the present study support and highlight the need to search diverse and unexplored habitats for

actinomycete isolates with novel antibacterial activity. Such an endeavor will undoubtedly lead to discoveries and new uses of secondary metabolites in other therapeutic areas such as cancer and immunosuppression, two areas where natural products from actinomycetes already have made substantial contributions.

REFERENCES

Berd D (1973). Laboratory identification of clinically important aerobic actinomycetes. Appl. Microbiol., 25: 665-681.

Demain AL, Sanchez S (2009). Microbial drug discovery: 80 years of progress. J. Antibiot., 62: 5-16.

Ellaiah P, Kalyan D, Rao VS, Rao BV (1996). Isolation and characterisation of bioactive actinomycetes from marine sediments. Hindustan Antibiot. Bull., 38: 48-52.

Ghanem NB, Sanry SA, El-Sherif ZM, El-Ela GAA (2000). Isolation and enumeration of marine actinomycetes from seawater and sediments in Alexandria. J. Gen. Appl. Microbiol., 46: 105-111.

Gordon RE (1966). Some strains in search of a genus - *Corynebacterium*, *Mycobacterium*, *Nocardia* or what? J. Gen. Microbiol., 43: 329-343.

Gordon RE (1967). The Taxonomy of soil bacteria. In: Gray TRG, Parkinson B (eds) The ecology of soil bacteria. Liverpool: Liverpool University Press, pp. 293-321.

Holt GJ, Krieg RN, Sneath AHP, Staley TJ, Williams TS (2000). Bergey's Manual of Determinative Bacteriology, 9th edn. Lippincott Williams, Wilkins. Philadelphia, USA.

Jensen PR, Dwight R, Fenical W (1994). Distribution of actinomycetes in near shore tropical marine sediments. Appl. Environ. Microbiol., 57: 1102-1108.

Kavitha A, Vijayalakshmi M, Sudhakar P, Narasimha G (2010). Screening of actinomycetes strains for the production of antifungal metabolites. Afr. J. Microbiol. Res., 4: 27-32.

Khan MR, Williams ST (1975). Studies on the ecology of actinomycetes in soil- Distribution and characteristics of acidophilic actinomycetes. Soil. Boil. Biochem., 7: 345-348.

Kharat KR, Kharat A, Hardikar BP (2009). Antimicrobial and cytotoxic activity of *Streptomyces* sp. from Lonar Lake. Afr. J. Biotechnol., 8: 6645-6648.

Kroppenstedt RM (1992). The genus *Nocardiopsis,* In: Balows A, Triiper HG, Dworkin M, Harder W and Schleifer KH (eds) The prokaryotes, 2nd ed. Springer Verlag, New York, pp 1139-1156.

Lakshmanaperumalsamy P, Chandramohan D, Natarajan R (1986). Seasonal variation of microbial population from sediments of Vellar estuary, South India. Colloque International de Bacteriologie Marine, Brest (France), 3: 43-54.

Lam KS (2006). Discovery of novel metabolites from marine actinomycetes. Curr. Opi. Microbiol., 9: 245-251.

Maiti SK (2003). Handbook of methods in Environmental studies. ABD publishers, Jaipur, India, pp.185-188.

Mansour SR (2003). The occurrence and distribution of soil actinomycetes in Saint Catherine area, South Sinai, Egypt. Pak. J. Biol. Scienc., 6: 721-728.

Moncheva P, Tishkov S, Dimitrova N, Chipeva V, Bogatzevska N (2002). Characteristics of soil actinomycetes from Antartica. J. Cult. Coll., 3: 3-14.

Olano C, Mendez C, Salas JA (2009). Antitumor Compounds from Marine Actinomycetes. Marine Drugs, 7: 210-248.

Oskay M, Tamer UA, Azeri C (2004). Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr. J. Biotechnol., 3: 441-446.

Pisano MA, Sommer MJ, Lopez MM (1985). Application of pretreatments for the isolation of bioactive actinomycetes from marine sediment. Appl. Microbiol. Biotechnol., 25: 285-288.

Saadoun I, Al-Momani F (1996). Bacterial and *Streptomyces* flora of some Jordan valley soils. Actinomycetes, 7: 95-99.

Schrempf H (2001). Recognition and degradation of chitin by *Streptomycetes*. Ant. Van. Leeuwenhoek, 79: 285-289.

Shiomi KT, Takeuchi (1990). New antibiotic produced by *Micromonospora globosa*. J. Biotech., 43: 1000-1005.

Shirling EB, Gottlieb D (1966). Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.

SPSS for windows, Rel. 11.X.X. 2001. Chicago: SPCC Inc.

Stach JE, Bull A (2005). Estimating and comparing the diversity of marine actinobacteria. Ant. Van. Leeuwenhoek, 87: 3-9.

Tsujibo H, Sakamoto T, Miyamoto K, Kusano G, Ogura M, Hasegawa T, Inamori Y (1990). Isolation of cytotoxic substance, kalafungin from an alkalophilic actinomycete, *Nocardiopsis dassonvillei subsp. prasina*. Chem. Pharm. Bull. Tokyo., 38: 2299-2300.

Waksman SA (1961). The actinomycetes, classification, identification and description of genera and species. Baltimore. The Williams and Wilkins Company, Baltimore, pp. 261-292.

Wawrik B, Kutliev D, Abdivasievna UA, Kukor JJ, Zylstra GJ, Kerkhof L (2007). Biogeography of actinomycete communities and Type II Polyketide synthase genes in soils collected in New Jersey and Central Asia. Appl. Envir. Microbiol., 73: 2982-2989.

Wilkins K (1996). Volatile metabolites from actinomycetes. Chemosphere, 32: 1427-1434.

Williams ST, Neilly T, Wellington EMH (1977). The decomposition of vegetation growing mineral mine waste. Soil. Biol. Biochem., 9: 271-275.

Wise R (2008). The worldwide threat of antimicrobial resistance. Curr. Sci., 95: 181-187.