International Journal of Agricultural Research

ISSN 1816-4897

Academic Journals Inc.

www.academicjournals.com

International Journal of Agricultural Research 7 (3): 152-159, 2012
ISSN 1816-4897 / DOI: 10.3923/ijar.2012.152.159

Studies on Actinomycetes Diversity in Eastern Ghats (Yercaud Hills) of Southern India for Secondary Metabolite Production

B. Nithya and P. Ponmurugan
Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 215, Namakkal District, Tamil Nadu, India

Corresponding Author: B. Nithya, Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode-637 215, Namakkal District, Tamil Nadu, India Tel: 04288-274741, 91-94422-93253 Fax: 04288-274745

ABSTRACT

A study was undertaken to isolate biologically diverse strains of actinomycetes from medicinal plants rhizosphere soil collected from different locations of Yercaud hills belonging to Eastern Ghats of southern India for the production of bioactive secondary metabolites. The results indicated that a total of 20 strains were obtained from soil samples and further correlated with soil nutrients status in which five novel strains were screened for further investigation based on *in vitro* performance. The bioactive antimicrobial compounds were extracted from the purified cultures and tested their efficacy against some human pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Serratia marcescens* and *Pseudomonas fluorescence* and beneficial microorganisms such as Phosphobacteria (PSB), Potassium solubilizing bacteria (KSB), *Azospirillum* and *Rhizobium* spp. including tea plant pathogens namely *Phomopsis theae* and *Tunstallia aculeate*. The results showed that all the strains were found to be of potential antagonists against the tested pathogens and thus providing the production of secondary metabolites.

Key words: Actinomycetes, *Streptomyces*, antimicrobial activity, secondary metabolites, tea plant pathogens

INTRODUCTION

Yercaud hills belonging to Eastern Ghats are otherwise called as Shevaroy range of hills situated at 1,500 m above mean sea level in Salem district in the state of Tamil Nadu, southern India. The hills have many diversified faunal and floral similarities to the Western Ghats. These are one to 4 km apart in the same semi-evergreen forest tract in which medicinal plants are growing in abundance. A total of 199 species belonging to 175 genera covering 85 families are recorded as wild or naturalized plant resources. These include 106 species as medicinal, 37 as of economic value, 8 as fuel, 12 having edible fruits, 26 having showy flowers which may be further developed and may be of immense horticultural importance and 28 species turned out to be exotic/aliens or naturalized (Kadavul and Parthasarathy, 1999). Yercaud forest environments provide diverse habitats for a unique range of flora and fauna which has been investigated for the presence of actinomycetes in extracting pharmaceutical, based active compounds (Thangapandian *et al*., 2007). Isolation and screening of antimicrobial compounds from such kind of biota will provide a significant contribution in the field of drug discovery.

Actinomycetes were one of the major groups of the soil population having high G+C content gram positive bacteria (Kumari *et al*., 2006; Khucharoenphaisan *et al*., 2012). Among the major group of Actinomycetes, *Streptomyces* spp. can produce an array of secondary metabolites having antibacterial or antifungal properties were applied for the human pharmaceutical use (Goodfellow *et al*., 1987; Wellington *et al*., 1992). It has been reported that most of the actinomycetes are widely used in industries due to their ability to produce numerous antibiotics (Raja and Prabakarana, 2011), enzymes, vitamins, growth hormones and anti-cancerous agents (Berdy, 1995). *Streptomyces* genus can also produce valuable metabolites, enzyme inhibitors commercially valuable enzymes like lipases, cellulases, amylase and proteases (Ravel *et al*., 2000). The most promising role for secondary metabolites relies upon defence mechanisms especially arresting the pathogens' growth. It has been reported that secondary metabolites extracted from actinomycetes exhibited antimicrobial (Muiru *et al*., 2008), anticandidal (Sanasam and Ningthoujam, 2010) or anticancerous/antiinflammatory activities (Ponmurugan and Nithya, 2008).

Actinomycetes have characteristics of both bacteria and fungi and are sometimes called as fungi-like bacteria. They are capable to grow both on rich substrates and on those containing a minimum or even an apparent lack of macro/micro nutrients (Wellington *et al*., 1992). The intention of the present study was to isolate and characterize the biologically diverse strains of actinomycetes from rhizosphere soil sales of medicinal plants at different locations of Yercaud hills for the production of bioactive secondary metabolites. The antimicrobial activity of the purified strains were studied by performing test using selected human and tea plant pathogenic microorganisms including some beneficial microorganisms under *in vitro* condition.

MATERIALS AND METHODS

Collection and analysis of soil samples: Soil samples were collected from rhizosphere regions of different medicinal plants at Yercaud hills belonging to Eastern Ghats of southern India from a depth of 6-10 cm. The study was carried out for a period of seven months from June 2011 to December 2011. The soil samples were allowed to air dry at room temperature and subsequently subjected to estimate various edaphic parameters like soil pH, total organic carbon (Walkley and Black, 1934) total nitrogen (AOAC, 1990) and available phosphorous (Jackson, 1973).

Isolation and characterization of actinomycetes: Enumeration and isolation of actinomycetes present in these soil samples were performed by serial dilution plate technique using Starch-casein Nitrate Agar (SCNA) medium (Sanasam and Ningthoujam, 2010). All the isolates of actinomycetes were grown on SCNA at room temperature and the growth rate was observed every day up to 5 days. Individual colonies were picked up, subcultured on SCNA and various colony characteristics such as colony colour, mycelial colour, margin, elevation, size, shape, exopolysacchride (EPS) and diffusible pigment production were recorded (Keiser *et al*., 2000; Ponmurugan *et al*., 2007; Dhananjeyan *et al*., 2010). The cover slip culture technique was employed to identify the actinomycetes cultures and morphological characterization methods such as Gram's staining, motility and cell wall amino acid analysis (Holt, 1994) were also determined to identify the isolated actinomycetes cultures up to genus level. Biochemical characterization tests such as pigment production, starch hydrolysis, casein hydrolysis, catalase test, nitrate reduction, indole production, gelatin hydrolysis and hydrogen sulphide production were carried out to identify the name of actinomycetes (Bergey and Holt, 1989; IMTECH, 1998).

Effect of biotic and abiotic factors on growth of actinomycetes: Influence of abiotic factors such as pH (4.0 to 6.5) and temperature (5 to 40°C) and nutrient factors such as carbon and nitrogen sources on growth of actinomycetes were studied. Six different carbon compounds such as glucose, maltose, sucrose (Kathiresan and Manivannan, 2007), fructose, starch and cellulose and four nitrogen compounds such as ammonium nitrate, sodium nitrate, potassium nitrate and casein hydrolysate were added by replacing starch and potassium nitrate respectively in the basal medium (starch-casein nitrate agar). The inoculated plates were inoculated for 10-12 days depending upon the nature of experiment (Ponmurugan *et al*., 2011).

Antifungal metabolite production (Bauer *et al*., 1966): In order to check the production of antibiotics by actinomycetes, antibiotic production medium was used. Antibiotic production medium (25 g starch, 10 g glucose, 2 g yeast extract, 3 g calcium carbonate and 1 mL of trace solution containing $ZnSO_4$, $MnCl_2$, $CuSO_4$, $FeSO_4$, pH 7.5) was used for extraction of antifungal compounds from actinomycetes cultures. It was inoculated into 25 mL of seed medium in 250 mL conical flask and kept in a rotary shaker at 220 rpm for 25 days. The culture filtrate was centrifuged at 11,000 rpm to get a clear solution and filter sterilized.

Study of antimicrobial activity of actinomycetes (Nichols *et al*., 1989): Antibacterial activity of the purified cultures was studied against human pathogenic organisms using Mueller Hinton agar medium and tea plant pathogens using PDA. For the present study, a lawn was made using standard strains of pathogenic organism such as *Staphylococcus aureus* (MTCC 740), *Escherichia coli* (MTCC 521), *Bacillus amyloliquefaciens* (MTCC 610) *Serratia marcescens* (MTCC 86) and *Pseudomonas fluorescence* (MTCC 645). Moreover, a few tea plant pathogenic microorganisms such as *Phomopsis theae* (UPASI 01) and *Tunstallia aculeate* (UPASI 22) and beneficial microorganisms such as Phosphobacteria (KSR PSB11), Potassium solubilizing bacteria (KSR KSB23), *Azospirillum* (KSR Azo01) and *Rhizobium* (KSR Rhi89) spp. were also selected for the present study. Sterilized filter paper discs impregnated with filter sterilized culture broth was placed onto the lawn made in Mueller Hinton agar plates. Antibiosis method was also followed to study the efficacy of culture filtrate of actinomycetes against tea pathogens (Dennis and Webster, 1971). The plates were incubated at 37°C for 24 and 72 h in the case of human and tea pathogens, respectively. They were observed for zone of inhibition which indicated a positive reaction for antimicrobial activities.

RESULTS AND DISCUSSION

Population density: Actinomycetes are the best and known for their ability to produce broad spectrum antibiotics which comprise a group of branching unicellular microorganisms. Many species of actinomycetes produce important antibiotics such as streptomycin, while others are pathogenic in humans and other animals, especially for skin diseases. In the present study, a survey was conducted to know the population diversity at Yercaud hill of Eastern Ghats which revealed that the total number of actinomycetes population was found to be 32.7×10^3 CFU g^{-1} soil dry weights in top station and 9.7×10^3 CFU g^{-1} soil dry weights in terrain region. The population density was positively correlated to soil nutrients like total organic carbon, total nitrogen and available phosphorous contents (Table 1). Similar observations were reported in tea soils by Baby *et al*. (2002) and Ponmurugan *et al*. (2007) who observed a correlation between beneficial microorganisms and nutrients in the soil. However, most of the isolates tend to grow in acidic soils which is an important characteristic feature of actinomycetes species (Stackebrandt *et al*., 1991).

Characterization of actinomycetes isolates: A total of 20 isolates of actinomycetes were obtained from medicinal plant rhizosphere soil samples and subjected to screen for their antimicrobial activity. Out of these, one isolate from each agroclimatic zone of Yercaud was selected based on the culture studies for further investigation. The isolates were designated as YER-11 to YER55 (Table 1). Morphological and biochemical characteristics of the isolates were studied and results were presented in the Table 2. The results indicated that the purified isolates of actinomycetes belonged to *Streptomyces* spp. as they showed good and vigorous sporulation with compact, chalk-like dry colonies of different colour variation from pink to white colour. This is the characteristic features of actinomycetes (Goodfellow *et al*., 1987). Most of the isolates were efficient in hydrolyzing starch, gelatin and casein. Indole production was strictly negative but catalase test was positive in all the isolates. Production of hydrogen sulphide and nitrite reduction showed positive result in majority of the isolates (Table 2). All the isolates were found to be gram-positive organism and showed a branched mycelium in their cell morphology similar to fungal characters (Bergey and Holt, 1989).

The growth of *Streptomyces* isolates on medium adjusted with different pH revealed that a better growth was recorded between pH 5.0 and 6.5. The optimum pH was 5.5 for all the isolates (Table 3). This pH level may be correlated with soil pH. The optimum temperature for the growth of *Streptomyces* isolates was 25°C followed by 20°C. The optimum temperature was 25°C for majority of the isolates. Among the different carbon sources tested, starch was found to be suitable

Table 1: Population density of actinomycetes and nutrient status in Yercaud hills of Tamil Nadu, Southern India

Name of agroclimatic zones	Designation of strains	Population density *	Soil pH	Organic carbon (%)	Total nitrogen (%)	Available phosphorous (ppm)
Top station	YER11	32.70	5.50	4.90	3825	20.60
Forest areas	YER22	22.50	6.00	4.30	2887	20.20
Terrain	YER33	9.70	5.50	3.80	2937	17.80
Low station	YER44	18.70	6.00	4.30	3037	17.80
Topographical area	YER55	22.40	5.50	4.60	3408	19.30
±SE		1.03	0.67	0.82	18.59	5.23
CD at p = 0.05		5.25	1.38	1.02	23.68	5.44

*CFU×10^3 CFU g^{-1} soil DW

Table 2: Morphological, physiological and biochemical characterizations of actinomycetes strains

	Isolates of actinomycetes				
Parameters	YER11	YER22	YER33	YER44	YER55
Cell morphology	Spiral spore chain	Rods	Cluster of spore chain	Coiled spore chain	Rods
Colour of the mycelium	Grayish white	Pinkish white	White	Creamy yellow	White
Gram's staining	++	++	++	++	++
Pigment production	++	++	++	++	-
Starch hydrolysis	++	-	-	++	++
Casein hydrolysis	++	-	-	++	++
Catalase test	+	++	++	+	++
Nitrate reduction	++	++	++	++	-
Indole production	-	-	-	-	-
Gelatin hydrolysis	++	+	++	+	++
Hydrogen sulphide production	++	++	++	++	-

++: Prominent growth, +: Moderate growth, -: No growth

for maximum growth followed by maltose. On the other hand, potassium nitrate and ammonium nitrate were found to be suitable for optimum growth followed by sodium nitrate and casein hydrolysate (Table 3). Production of antifungal metabolites has been known to be influenced by components of medium and cultural conditions such as pH, temperature, carbon and nitrogen sources (Augustine *et al*., 2004).

Antibiotic potential of *Streptomyces* spp.: The results on the antifungal activity against tea pathogens showed that the well-developed inhibition zone was formed around paper discs impregnated with *Streptomyces* broth culture. The linear growth of *P. theae* was 38.0 mm on 4th day and it was 36.7 mm on 8th day by *T. aculeata* (Table 4). The results coincided with the report of Zahner *et al*. (1979). The formation of inhibition zone around the pathogenic strain is due to the

Table 3: Effect of biotic, abiotic and nutrient factors on the growth of actinomycetes isolates

	Isolates of actinomycetes				
Parameters	YER11	YER22	YER33	YER44	YER55
Optimum pH	5.0	5.0	5.5	5.5	5.0
Optimum temperature (°C)	25	25	20	20	25
Glucose*	++	+	+	++	++
Fructose*	++	+	+	++	++
Maltose*	++	++	++	++	++
Sucrose*	++	++	-	-	++
Starch*	++	++	++	++	++
Cellulose*	++	++	-	-	++
Ammonium nitrate**	++	++	++	++	++
Sodium nitrate**	++	+	+	+	++
Potassium nitrate**	++	++	++	++	++
Casein hydrolysate**	++	+	+	+	++

++: Prominent growth, +: Moderate growth, -: No growth, *Carbon sources, **Nitrogen sources

Table 4: Antagonistic effect of *Streptomyces* spp. on tea plant pathogens such as *P. theae* and *T. aculeata*

	Antibiosis method (radial growth in mm)		Paper disc method (linear growth in mm)	
Days after inoculation	*P. theae*	*T. aculeata**	*P. theae*	*T. aculeata**
1	0.0 (100)	0.0 (100)	28.3	13.7
2	0.0 (100)	0.0 (100)	33	16.3
3	0.0 (100)	0.0 (100)	35.7	21.4
4	0.0 (100)	0.0 (100)	38	24
5	1.8 (93)	0.0 (100)	-	27.3
6	3.3 (88)	0.0 (100)	-	30
7	5.8 (81)	1.5 (95)	-	33.3
8	10.1 (71)	2.7 (90)	-	36.7
9	13.3 (65)	3.9 (86)	-	-
10	10.0 (53)	5.5 (80)	-	-
±SE	0.77	0.67	1.43	1.2
CD at p = 0.05	2.38	1.3	3.25	2.51

*Since the pathogen is slow growing, the experiment was completed within 10 days, Values in parentheses denotes% growth inhibition on radial growth

Table 5: Antimicrobial activity of *Streptomyces* spp. against human pathogenic and beneficial microorganisms

	Streptomyces spp. used				
Culture used	YER11	YER22	YER33	YER44	YER55
Staphylococcus aureus	+	-	++	++	+
Escherichia coli	-	++	-	-	+
Bacillus amyloliquefaciens	++	-	++	-	+
Serratia marcescens	+	-	+	++	-
Pseudomonas fluorescence	+	++	+	++	++
Phosphobacteria	-	-	-	-	
Potassium solubilizing bacteria	-	-	-	-	-
Azospirillum	-	-	-	-	-
Rhizobium	-	-	-	-	-

++: Positive reaction for zone of inhibition, +: Weakly positive reaction for zone of inhibition, -: Negative reaction for zone of inhibition

production of secondary metabolites by *Streptomyces* spp. (Sanglier *et al*., 1993). According to Demain and Fang (1995), the most widely accepted theory is that antibiotics are used to compete with other organisms in nutrient depleting environment. Thus, the formation of inhibition zone around the tea plant pathogenic strains is purely based on the antifungal properties of *Streptomyces* spp. The growth of fungal pathogens such as *Aspergillus niger*, *Fusarium oxysporum*, *Candida albicans* and *Cryptococcus humicolus* was suppressed significantly using antifungal metabolites obtained from *Streptomyces* spp. (Augustine *et al*., 2004). The *Streptomyces* isolated from the polyherbal products had the potential antagonistic activity against the fungal pathogens (Mohd-Fuat *et al*., 2010). According to Anita and Ponmurugan (2011), the growth of the *P. theae* was inhibited by the culture filtrate of the eminent strain *T. atroviride* Tv1. In the present study, both *P. theae* and *T. aculeata* pathogens failed to germinate in the medium amended with antifungal substance extracted from *Streptomyces* (Table 4).

Similarly, an array of antibacterial compounds were extracted and tested against some human pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus amyloliquefaciens*, *Serratia marcescens* and *Pseudomonas fluorescence* as well as beneficial microorganisms like Phosphobacteria, PSB, KSB, *Azospirillum* and *Rhizobium* spp. (Table 5). The results showed that the growth of pathogenic microorganisms was significantly suppressed whereas beneficial microorganism's growth was not inhibited due to synergetic effect. In this study, most of the isolates of *Streptomyces* spp. were found to be of potential antagonists against plant and human pathogens and thus proving the production of secondary metabolites that has the potential to control variety of pathogens (Ponmurugan *et al*., 2011). Devi *et al*. (2006) has also reported that the extract obtained from the marine actinomycetes showed good antimicrobial activity against human pathogens. Similarly the study made by Aghighi *et al*. (2004) observed the new strain of *Streptomyces plicatus* 101 had an effective antifungal activity besides *Verticillium dahliae*. Based on these results it can be inferred that *Streptomyces* spp. can be used as soil inoculants to prevent the growth of soil-borne pathogens like *P. theae* and *T. aculeate* in tea soils as well as some human pathogens under *in vitro* condition.

To conclude, most of the strains of *Streptomyces* spp. were found to be potential antagonists against human and plant pathogens and thus proving the production of secondary metabolites that has the potential to control the pathogens.

ACKNOWLEDGMENTS

The authors are thankful to the Principal and the Management of K.S.R. College of Educational Trust, Tiruchengode, for providing necessary facilities and constant encouragement to carry out this study.

REFERENCES

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Aghighi, S., G.H.S. Bonjar and I. Saadoun, 2004. First report of antifungal properties of a new strain of *Streptomyces plicatus* (Strain 101) against four Iranian phytopathogenic isolates of *Verticillium dahliae*, a new horizon in biocontrol agents. Biotechnology, 3: 90-97.

Anita, S. and P. Ponmurugan, 2011. *In vitro* evaluation of *Trichoderma atroviride* against *Phomopsis theae* a casual agent of collar canker disease in tea plants. Int. J. Agric. Res., 6: 620-631.

Augustine, S.K., S.P. Bhavsar, M. Baserisalehi and B.P. Kapadnis, 2004. Isolation, characterization and optimization of antifungal activity of an actinomycete of soil origin. Indian J. Exp. Biol., 42: 928-932.

Baby, U.I., N. Tensingh-Baliah, P. Ponmurugan and R. Premkumar, 2002. Population level of certain of certain beneficial microorganisms in tea soils. UPASI Tea Res. Found. Newslett., 12: 3-3.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Berdy, J., 1995. Are actinomycetes exhausted as a source of secondary metabolites? Biotechnologia, 7-8: 13-34.

Bergey, D.H. and J.G. Holt, 1989. Bergey's Manual of Systemic Bacteriology, Volume 4. 9th Edn., Williams and Wilkins, UK., ISBN-13: 9780683090611, Pages: 2648.

Demain, A.L. and A. Fang, 1995. Emerging concepts of secondary metabolism in actinomycetes. Actinomycetologica, 9: 98-117.

Dennis, C. and J. Webster, 1971. Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc., 57: 41-48.

Devi, N.K.A., M. Jeyarani and K. Balakrishnan, 2006. Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. Pak. J. Biol. Sci., 9: 470-472.

Dhananjeyan, V., N. Selvan and K. Dhanapal, 2010. Isolation, characterization, screening and antibiotic sensitivity of actinomycetes from locally (Near MCAS) collected soil samples. J. Biol. Sci., 10: 514-519.

Goodfellow, M., J. Lacey and C. Todd, 1987. Numerical classification of thermophilic *Streptomyces*. J. Gen. Microbiol., 133: 3135-3149.

Holt, J.G., 1994. Bergay's Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore, USA.

IMTECH, 1998. Actinomycetes: Isolation, Screening, Identification and Gene Cloning in *Streptomyces*, Laboratory Manual. Institute of Microbial Technology, Chandigarh, India, Pages: 94.

Jackson, M.L., 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India, pp: 498-516.

Kadavul, K. and N. Parthasarathy, 1999. Plant biodiversity and conservation of tropical semi-evergreen forest in the Shervarayan hills of Eastern Ghats, India. Biodivers. Conserv., 8: 419-437.

Kathiresan, K. and S. Manivannan, 2007. Production of alkaline protease by *Streptomyces* sp., isolated from coastal mangrove sediment. Res. J. Environ. Sci., 1: 173-178.

Keiser, T., M.J. Bibb, M.J. Buttner, F.K. Chater and D.A. Hopwood, 2000. General Introduction to Actinomycetes Biology. In: Practical Streptomyces Genetics, Kieser, T. (Ed.). John Innes Foundation, UK., ISBN-13: 9780708406236, pp: 1-21.

Khucharoenphaisan, K., N. Sripairoj and K. Sinma, 2012. Isolation and identification of actinomycetes from termite's gut against human pathogen. Asian J. Anim. Vet. Adv., 7: 68-73.

Kumari, K.K., P. Ponmurugan and N. Kannan, 2006. Isolation and characterization of *Streptomyces* sp. for secondary metabolite production. Biotechnology, 5: 478-480.

Mohd-Fuat, A.R., A.R.B. Amini and O.M. Hasyima, 2010. Isolation of *Streptomyces* with broad spectrum antifungal activity from polyherbal products. Int. J. Botany, 6: 259-265.

Muiru, W.M., E.W. Mutitu and D.M. Mukunya, 2008. Identification of selected actinomycete isolates and characterization of their antibiotic metabolites. J. Boil. Sci., 8: 1021-1026.

Nichols, W.W., M.J. Evans, M.P. Slack and H.L. Walmsley, 1989. The penetration of antibiotics into aggregates of mucoid and non-mucoid *Pseudomonas aeruginosa*. J. Gen. Microbiol., 135: 1291-1303.

Ponmurugan, P., C. Gopi and A. Maripandi, 2007. Studies on Actinomycetes diversity in Southern Indian tea soils for antifungal activity. J. Plant. Crops, 35: 28-32.

Ponmurugan, P. and B. Nithya, 2008. Plasmid DNA of antibiotic producing strains of *Streptomyces sannanensis* isolated from different states in Southern India. Biotechnology, 7: 487-492.

Ponmurugan, P., V. Elango, S. Marimuthu, T.C. Chaudhuri, D. Saravanan, B.M. Gnanamangai and K.M. Karunambika, 2011. Evaluation of actinomycetes isolated from southern Indian tea plantations for the biological control of tea pathogens. J. Plant. Crops, 39: 239-243.

Raja, A. and P. Prabakarana, 2011. Actinomycetes and drug-An overview. Am. J. Drug Discovery Dev., 1: 75-84.

Ravel, J., E.M.H. Wellington and R.T. Hill, 2000. Interspecific transfer of *Streptomyces* linear plasmids in sterile amended soil microcosms. Applied Environ. Microbiol., 66: 529-534.

Sanasam, S. and D.S. Ningthoujam, 2010. Screening of local actinomycete isolates in Manipur for anticandidal activity. Asian J. Biotechnol., 2: 139-145.

Sanglier, J.J., H. Haag, T.A. Huck and T. Fehr, 1993. Novel bioactive compounds from actinomycetes: A short review. Res. Microbiol., 144: 633-642.

Stackebrandt, E., D. Witt, C. Kemmerling, M. Kroppenstedt and W. Liesack, 1991. Designation of *Streptomyces* 16S and 23S rRNA based target regions of oligonucleotide probes. Applied Environ. Microbiol., 57: 1468-1477.

Thangapandian, V., P. Ponmurugan and K. Ponmurugan, 2007. Actinomycetes diversity in the rhizosphere soils of different medicinal plants in Kolly Hills-Tamilnadu, India, for secondary metabolite production. Asian J. Plant Sci., 6: 66-70.

Walkley, A. and I.A. Black, 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.

Wellington, E.M.H., E. Stackebrandt, D. Sanders, J. Wolstrup and N.O.G. Jorgensen, 1992. Taxonomic status of Kitasatosporia and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and Enrici 1943, 339AL. Int. J. Syst. Bacteriol., 42: 156-160.

Zahner, H., W. Weber, J. Siebers, K. Schroder and A. Zeeck, 1979. *Streptomyces*. Arch. Microbiol., 124: 111-116.