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Isolation of actinobacteria and its antagonistic effects on native organisms from *Northern black soils* of Katheru (A.P), India

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ABSTRACT

Actinobacteria are a group of prokaryotic organisms belonging to Gram-positive bacteria and play an important ecological role in recycling substances in the nature. The main objective of the present study was to isolate and identify Actinobacteria from *Northern black soils* of Katheru, Rajahmundry district, Andhra Pradesh, INDIA, where tobacco is being cultivated on a large scale. The isolates were identified using morphological characters and identified as *Streptomyces*. Further the isolates were examined for antimicrobial activity. The results obtained indicate that *Streptomyces II* possess positive antagonistic effect on both gram positive *Bacilli and Cocci*, where as *Streptomyces I* possess negative antagonistic effect. They were further tested for their antagonistic effect on different fungi viz., *Aspergillus flavus, Aspergillus niger, Pythium, Rhizoctonia solani and Sclerotium*. *Streptomyces II* showed antagonistic effect on all fungi except *Sclerotium* where as *Streptomyces I* showed negative antagonistic effect. Biochemical studies revealed that both species of *Streptomyces* are amylase positive. Thus, *Streptomyces II* may be used for the production of polymeric anti-fungal antibiotics and to treat some of bacterial diseases of tobacco. ! 2013 Trade Science Inc. - INDIA

KEYWORDS

Actinobacteria;
Antagonist;
Bacilli;
Cocci;
Antibiotics.

INTRODUCTION

Actinobacteria comprise group of gram positive filamentous bacteria. They are unicellular microorganisms, 1µM in diameter, filamentous, branching monopodial, seldom dichotomous, producing colonies of radiating structure. Actinobacteria are the most economically and biotechnologically valuable prokaryotes which are well known to produce chemically diverse metabolites with wide range of biological activity[1]. In Recent days the discovery of known metabolites and actinobacteria are increasing due to the exploitation of natural ecosystems. Exploitation of less and unexplored ecosystems for actinobacteria is highly necessary for the discovery of novel bioactive metabolites. Actinobacteria are important sources of new bioactive compounds such as antibiotics and enzymes[2-5] which have diverse clinical effects and are active against many organisms [Bacteria, Fungi, Parasites *etc*.]. In fact more than 50% of the known natural antibiotics are produced from

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actinobacteria[6]. The most striking feature of the actinobacteria is their ability to produce a wide variety of secondary metabolites. These natural products have been extraordinary sources of lead structures in the development of newer drugs[7-9]. Tobacco, an important cash crop all over the world, is greatly influence by temperature and soil conditions. Several improved varieties in all the types of tobacco have been evolved and released. Improved varieties evolved at the Central Tobacco Research Institute, Rajahmundry (Andhra Pradesh) and its research stations all over the country are furnished. These have become very popular among the farmers because of their superior yield, quality and resistance to diseases in the specific diseases prone areas. The fungi *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and species of the genus *Pythium* are the most important pathogens that cause damping-off diseases in tobacco seed-beds of the area. Besides, the same fungi are the causes of damping-off diseases in tomato seedbeds, and also the causes of root and collar rots of seedlings in the first stages of their development after the transplantation in the greenhouse. The actinomycetes are known to decompose organic matter, especially polymers such as lignocellulose, starch, and chitin, in soil[10-12]. Microbiologists are becoming interested in using actinomycetes as agents for biological control of soil-borne root diseases of crop plants. Streptomyces species and a few other actinomycetes have been shown to protect several different plants to various degrees from soil-borne fungal pathogens, primarily in glasshouse experiments. In the present study we have isolated actinobacteria from the Northern black soils of Katheru where tobacco is cultivated and studied for their anti microbial activity.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from *Northern black soils* (Katheru), where tobacco is cultivated. The samples were dried in open air, finely ground and sieved to separate large particles. The samples were stored in cloth bags and used whenever required.

Isolation of actinobacteria

One gram of Northern black soil was weighed and transferred to an Erlenmeyer's flask containing 99 ml of sterile distilled water. The soil suspension was further diluted to 10^{-5} levels. One ml of the diluted suspension was spread over the surface of starch casein agar medium. The pH of the media was adjusted to 7.2. To prevent the fungal and bacterial contaminants, Cycloheximide [100mg/1] and Nalidixic acid [20mg/1] were prepared separately in sterile water and mixed with the medium just before use. The petriplates were then incubated at room temperature for 7-11 days and the colonies were observed from third day onwards and up to one month. Strains of actinobacteria were picked out and purified by repeated streaking on Nutrient agar medium.

Screening for antagonistic activity on the native micro organisms

To screen for the antagonistic activity of the isolated bacteria, a loop full of inoculum was streaked in the middle of the petridish containing nutrient agar medium. After inoculation, petridishes were incubated at room temperature for 3 days for growing actinobacteria and then 24hrs old pathogenic bacteria were inoculated near the growth line of actinobacteria in the same petridish. The cross streaked plates were incubated at room temperature for 24 hrs. The inhibition zone [Clearing zone] produced between the actinobacteria and the pathogenic bacteria were measured. In the same way potato dextrose agar medium was used to screen for antagonistic activity on fungi. Fungal mycelial mat was placed near the growth line of actinobacteria in the same petridish to look for antagonistic effect.

RESULTS

Isolation of actinobacteria

In the present study, several actinobacteria strains were isolated from Northern black soils of tobacco cultivated area and they were identified as Streptomyces based on their morphological and biochemical characteristic features. When 200µl of the soil sample exttract was plated on to petridishes containing starch casein agar medium, actinobacteria growth was observed after 11 days of incubation (Figure 1). The plates show two different types of colonies, which can be distinguished very easily based on their pigmentation. Out of 47 isolates obtained, we have selected 2 strains, one with pigmentation (*Streptomyces I*) and the other with

no pigmentation (*Streptomyces II*) (Figure 2 a and b)

Figure 1 : Soil sample, 1 gm [w/v] was suspended in 100 ml of distilled water and vortexed thoroughly for 10 minutes. The suspension was centrifuged at 5000 rpm for 5 min and the supernatant was subjected to serial dilution up to 10^{-5}. 200µl of sample was plated on to petridishes containing starch casein agar medium. Actinobacteria growth was observed in starch casein agar plates after 11 days of incubation. The plates show two different types of *Streptomyces* colonies.

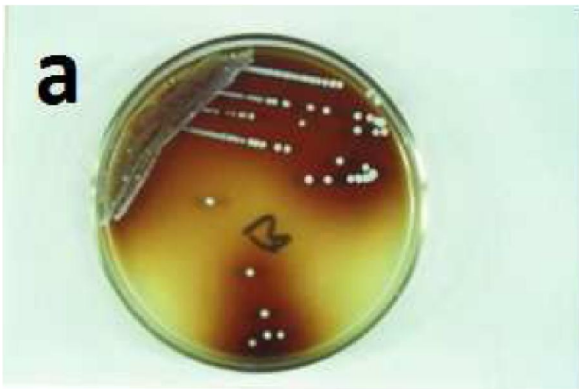
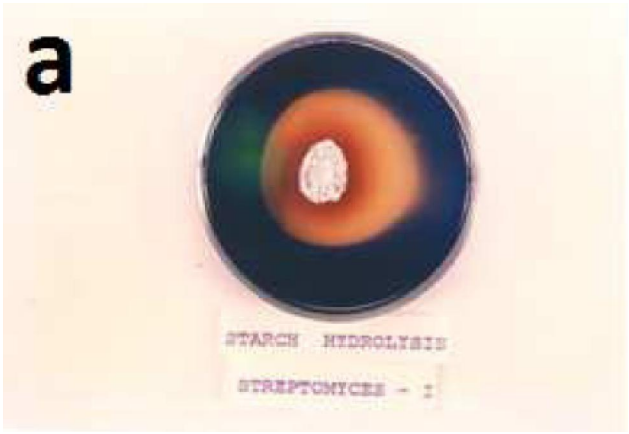


Figure 2 : Actinobacteria colonies from starch casein agar plates was subcultured by streak plate method to obtain pure culture. It was done by picking a colony using a sterilized loop and then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks. The colonies showed pigmentation were named as *Streptomyces I*. The colonies which showed no pigmentation were named as *Streptomyces II*.

Biochemical activities of *Streptomyces I & II*

A typical positive starch hydrolysis reaction [i.e., Clear zone surroundings the microbial colonies] is shown by *Streptomyces I* & *II* by the production of exoenzyme amylase which is diffused into the medium surrounding the growth (figure 3 a and b).



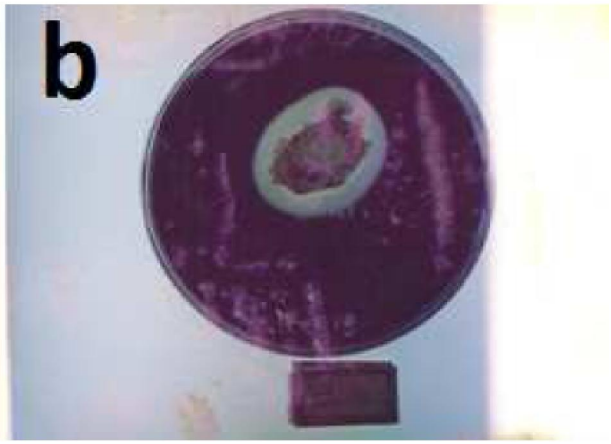


Figure 3 (a & b) : *Streptomyces I* [3a] & *Streptomyces II* [3b] were inoculated on starch agar medium and incubated at room temperature. Amylase activity of *Streptomyces I & II* was deteremined by addition of iodine to the starch agar medium, which indicates utilization of starch by production of zone of clearance.

Antagonistic activity of *Streptomyces I & Streptomyces II* on Gram positive *Bacilli and Cocci*

Among the 2 isolates that were selected for the further antagonistic activity studies, *Streptomyces I* showed negative antagonistic effect on both Gram positive *Bacilli and Cocci*, where as *Streptomyces II* showed positive antagonistic effect on both positive *Bacilli and Cocci* [TABLE 1, figure 4].

Antagonistic activity of *Streptomyces I & Streptomyces II* on Fungi

Similarly, antagonistic effect was also studied on

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TABLE 1 : Antagonistic effect of actinobacteria showing inhibition zone against Gram positive bacilli and cocci isolated from Northern black soils.

Bacteria	Streptomyces I	Streptomyces II	Photograph number
Gram Positive bacilli	[-] ve	[+] ve	Figure 4
Gram Positive cocci	[-] ve	[+] ve	

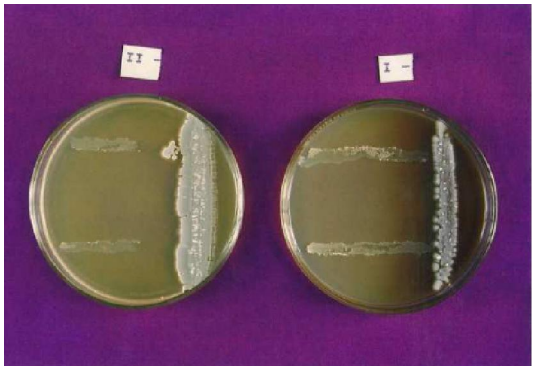

Figure 4 : In order to check tha antagonistic activity against bacteria, *Streptomyces I & II* were streaked in the middle of the petridish containing nutrient agar medium and incubated at room temperature for 3 days for growing actinobacteria. 24hrs old pathogenic bacteria were inoculated near the growth line of actinobacteria in the same petridish. The cross streaked plates were further incubated at room temperature for 24 hrs. The inhibition zone [Clearing zone] produced between the actinobacteria and the pathogenic bacteria indicates that *Streptomyces II* shows antagonistic effect on both *gram +ve bacilli and cocci*. *Streptomyces I* shows negative antagonistic effect on both both *gram +ve bacilli and cocci*.

various of fungal cultures viz., *Aspergillus flavus, Aspergillus niger, Pythium* and *Rhizoctoina solani*. *Streptomyces* ^{2} showed negative antagonistic activity towards all fungal isolates. Whereas *Streptomyces* ^{22} showed positive effect on all fungi except *Sclerotium* [TABLE 2, figure 5 a to e].

TABLE 2 : Antagonistic effect of actinobacteria showing inhibition zone against different fungal isolates of Northern black soils.

Fungal species	Streptomyces I	Streptomyces II	Photograph number
Aspergillus flavus	[-] ve	[+] ve	Figure 5a
Aspergillus niger	[-] ve	[+] ve	Figure 5b
Pythium	[-] ve	[+] ve	Figure 5c
Rhizoctoina solani	[+] ve	[+] ve	Figure 5d
Sclerotium	[-] ve	[-] ve	Figure 5e

DISCUSSION

Actinobacteria comprise a large number of organ-

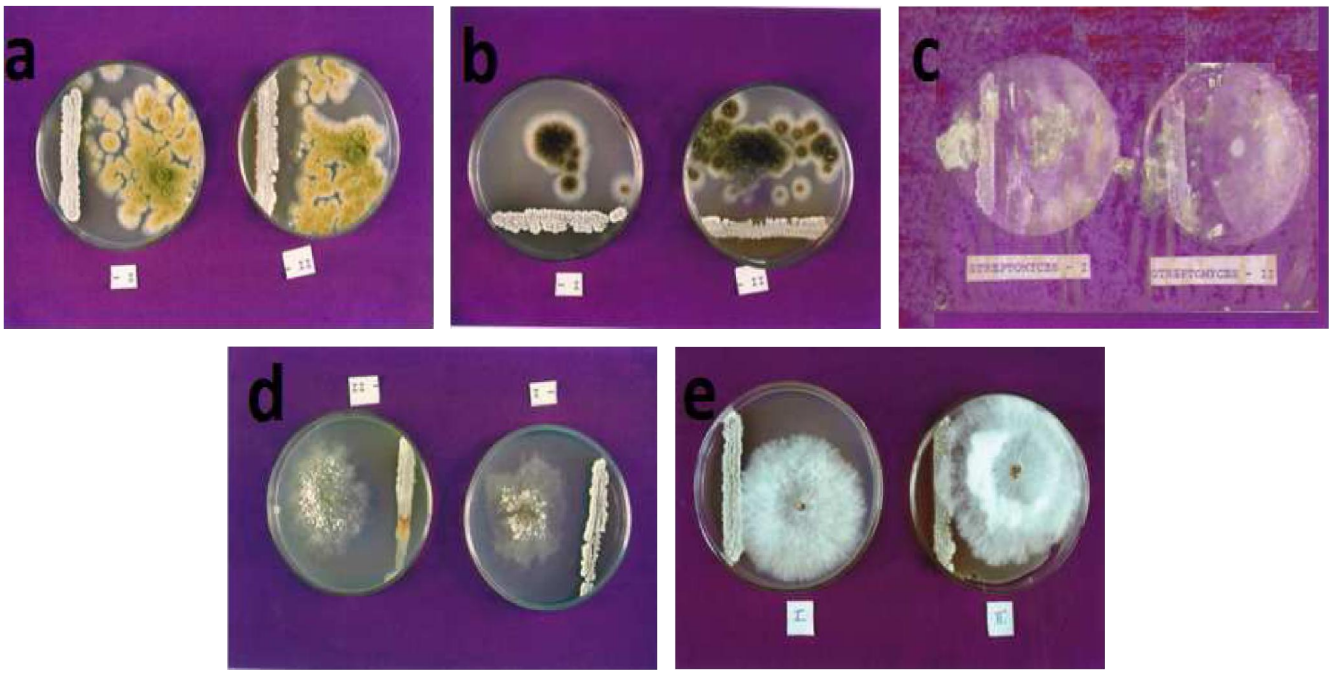

Figure 5 (a to e) : To determine the antagonistic activity towards fungi, *Streptomyces I & II*, were plated on PDA medium and petridishes were incubated at room temperature for 3 days. Pre grown (72 hrs old) fungal mycelial mat was then placed near the growth line of actinobacteria in the same petridish to check for antagonistic effect. *Streptomyces II* showed positive antagonistic activity towards *Aspergillus flavus* [a], *Aspergillus niger* [b], *Pythium* [c] and *Rhizoctoni solani* [d], whereas negative antagonistic activity towards *Sclerotium* [e]. *Streptomyces I* showed negative antagonistic towards all the above mentioned fungi.

isms which are responsible for the production of about half of the discovered bioactive secondary metabolites[24], notably antibiotics[25], anti tumour agents[26], immunosuppressive agents[27] and enzymes[28]. Gasperine was the first to demonstrate the antagonistic action of actinobacteria. They grow on starch casein agar medium. This medium was first used by Kuster and Williams, in 1964[29]. The growth inhibiting effect of actinobacteria upon bacteria and fungi is largely through the production of Antibiotics. Approximately 60% of antibiotics developed for agricultural use were isolated from *Streptomyces sp*[30]. Since tobacco crops can be affected by various bacteria and fungi, studies for isolating actinobacteria species from the nearby tobacco cultivated soils provides a way to isolate polymeric antifungal and anti bacterial antibiotics which may be useful in treating some of the bacterial and fungal diseases of tobacco plants. The fungal diseases include Anthracnose caused by *Colletotrichum destructivum*, Bikini disease by *Phytopthera sps*, Downy mildew disease by *Peronospora tabacina*, Collar rot by *Sclerotinia sclerotiorum*, Damping off disease by *Pythium spp*, Fusarium wilt by *Fusarium oxysporum* etc. The antagonistic activities can easily be demonstrated by the agar-cross-streak method. Mustafa *et al.*, [2004] reported the isolation of Actinobacteria from farming soils[31]. Anka Lukic et al showed antifungal spectra of actinobacteria isolated from Tobacco[32]. The present study is an attempt to isolate novel and newer antibiotic producer from the Northern black soils of katheru where tobacco is cultivated. The antibacterial activity of the test isolates was varied. One out of the two selected actinobacteria isolates were shown to have very potent *in vitro* antibacterial activity against both *G +ve Bacilli, Cocci* and anti fungal activity against *Aspergillus flavus, Aspergillus niger, Pythium* and *Rhizoctoina solani*.

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REFERENCES

[1] R.Balagurunathan, M.Radhakrishnan; Actinobacteria: Diversity and their importance. In: Microbiology - Applications and current trends. P.C.Trivedi, (Ed); Pointer publishers, Jaipur, India, 297-329 **(2007)**.

[2] L.C.Vining; Secondary metabolism, inventive evolution and biochemical diversity - A review, Gene, **115**, 135-140 **(1992)**.

[3] C.Edwards; Isolation, properties and potential applications of thermophilic actinobacteria. Appl.Biochem.Biotechnol., **42**, 161-179 **(1993)**.

[4] A.L.Demain; Why do microorganisms produce antimicrobials? In: P.A.Hunter, G.K.Darby, N.J.Russell, (Eds); Fifty years of antimicrobials: Prospective and future trends - Symposium 53. Society of General Microbiology, Cambridge University Press, 205-228 **(1995)**.

[5] L.H.Xu, Y.Jiang, W.J.Li, M.L.Wen, *et al.*; *Streptomyces roseoalbus* sp. Nov., an actinomycete isolated from soil in Yunnan, China. Antoine van Leeuwenhoek., **87**, 189-194 **(2005)**.

[6] S.Miyadoh; Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. Actinomycetologica, **9**, 100-106 **(1993)**.

[7] K.J.Kutzner; The family S*treptomycetaceae*. In: M.P.Starr, H.Stolp, H.G.Trper, A.Balows, H.G.Schlegel, (Eds); The prokaryotes, A Handbook on Habitats, isolation and identification of bacteria. Spriner-verlag, New York, **2**, 2028-2090 **(1986)**.

[8] S.T.Williams, M.Goodfellow, G.Alderson; Genus *Streptomycetes*. In: S.T.Williams, M.E.Sharpe, J.G.Holt, (Eds); Bergey's manual of systematic Bacteriology, Williams and Wilkins, Baltimore, **4**, 2452-2492 **(1989)**.

[9] T.Weber, K.Welzel, S.Pelzer, A.Vente, *et al.*; Exploiting the genetic potential of polyketode producing *Streptomycetes*. J.Biotechnol., **106**, 221-232 **(2003)**.

[10] M.P.Lechevalier; Actinomycetes in agriculture and forestry, In M.Goodfellow, S.T.Williams, M.Mordarski, (Eds); Actinomycetes in biotechnology. Academic Press, Inc., New York, 327-358 **(1989)**.

[11] M.C.Srinivasan, R.S.Laxman, M. V. Despharde; Physiology and nutritional aspects of actinomycetes: An overview. World J.Microbiol.Biotechnol., **7**, 171-184 **(1991)**.

[12] W.Zimmerman; Degradation of lignin by bacteria. J.Biotechnol., **13**, 199-130 **(1990)**.

[13] A.T.Bolton; Effects of amending soilless growing mixtures with soil containing antagonistic organisms

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on root rot and black leg of geranium (Pelargonium hortorum) caused by Pythium spendens. Can.J.Plant Sci., **58**, 379-383 **(1978)**.

[14] A.B.Filnow, J.L.Lockwood; Evaluation of several actinomycetes and the fungus Hypochytrium catenoides as biocontrol agents of Phytophthora root rot of soybean. Plant Dis., **69**, 1033-1036 **(1985)**.

[15] S.Hussain, A.Ghaffar, M.Aslam; Biological control of Macrophomina phaseolina charcoal rot of sunflower and mung bean. J.Phytopathol., **130**, 157-160 **(1990)**.

[16] P.R.Merriman, R.D.Price, K.F.Baker, J.F.Kollmorgen, T.Piggott, E.H.Ridge; Effect of bacillus and streptomyces spp. applied to seed, In E.Bruehl (Ed); Biology and control of soil-borne plant pathogens. American Phytopathological Society, St. Paul, Minn., 130-133 **(1977)**.

[17] Z.K.Mohamed; Physiological and antagonistic activities of streptomycetes in rhizosphere of some plants. Egypt J.Phytopathol., **14**, 121-128 **(1982)**.

[18] G.S.Reddi, A.S.Rao; Antagonism of soil actinomycetes to some soil-borne plant pathogenic fungi. Indian Phytopathol., **24**, 649-657 **(1971)**.

[19] I.L.Stevenson; Antibiotic activity of actinomycetes in soil as demonstrated by direct observation techniques. J.Gen.Microbiol., **15**, 372-380 **(1956)**.

[20] E.D.Sutherland, G.C.Papavizas; Evaluation of oospore hyperparasites for the control of Phytophthora crown rot of pepper. J.Phytopathol., **131**, 33-39 **(1991)**.

[21] J.C.Tu; Hyperparasitism of streptomyces albus on a destructive mycoparasite nectria inventa. J.Phytopathol., **117**, 71-76 **(1986)**.

[22] G.Turhan; A new race of streptomyces ochraceiscleroticus in the biological control of some soil-borne plant pathogens. II. In vivo studies on the possibilities of using C/2-9 against some important diseases. J.Plant Dis.Prot., **88**, 422-434 **(1981)**.

[23] G.Turhan, F.Grossmann; Investigation of a great number of actinomycete isolates on their antagonistic effects against soil-borne fungal plant pathogens by an improved method. J.Phytopathol., **116**, 238-243 **(1986)**.

[24] J.Berdy; Bioactive microbial metabolites. J.Antibiot. [TOKYO], **58**, 1-26 **(2005)**.

[25] W. R.Strohl; Antimicrobials. In: Microbial diversity and bioprospecting. A.T.Bull, (Ed); ASM Press, 336-355 **(2004)**.

[26] G.M.Cragg, D.G.I.Kingston, D.J.Newman, (Eds); Anti cancer agents from natural products. Brunner-Routledge Psychology Press, Taylor and Francis. 58-61 **(2005)**.

[27] J.Mann; Natural products as immunosuppressive agents. Nat.Prod.Rep., **18**, 417-430 **(2001)**.

[28] C.Oldfield, N.T.Wood, S.C.Gilbert, F.D.Murray, *et al.*; Desulphurisation of benzothiophene and dibenzothiophene by actinomycete organisms belonging to the genus rhodococcus and related taxa. Antonie van leeuwenhoek, **74**, 114-132 **(1998)**.

[29] G.Gasperini; Recherches morphologiques et biologiques sur un microorganisme de l'atmosphere, Streptothrixfoer8teri Cohn.Ann.Microgr., **10**, 449-474 **(1890)**.

[30] Y.Okami, K.Hotta; Search and discovery of new antibiotics. Int.J.Syst.Bacteriol., **29**, 51-55 **(1988)**.

[31] A.Mustafa Oskay, Üsame Tamer., Cem Azeri; Antibacterial activity of some actinobacteria isolated from farming soils of Turkey. Afr.J.Biotechnol., **3(9)**, 441-446 **(2004)**.

[32] R.E.Anka Lukic, G.B.Welty, Lucas; Antifungal spectra of actinobacteria isolated from tobacco. Antimicrobial Agents and Chemotherapy, **1(4)**, 363-365 **(1972)**.