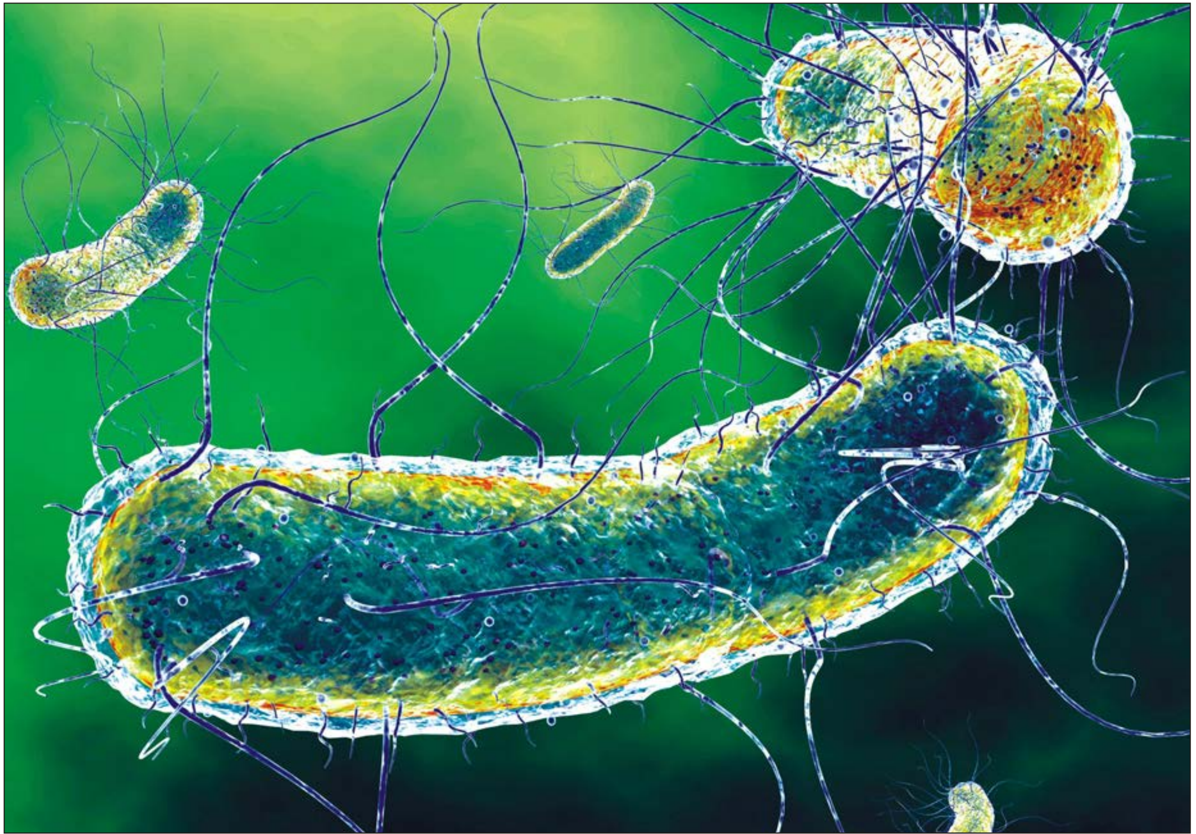

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First Report of Antimicrobial Spectra of Novel Strain of *Streptomyces tritolerans* (Strain AS1) Isolated from Earthworm Gut (*Eisenia foetida*) Against Plant Pathogenic Bacteria and Fungi

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Abstract: This is the first report on antagonistic activity of a new strain of *Streptomyces tritolerans* (strain AS1) isolated from earthworm gut (*Eisenia foetida*) against plant pathogenic bacteria and fungi. Actinomycetes particularly *Streptomyces* sp. enhance soil fertility and have antagonistic activity against wide range of soil borne plant pathogens which had received special attention. The main objective of the study was to isolate and screen for antagonistic activity exhibiting actinomycetes from an earthworm gut. In search for metabolites of Actinomycetes having antibacterial and antifungal activity against cosmopolitan plant pathogens 20 isolates were screened. Among all, strain AS1 that was identified as a new strain of *S. tritolerans*, showed high level of activity in dual culture bioassay and well diffusion methods. AS1 was discovered to be a novel strain of *Streptomyces tritolerans* sp. and the 16S rRNA gene sequence was deposited at GenBank with accession no EU623448. Ethyl acetate extract obtained from AS1 strain was tested for its antagonistic potentiality against test organisms (phytopathogenic bacteria and fungi) and showed good inhibition levels (20-40 mm). Due to its Antimicrobial potentiality on wide range of plant pathogens, *Streptomyces tritolerans* (strain AS1) would play an important role in integrated biocontrol programmes.

Key words: *Eisenia foetida*, *Streptomyces* sp., 16S rRNA analysis, bioassay, antifungal activity

INTRODUCTION

Investigation of the interaction of actinomycetes with soil invertebrates is one of the ways to study the development in biogeocenoses. At present the literature contains sufficient evidence of the presence of actinomycetes in the gut of soil invertebrates (in particular earthworms). Many scientists have studied the microbial community in the gut of earthworms (Fischer *et al*., 1995; Karsten and Drake, 1995). Mostly the population density of actinomycetes in the intestinal tract of invertebrates was studied by Szabo (1974), Chu *et al.* (1987) and Toyota and Kimura (2004) studied widely on microbial community indigenous to the earthworm *Eisenia foetida*.

To better understand the role of earthworms in nature and their potential usage, the role of earthworm intestinal microorganisms must be rigorously defined. However, little attention has been paid on the role of earthworm intestinal microorganisms that were not exploited and elucidated.

At the present time, with several thousands of described microbial metabolites, strategies must be introduced into the screening programmes to increase the chances of discovering the novel

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compounds (Bull *et al*., 2000; Lacini *et al*., 1995; Busti *et al*., 2006). The filamentous actinomycetes account for a significant fraction of microbial metabolites and among them, *Streptomyces* is so far the most prolific genus (Watve *et al*., 2001).

Streptomyces species generally synthesize a sizeable number of diverse natural secondary metabolites, the best known of which are antibiotics currently used world wide as pharmaceutical and agricultural products (El-Naggar *et al*., 2003; Pamboukian and Facciotti, 2004; Ben-Fguria *et al*., 2005; El-Naggar *et al*., 2006). Two thirds of commercially available antibiotics (Miyadoh, 1993) and approximately 60% of those used for agricultural purposes were isolated originally from *Streptomyces* species (Tanaka and Mura, 1993).

There are several theories, which may explain antibiotic production; the most widely accepted one being that Antimicrobial compounds help the organism to compete with other organisms in relatively nutrient-depleted environment by reducing competition. Over 50 main antibiotics have been isolated from *Streptomycetes* species, including streptomycin, neomycin, chloramphenical and tetracyclines (Keiser *et al*., 2000).

In modern agriculture, pesticide application is still an invaluable and effective method to control plant diseases. However, since the use of agrochemicals is falling in to disfavor because of environmental pollution and detrimental effects on a variety of non target organisms, potential use of microbes based biocontrol replacement or supplements for agrochemicals has been addressed in many reports (Shimizu *et al*., 2000; Zamanian *et al*., 2005).

With the increased concern about conserving natural resources as air, soil and water, natural or biological control of plant diseases has received increased emphasis (Dingra and Sinclair, 1995; Aghighi *et al*., 2004).

With respect to their role in biological control presently we focused on the isolation and screening of Actinomycetes exhibiting broad range of antagonistic activity against plant pathogenic bacteria and plant pathogenic fungi from earthworm gut (*Eisenia foetida*).

MATERIALS AND METHODS

Collection of Earthworms and Sampling Procedure

Earthworms (*Eisenia foetida*) were collected from Farmyard Manure (FYM) around Tirupati region, Andhra Pradesh, India, 2006. Earthworms were washed with sterile tap water and then placed on a sterile petriplate moistened with filter paper and subjected to starvation for 24 h, further they were disinfected with 70% ethanol, gut content was dissected out, weighed and homogenized (for 5 min with a vortex mixture) in sterile 0.85% NaCl solution for dilution plate method.

Isolation of Actinomycetes from Earthworm Gut

For isolation of Actinomycetes, the gut content (1 g) was suspended in 10 mL of sterile 0.85% NaCl solution, serially diluted (10^{-1} to 10^{-5}) and subjected to centrifugation at 500 rpm for 20 min to disperse the spore chains if any. Then the resultant suspension was allowed to settle for almost 1 h. The clear culture supernatant was plated on to Starch Casein Agar (SCA) (Okazaki and Okami, 1972) supplemented with cycloheximide, streptomycin and nystatin (50 µg mL^{-1}) to inhibit the normal bacterial and fungal microflora. The plates were then incubated at 28°C for 7-14 days until sporulation of culture occur. Three replicates were considered for each dilution. The isolated strains were then screened for their antagonistic activity. Pure cultures were maintained on Glucose Yeast Extract-Malt extract Agar (GYMA) (ISP 2) (International Streptomyces Project (ISP; Shirling and Gottlieb, 1966) and ISP5 (Glycerol Aspargine Agar) agar at 4°C and as glycerol suspensions (20%W/V) at -20°C.

Test Organisms

Two bacterial cultures *Bacillus cereus* (MTCC 430), *Xanthomonas capsicii*, were used to determine the antibacterial activity of the isolated *Streptomyces* strains. Cultures were obtained from IMTECH, Chandigarh, India.

Nine fungal cultures (*Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliformae*, *Fusarium udum*, *Macrophomena phaseolina*, *Rhizoctonia solani*, *Colletotricum capscii*, *Aspergillus flavus*) were used to determine the antifungal activity of the isolated *Streptomyces* strains. Cultures were obtained from D.O.R Agricultural University, Rajendranagar, Hyderabad.

***In vitro* Screening of the Isolates for Antagonism**

Dual Culture Bioassay

Screening for the antifungal activity of antagonistic strains was carried out following the dual culture bioassay. For this fungal mycelial disks (diameter of 6 mm) prepared from growing margin of cultures of test fungal isolates were placed in the center of PDA plates and allowed to grow for 2-3 cm in diameter. A loop full of actinomycetes culture was taken from well-grown streaks of culture and placed at the periphery of the plate and incubated at 28°C for 4-5 days (modified method of Dhingra and Sinclair, 1995).

The level of inhibition at dual cultures was calculated by subtracting the distance (mm) of fungal growth in the direction of antagonistic colony (D) from the growth radius (R) of control culture to give $I = D - R$ (Aghighi *et al*., 2004).

Where, 5-9 mm (+) weak inhibition 10-19 mm, (++) (moderate inhibition);
>20 mm, (+++) strong inhibition.
Controls included fungal mycelial plugs in center of PDA plates.

Well Diffusion

The actinomycetes isolates were cultivated for 7-14 days using ISP2 (GYMA) medium at 28°C. A disk of 0.4 cm diameter of this agar culture was transferred to 250 mL Erlenmeyer flasks containing 50 mL of GYMA liquid medium. The inoculated flasks were kept on a rotary shaker (200 rpm) at 28-30°C/5 days. The broth was filtered through whatman filter paper No. 1.

The plates seeded with test organisms were punctured with sterile cork borer to make wells (8 mm in diameter). Culture filtrate suspension (0.1 mL) was transferred to each well under aseptic conditions and incubated at 28°C. The incubation period was 2 days in the case of bacteria and 3 days in the case of fungi. The antimicrobial activity of the actinomycetes isolated was detected as clear zone of inhibition around wells and it was measured in millimeters (mm) (Rifaat *et al*., 2006).

Identification and Characterization of Active Isolates

The cultural and morphological characteristics of the isolates were studied in accordance with the guidelines established by the International Streptomyces Project (Shirling and Gottlieb, 1966) and Bergey's Manual of Systemic Bacteriology (Locci, 1989). Morphological properties were examined by light microscopy (Olympus microscope) and Scanning Electron Microscopy following desiccation of cells and gold coating, using JEOL model JSM 5600, JAPAN (Bozzola and Russell, 1998). A range of phenotypic properties was examined using standard procedures (Williams *et al*., 1983). The utilization of sole carbon and nitrogen sources was carried using the media and methods described by Shirling and Gottlieb (1966). Resistance to antibiotics was examined by incorporating Rifampicin (5 μg mL^{-1}, PencillinG (10 μg mL^{-1}), Ampicillin (10 μg mL^{-1}) and Amoxicillin (10 μg mL^{-1}) in to glucose yeast extract agar (Lechevalier and Lechevalier, 1970; Lechevalier *et al*., 1971) as basal medium. Other physiological and biochemical characteristics were determined by the methods described by Shirling and Gottlieb (1966).

16S rRNA Gene Sequencing

The genomic DNA was isolated from the strain AS1 according to Takagi *et al.* (1993). The 16S rRNA gene was amplified by PCR using universal primers and procedures described by Weisburg *et al.* (1991). Amplification reactions were performed on an automated thermocycler (Biorad, USA) using 1 μL Taq polymerase and the recommended buffer system. In a final volume of 50 μL, containing 5 μL of 10X Taq buffer, 1 μL of dNTP (10 mM), 2 μL of primer 1 and 2, 2 μL of template DNA, 1 μL Taq polymerase and distilled water (39 μL) made up to 50 μL, reactions were carried out. Amplification profile is as follows: 94°C for 5 min followed by 30 cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min and 30 sec and a final extension of 15 min. Amplified DNA was purified using QIAGEN PCR purification kit and was sequences at Genie (Banglore, India). Multiple alignments with a selection of 16SrRNA gene sequences were used and calculation of levels of sequence similarity were carried out using CLUSTALW. Similarity searches of the sequences obtained were performed using BLAST (Altschul *et al.*, 1990). A phylogenetic tree was constructed using the neighbor-joining method of Saitou and Nei (1987).

Nucleotide Sequence Accession Number

The 16S rRNA gene sequence of strain AS1 determined in this study has been deposited in GenBank under the accession number EU623448.

Antagonistic Activity of Cultures Against Various Fungal Pathogens in Liquid Medium

The potential antagonistic activity of the cultures was also tested in liquid media. For this fungal culture were grown along with the antagonist strain in 50 mL of Czapek's Broth (CZB) in 250 Erlenmeyer flasks. For this fungal agar disk of 5 mm diameter and 0.5 mL of mycelial suspension of antagonist were inoculated into 50 mL of CZA and incubated for 4 days at 28°C. The difference in dry weight between the mycelia grown with and without the actinomycete culture was measured according to Yuan and Crawford (1995). For this, cultures were passed through pre weighed whatman No. 1 filter paper and were dried overnight in an oven at 60°C and re weighed. Dry weights of fungal cultures were calculated and compared.

All the above experiments were carried out in triplicates.

Extraction of Compound and Testing its Antagonistic Activity

The fresh actinomycete culture (AS1) showing high antagonistic activity was inoculated in to starch casein broth and incubated for 7-12 days at 28°C shaking at 150 rpm. The liquid was separated through filtration with whatman No. 1 filter paper. Then the filtrate was extracted twice with ethyl acetate. The filter paper disks (8 mm diameter) were impregnated with ethyl acetate extract dried and was placed on to the plates seeded with test organisms and the activity was detected by disc diffusion assay (Bauer *et al.*, 1966). The plates were incubated at 28°C for 24-48 h and zone of inhibition was observed.

RESULTS AND DISCUSSION

Screening for Antagonistic Activity Exhibiting Strain

Of the 20 actinomycetes obtained from the earthworm gut, 10 isolates (50%) of the total strains exhibited antimicrobial activity after screening against test organisms by well diffusion and dual culture bioassay. Among them AS1 isolate showed high antifungal and antibacterial activity against test organisms in comparison to other isolates exhibiting antimicrobial activity.

Isolate AS1 showed good antibacterial activity against, *Xanthomonas capsicii* and *Bacillus cereus*, the zone of inhibition was observed to 32 and 35 mm, respectively. Antifungal activity was effective against all the test fungal pathogens tested (Table 1).

Table 1: Antifungal activity of antagonists against phytopathogenic fungi by dual culture bioassay

	Isolates								
Test organism	AS1	AS2	AS3	AS4	AS5	AS6	AS7	AS8	AS9
F. oxysporum	+++	++	+	-	++	+	+++	+	++
F. udum	+++	++	++	-	+	++	-	++	+
F. solani	+++	++	++	-	++	+	++	+++	-
F. moniliformae	++	+++	+	-	+	-	+	++	+
A. alternata	+++	++	-	+++	++	+	-	+	++
R. solani	+++	++	+++	++	-	++	++	+	+++
M. phaseolina	+++	++	-	+	++	+++	+	-	++
A. flavus	++	+++	++	++	+	++	++	+	-
C. capscii	+++	++	+	-	++	+	++	-	-

5-9 mm, +: Weak inhibition, 10-19 mm, ++: Moderate inhibition and I >20 mm, +++: Strong inhibition

Identification and Characterization of Active Isolates

Actinomycetes were isolated from an earthworm gut (*Eisenia foetida*) and all the 10 isolates showing antagonistic activity have shown the morphological and physiological characteristics typical of *Streptomyces* (Anderson and Wellington, 2001; Locci, 1989; Williams *et al*., 1989; Shirling and Gottlieb, 1966). The potent isolate AS1 showing broad-spectrum antagonistic activity was further characterized. The detailed physiological and biochemical properties of AS1 strain were shown in Table 2 and Fig. 2A and B. The isolate AS1 is a new strain of *S. tritolerans* sp. isolated from earthworm gut which exhibits broad spectrum of antagonistic activity against wide range of plant pathogenic fungi and bacteria.

16S rRNA Gene Sequencing

16S rRNA analysis (partial sequencing) was performed and the results indicated it to be *Streptomyces tritolerans* sp which was close homologue of *S. tendae*. The 16srRNA sequence of AS1 was deposited at GenBank with accession number EU623448 (Fig. 1).

The 16S rRNA gene sequence of the strain AS1 was genotypically similar to *Streptomyces tritolerans* DAS165^T 16S rRNA gene (Syed *et al*., 2007) but showed some unique phenotypic features, which were distinct from strain *Streptomyces tritolerans* DAS165^T such as production of diffusible pigments with varied colors in different medias, morphological (Spore chain morphology-Retinaculiperti-spirals, Spore surface-smooth to warty, Reverse side pigments-olive green to dark green) physiological (pHrange 6-10) and biochemical properties (Urease + ve, L-arginine was utilized, Denitrification +ve). Based on characteristic features observed it was assigned as new strain of *Streptomyces tritolernas* sp. named as *Streptomyces tritolerans* AS1.

Inhibition of Fungi in Liquid Medium by Active Isolates

The effect of AS1, AS2 and AS6 strains in inhibiting the fungal growth was also studied in terms of dry weight. After 4 days of incubation the dry weight of fungal mycelium in three cultures was calculated according to Yuan and Crawford (1995). The dry weight of mycelia grown without any actinomycete culture was taken as control. All the fungal cultures showed reduction in dry weight when grown with AS1, AS2 and AS6 strains. AS1 showed good reduction in the dry weight of mycelium tested when compared to other strains AS2 and AS6 (Table 3). There was more than 60% of reduction in dry weight of the test fungal culture grown with AS1 strain when compared to control. Substantial reduction in dry weights of fungi by AS1 strain must be due to antagonistic nature of isolate and it proves its strong antagonistic activity against fungal pathogens tested.

Activity of Ethyl Acetate Extract on Test Fungal Isolates

Ethyl acetate extract of AS1 strain was tested for its antagonistic activity and showed high activity against *F. udum*, *F. oxysporum*, *R. solani*, *F. solani*, *A. alternata*, *C. capscii* and moderate

Table 2: Morphological, physiological and biochemical characteristics of *Streptomyces tritolerans* strain AS1

Characteristics	
Spore mass	White to Grey
Spore chain morphology retinaculiperti-spirals	
Spore surface	Smooth to warty
Reverse side pigments	Olive green to Dark Green
Diffusible pigments	+
Melanin pigments production	+
Growth at	
15°C	-
45°C	+
50°C	+
NaCl (7%)	+
PH range	6.0-10.0
Hydrolysis of	
Casein	+
Cellulose	+
Pectin	-
Starch	+
Gelatin	+
Chitin	+
Catalase activity	+
Oxidase activity	+
Lipase activity	+
Urease activity	+
Denitrification activity	+
Indole test	+
Methyl red test	+
Voges-prausker test	-
Citrate utilization	+
Arginine dehydrolase test	+
Antagonistic to	
B. subtilis	+
S. aureus	+
M. luteus	+
C. albicans	ND
Resistance to	
Rifampicin	+
Pencillin G	+
Ampicillin	+
Amoxicillin	-
Utilization of	
Sucrose	+
Raffinose	+
D-Mannitol	+
L-Rhamnose	-
Maltose	+

Table 3: Ability of *Streptomyces tritolerans* AS1 to inhibit the mycelial growth of fungi in liquid medium

	Average dry weight[a] (mg) ±SD	
Test fungus	Control	AS1
M. phaseolina	46.6±6.05	18.0±2.41[b]
Mucor	37.6±6.93	11.8±1.41[b]
A. alternate	45.1±3.61	24.9±2.75[b]
A. flavus	28.3±3.56	19.9±5.44[b]

[a]: The value of each treatment is the average dry weight of three mycelial mats. [b]:Significant reduction (p<0.05 in dry weight of fungal mycelia between the control and AS1

activity against *F. moniliformae*, *M. phaseolina*, *A. flavus*. The inhibition values observed were ranged from 20-40 mm. Compound extracted from this strain found to have a potential impact on biocontrol of plant pathogens. Antifungal activities of AS1 strain against all the tested plant pathogenic bacteria and fungi highlights it as useful candidate for further investigation in biocontrol of plant pathogens.

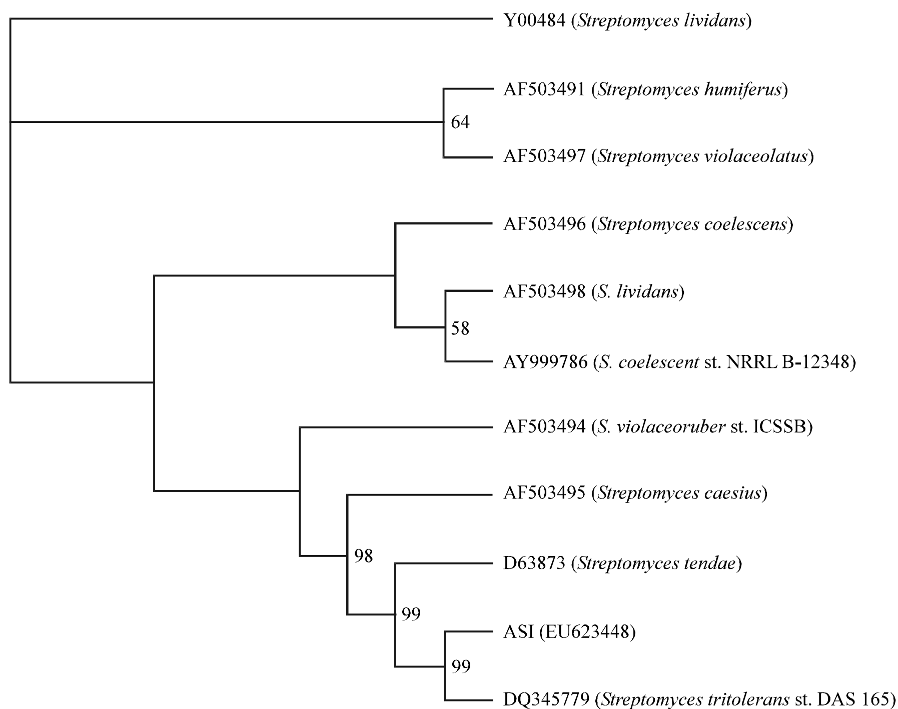

Fig. 1: Phylogenetic tree of AS1 isolate by Neighbour Joining method

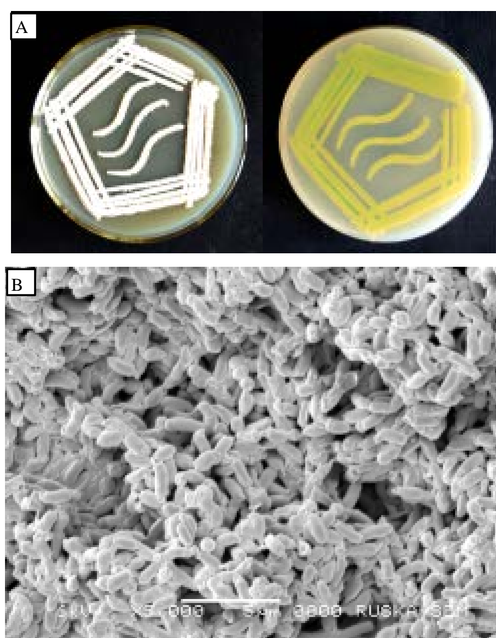

Fig. 2: (A) Aerial and substrate mycelium of *Streptomyces tritolerans* AS1 isolate. (B) Scanning electron micrograph of *Streptomyces tritolerans* AS1 isolate

The data presented here provide the first conclusive evidence for the widespread and persistent occurrence of indigenous *Streptomycetes* population in earthworm gut exhibiting antimicrobial activity. In the present study we have screened for actinomycetes exhibiting antimicrobial activity from the earthworm gut (*Eisenia foetida*). The earthworm gut is favorable for the development of actinomycetes due to neutral pH and optimal humidity and temperature. Our study of interaction of actinomycetes with soil invertebrates is significant. In addition, the increased organic carbon and nitrogen content in earthworm gut may also stimulate microbial activity (Karsten and Drake, 1995).

Biological control methods offer an alternative to hazardous chemicals for controlling plant diseases. Chemical measures have lost their attractiveness because of development of resistant strains of pathogens and due to undesirable effects on environment.

Thus it may be assumed that *Streptomyces tritolerans* sp. strain AS1 may be a useful candidate for agriculturally important crop plants for increased tolerance against the tested cosmopolitan fungal pathogens. With this worldwide perspective, to help the soil ecosystem environmentally safe and non-chemical measures are to be developed for combating plant diseases.

CONCLUSION

In conclusion, this is the first report on antagonistic activity of new strain *Streptomyces tritolerans* sp. AS1 isolated from earthworm gut having wide application in agriculture. The obtained results also indicated that earthworm gut is promising and can be an important source of habitat adopted possessing antimicrobial activity. They should receive higher attention in research for biological controls worldwide.

REFERENCES

Aghighi, S., Shahidi, G.H. Bonjar and I. Saadoun, 2004. First report of antifungal properties of a new strain of *Streptomyces plicatus* (Strain101) against four Iranian phytopathogenic isolates of *Verticillium dahliae*, a new horizon in biocontrol agents. Biotechnology, 3: 90-97.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman, 1990. Basic local alignment search tool. J. Mol. Biol., 215: 403-410.

Anderson, A.S. and M.H.E. Wellington, 2001. The taxonomy of *Streptomyces* and related genera. Int. J. Syst. Evol. Microbiol., 51: 797-814.

Bauer, A.W., W.M.M. Kirby, J.M. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-497.

Ben-Fguria, L.F., S. Fosto, R.B. Mehdi, L. Mellouli and H. Laatsch, 2005. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated, *Streptomyces* sp. strain US80. Microbiol. Res., 156: 341-347.

Bozzola, J.J. and L.D. Russell, 1998. Electron Microscopy Principles and Techniques for Biologists. 2nd Edn. Jones and Bartlett Publishers, Sudbury, MA. ISBN:0763701920.

Bull, A.T., A.C. Ward and M. Goodfellow, 2000. Search and discovery strategies for biotechnology: The paradigm shift. Microbiol. Mol. Biol. Rev., 64: 573-606.

Chu, T.L., I.M. Szabo and I. Szabo, 1987. Nocardioform gut actinomycetes of Glomeris-Hexasticha Brandt (*Diplopoda*). Biol. Fert. Soils, 3: 113-116.

Dhingra, O.D. and J.B. Sinclair, 1995. Basic Plant Pathology Methods. 1st Edn. CRC Press, USA, ISBN 978-0-387-24145-6.

El-Naggar, M.Y., M.A. Hassan, W.Y. Said and S.A. El-Assar, 2003. Effect of support materials on antibiotic MSW2000 production by immobilized *Streptomyces violatus*. J. Gen. Applied Microbiol., 49: 235-243.

El-Naggar, M.Y., S.A. El-Assar and S.M. Abdul-Gawad, 2006. Maeroparamycin production by newly isolated *Streptomyces* sp. strain MAR01: Taxonomy, Fermentation, Purification and structural elucidation. J. Microbiol., 44: 432-438.

Fischer, K., D. Hahn, R.I. Amann, O. Daniel and J. Zeyer, 1995. *In situ* analysis of the bacterial community in the gut of earthworm *Lumbricus terrestris* L. by whole-cell hybridization. Can. J. Microbiol., 41: 666-673.

Karsten, G.R. and H.I. Drake, 1995. Comparative assessment of the aerobic and anaerobic microfloras of earthworm guts and forest soils. Appl. Environ. Mirobiol., 61: 1039-1044.

Keiser, T., M.J. Bibb, M.J. Buttner, K.F. Chater and D.A. Hopwood, 2000. General Introduction to Actinomycete Biology. Practical *Streptomyces* Genetics. 1st Edn., John Innes Foundation, England, ISBN 0-7084-0623-8, pp: 1-21.

Lacini, G., F. Parenti and G.G. Gallo, 1995. Antibiotics: A multidisciplinary Approach. 1st Edn. Plenum, New York, ISBN: 0306449242.

Lechevalier, A. and M.P.A. Lechevalier, 1970. Critical Evaluation of the Genera of Aerobic Actinomycetes. In: The *Actinomycetales*, Prasuer, H., (Eds.). Glustal Fischer, Verlag, Jena, pp: 393-405.

Lechevalier, H.A., M.P. Lechevalier and N.N. Gerber, 1971. Chemical composition as a criterion in the classification of actinomycetes. Adv. Applied Microbiol., 14: 47-72.

Locci, R., 1989. Streptomyces and Related Genera. In: Bergey's Manual of Systemic Bacteriology. Williams, S.T. (Ed.). Williams and Williams Company, Baltimore, pp: 2431-2508.

Miyadoh, S., 1993. Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. Actinomycetologica, 9: 100-106.

Okazaki, T. and Y. Okami, 1972. Studies on marine microorganisms. II. Actinomycetes in Sagami Bay and their antibiotic substances. J. Antibiot., 25: 461-466.

Pamboukian, C.R.D. and M.C.R. Facciotti, 2004. Production of the antitumoral retamycin during continuous fermentation of *Streptomyces olindensis*. Process Biochem., 39: 2249-2255.

Rifaat, H.M., N.H. Abd El Naser, S.M. Helmy and A.M. Ali, 2006. Taxonomical studies on certain streptomyces exhibiting antimicrobial activity isolated from Egyptian soils. J. Cult. Collections, 5: 16-24.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Shimizu, M., Y. Nakagawa, Y. Sato, T. Furumai, Y. Igarashi, H. Onaka, R. Yoshida and H. Kunoh, 2000. Studies on endophytic actinomycetes (I) *Streptomyces* sp. isolated from *Rhododendron* and its antifungal activity. J. Genet. Plant Pathol., 66: 360-366.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 313-340.

Syed, D.G., D. Agasar, C.J. Kim, W.J. Li, J.C. Lee, D.J. Park, L.H. Xu and X.P. Tian, 2007. *Streptomyces tritolerans* sp. nov., a novel actinomycete isolated from soil in Karnataka, India. Antonie van Leeuwenhoek, 92: 391-397.

Szabo, I.M., 1974. Microbial Communities in a Forest-Rendzina Ecosystem: The Pattern of Microbial Communities. 1st Edn., Akademiai Kiado, Budapest, ISBN 10: 9630500698, pp: 415.

Takagi, H., O. Shida, K. Kadowaki, K. Komataga and S. Vadka, 1993. Characterization of Bacillus brevis, with descriptions of *Bacillus migulansus* sp. nov., *Bacillus choshinensis* sp. nov., *Bacillus parabrevis* sp. nov and *Bacillus galactophiles* sp. nov. Int. J. Syst. Bacteriol., 43: 221-231.

Tanaka, Y.T. and S.O. Mura, 1993. Agroactive compounds of microbial origin. Ann. Rev. Microbiol., 47: 57-87.

Toyota, K. and M. Kimura, 2004. Microbial community indigenous to the earthworm *Eisenia foetida*. Biol. Fertil. Soils, 31: 187-190.

Watve, M., R. Tickoo, M.M. Jog and B.W. Bhole, 2001. How many antibiotics are produced by the genus streptomyces? Arch. Microbiol., 176: 386-390.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol., 173: 697-703.

Williams, S.T., M. Goodfellow and G. Alderson, 1989. Genus *Streptomyces* Waksman and Henrici 1943. 339[AL]. In: Bergey's Manual of Systematic Bacteriology. Williams, S.T., M.E. Sharpe and J.G. Holt (Eds.). Williams and Wilkins Company, Baltimore, ISBN 0-683-09061-5 pp: 2453-2492.

Williams, S.T., M. Goodfellow, G. Alderson, E.M.H. Wellington, P.H.A. Sneath and M.J. Sackin, 1983. Numerical classification of *Streptomyces* and related genera. J. Gen. Microbiol., 129: 1743-1813.

Yuan, W.M. and D.L. Crawford, 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. Applied Environ. Microbiol., 8: 3119-3128.

Zamanian, S., G.H. Shahidi Bonjar and I. Saadoun, 2005. First report of Antibacterial properties of a New strain of *Streptomyces plicatus* (strain 101) against erwinia carotovora from Iran. Biotechnology, 4: 114-120.