Science Arena Publications
Specialty Journal of Biological Sciences
Available online at www.sciarena.com
2017, Vol, 3 (2): 32-39

Streptomyces Banglaensis Sp.Nov., Isolated from Soils of Rajshahi, Bangladesh Producing Actinomycin D

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Abstract : The taxonomic position of a novel actinomycete strain, ANTS-1^T isolated from soil was clarified using a polyphasic approach. The strain was assigned to the genus *Streptomyces* based on its phenotypic and cultural characteristics. The isolate produced actinomycin D, an antitumor agent as a principal secondary metabolite. A partial sequence of 16S rDNA of this strain was determined and compared with other *Streptomyces* species in the NCBI database. Phylogenetic analysis based on partial 16S rDNA sequence indicated that strain ANTS-1^T was most closely related to *Streptomyces griseoplanus* NBRC 12779 (98.93%), *Kitasatospora arboriphila* HKI 0189 (97.35%) and *Streptomyces paucisporeus* 1413 (97.35%). However, the phenotypic properties and the nature of secondary metabolite produced by the strain ANTS-1^T differed from closely related species of *Streptomyces*. Thus, the strain ANTS-1^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces banglaensis* sp. nov. is proposed.

Keywords: Actinomycetes, *Streptomyces*, actinomycin D, novel species

INTRODUCTION

Streptomyces is the type genus of the family Streptomycetaceae covering around 600 known species (Anderson & Wellington, 2001). The genus was proposed by Waksman and Henrici (1943) for aerobic, Gram-positive, filamentous bacteria producing well developed vegetative hyphae (between 0.5-2.0 μm in diameter) with branches. The species belong to the genus Streptomyces constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immunomodulators, anticancer drugs, antiviral drugs, herbicides and insecticides (Rahman et al., 2011). Due to the technical improvement of screening methods for bioactive compounds from natural sources, thousands of compounds have been discovered and among them, majority were isolated from microbes (Demain & Sanchez, 2009). From the 22,500 biologically active compounds obtained from microbes, 45% are produced by actinomycetes (mainly *Streptomyces*), 38% by fungi, and 17% by unicellular bacteria (Berdy, 2005).

During the screening for antibiotic-producing organisms in collected soil samples of different parts of Rajshahi city, an actinomycete isolate was isolated producing a potent antitumor compound, actinomycin D. The strain was designated as ANTS-1^T, and identified as *Streptomyces* based on its distinctive morphologic

characteristics. This study is aimed to determine the taxonomic position of the bacterial strain ANTS-1^T by a polyphasic approach.

Materials and Methods

The strain was isolated on starch-casein nitrate-agar medium supplemented with cycloheximide (Demain & Davies, 1999) using serial dilution technique and maintained on yeast-extract glucose agar (Shirling & Gottlieb, 1966) slants at 4 °C. Spores from pure cultures were stored in 20% glycerol (v/v) at -20°C.

Morphology of the strain ANTS-1^T grown on yeast-extract glucose agar (YEGA) at 28°C was examined under light and scanning electron microscopy for the organization of mycelium and spore surface ornamentation. Growth and sporulation of strain ANTS-1^T were observed on standard media (Table 1); aerial spore-mass color, pigmentation of substrate mycelium and the production of diffusible pigments were recorded following incubation of the strain at 28°C after 7, 14 and 21 days. Peptone/yeast extract/iron agar and tyrosine agar (Shirling & Gottlieb, 1966) were used to score the production of melanin pigments.

Basal mineral salts agar (Hopwood, 1967) with 1% (w/v) sole carbon sources was used to assess for carbon utilization. Antibiotic resistance was determined at 28°C on YEGA medium (Shirling & Gottlieb, 1966), using the disc method (Al-Tai et al., 1999). The ability of strain ANTS-1^T to inhibit the growth of different bacteria was detected by using the modified cross-streak method (Williston et al., 1947) on yeast-extract-glucose-agar medium. Briefly, the isolate was streaked agar plates in a single line and the plates were incubated at 28ºC for 5 days to allow the isolate to secrete antibiotics into the medium. Properly diluted test organisms were cross-streaked along the line and incubated for 12 hrs at 37ºC.

The genomic DNA was isolated from the strain ANTS-1^T according to the method described by Hunter (1988). The 16S rDNA was amplified by PCR using the forward (5´-ACCATGTGATCCTGGCTCAG-3´) and reverse (5´-AAGGAGGTGATCCAGCCGCA-3´) 16S primers. PCR products were detected by agarose gel electrophoresis and the band was excised and isolated using TAE buffer followed by purification by phenol chloroform extraction and ethanol precipitation. The purified DNA was then sequenced directly by dideoxy terminator cycle sequencing kit (Applied Biosystems) in an Applied Biosystems DNA sequences.

The partial sequence (568 bp) of the 16S rRNA gene of the strain ANTS-1^T was aligned with those of phylogenetically close reference strains retrieved from the GenBank (16S rRNA) and Bioinformatic Bacterial Identification (BIBI) database (Devulder et al., 2003) by using the program CLUSTAL_X (Thompson et al., 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura et al., 2007) with two treeing algorithms, maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods using bootstrapping with 1000 replicates. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There was a total of 561 positions in the final dataset. The nucleotide frequencies for the 16S rRNA gene sequence dataset were 0.218 (A), 0.174 (T), 0.248 (C), and 0.36 (G).

Submission of the 16S rDNA sequence to GenBank:

The partial sequence of the 16S rDNA of ANTS-1^T was submitted to GenBank and an accession no. JF812169 was assigned.

Results and Discussion:

The colonial morphology of strain ANTS-1^T conformed to the genus *Streptomyces* (Williams et al., 1989). The strain formed a highly-branched substrate mycelium and aerial hyphae that differentiated into long spiral

spore-chains (Fig.1). On standard media, the color of the substrate mycelium was grayish orange and that of the aerial spore mass was moderate brown (Table 1).

From the phylogenetic tree (Fig. 2) of the strain ANTS-1^T, it is evident that the strain was closely related to species of the family *Streptomycetaceae*. The highest similarity was found to *Streptomyces griseoplanus* NBRC 12779 with 98.93% nucleotide sequence identity. Other species that showed significant sequence identity include *Kitasatospora arboriphila* HKI 0189 (97.35%) and *Streptomyces paucisporeus* 1413 (97.35%). These values correspond to 4-12 nt differences out of 561 positions. The maximum-parsimony tree showed that strain ANTS-1^T is a sister species of *S. griseoplanus* with a bootstrap value of 68%. The tree resulting from the neighbour-joining (NJ) method yielded the same relationship between *Streptomyces banglaensis* ANTS-1^T and *S. griseoplanus* with a high bootstrap value of 89% (Fig. 2). The neighbor-joining dendogram showed that the clade composed by both species was a sister clade to the species *Streptacidiphilus oryzae* TH49 and *Streptomyces paucisporeus* 1413. Thus, the results indicate that the strain ANTS-1^T may be type strain of *S. griseoplanus.*

However, the strain had a number of phenotypic characteristics that distinguish it from the most closely related organism *S. griseoplanus*. As can be seen in Table 2, strain ANTS-1^T differs from *S. griseoplanus* by spore chain morphology, mature spore chains, utilization of carbon sources such as L-arabinose, D- xylose, raffinose, mannitol, inositol and sucrose as sole carbon sources and growth on standard medias such as ISP-4 and ISP-5. Moreover, these two organisms are different from each other by the production of secondary metabolite and soluble yellow pigments. Thus, this polyphasic taxonomic study suggest that strain ANTS-1^T should be recognized as a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces banglaensis* sp. nov.

Description of Streptomyces banglaensis sp. nov.

Streptomyces banglaensis (ban.gla.ensis. N.L. masc. adj. banglaensis belonging to Bangladesh, the source of the soil from which the organism was isolated).

Aerobic, Gram-positive actinomycete. Forms highly branched substrate mycelium and aerial hyphae that differentiate into long spiral chains containing more than 20 spores. Aerial spore-mass colour is moderate brown and substrate mycelium is grayish orange on standard media. Yellowish-orange diffusible pigments are formed. Melanin pigments are not produced on peptone/iron or tyrosine agars. Utilizes glucose, sucrose, inositol, mannitol, lactose, starch and methyl cellulose as sole carbon sources. Utilizes L-aspargine and L-tyrosine as sole nitrogen source. Growth occurs in the presence ampicillin, penicillin**,** cephradine**,** ceftazidime and ceftriaxone and produces actinomycin D, an antitumor compound as principle metabolite. The secondary metabolite produced by the strain exhibits strong antibacterial activity against *Bacillus cereus*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Escherichia coli* but weak activity against yeast *Candida albicans* and mold *Aspergillus flavus*.

The type strain is ANTS-1, was isolated from soil samples collected from Rajshahi, Bangladesh.

Acknowledgements

This work was partly supported by the National Science and Information and Communication Technology (NSICT) fellowship funded by The Ministry of Science and Communication Technology, Govt. of Bangladesh. We are very grateful to Dr. Apurbo Kumar Roy for the light microscopy images.

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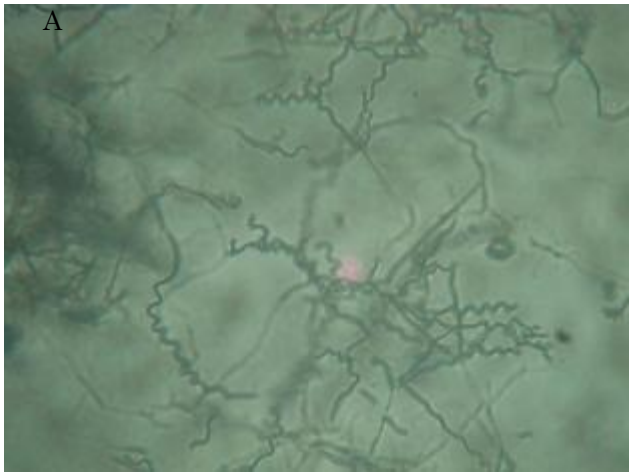
A



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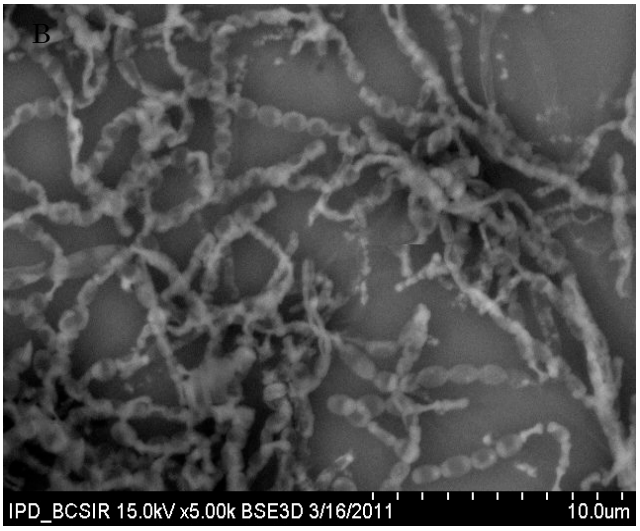

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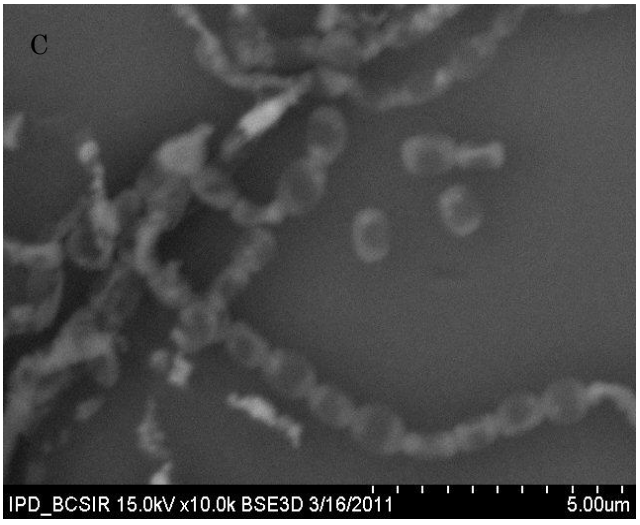

Fig. 1. Optical (A) and scanning electron micrograph (B and C) of the strain ANTS-1 showing spiral aerial mass with rounded, smooth spore chains. The organism was grown on yeast-extract glucose agar for 21 days at 28°C.

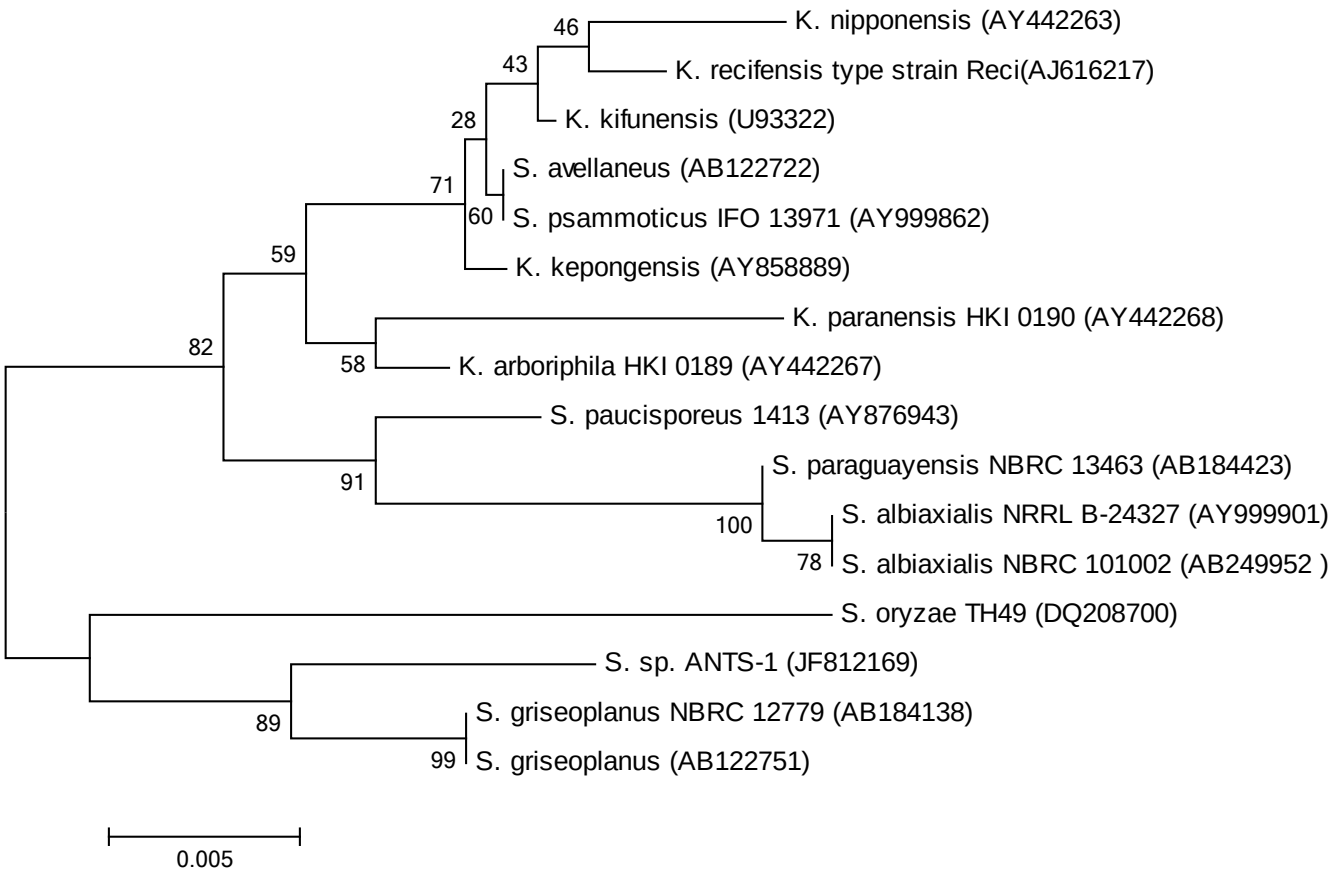

Fig. 2. Phylogenetic tree derived from 16S rRNA gene sequences showing the relationships between strain ANTS-1 and closely related species of the genus *Streptomyces*. The tree was constructed by using the neighbour-joining method and was based on a comparison of 561 nucleotides. Bootstrap values are expressed as percentages of 1000 replications. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Bar, 0.005 substitutions per nucleotide position.

Table 1. Growth and some phenotypic characteristics of strain ANTS-1 on different media

Agar medium	**Growth**	**Aerial mycelium**	**Substrate mycelium**	**Yellow Pigmentation**
YEME (ISP 2)	+++	Grayish orange	Moderate brown	+++
Oatmeal (ISP 3)	++	Medium bluish gray	Dark yellowish orange	++
Starch (ISP 4)	+++	Medium bluish gray	Dark yellowish orange	+++
Glycerol/asparagine (ISP 5)	+	Grayish yellow	Moderate yellow	+
Tyrosine (ISP 7)	++	White	Beige	-
TYE (ISP 1)	++	Light gray	Dusky yellow	+
Nutrient	+++	Light gray	Dusky yellow	-
Czapek-Dox (acidic)	+++	Deep gray	Moderate brown	+++
Czapek-Dox (basic)	++	Medium bluish gray	Dark yellowish orange	++
YEGA)	+++	Gray	Moderate brown	+++

+++, Good growth; ++, Moderate growth; +, poor growth

Table 2. Comparison of phenotypic properties of strain ANTS-1 and *Streptomyces griseoplanus*

Property	ANTS-1	*Streptomyces griseoplanus*
Spore chain morphology	Spirales	Flexuous to spiral
Mature spore chains	More than 20 spores per chain	Generally, 10 to 50 spores per chain
Spore surface	Smooth	Warty
Secondary -metabolite	Actinomycin D	Anticapsin, Alazopeptin
Diffusible pigment production		-
ISP-2	Yellowish Orange	-
ISP-3	Yellowish Orange	-
ISP-4	Yellowish Orange	
Growth on sole carbon sources		
L-arabinose	-	+
D-xylose	-	+
Raffinose	-	+
D-fructose	-	+
Mannitol	+	-
Inositol	+	-
Sucrose	+	-