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Original Research Article

Isolation and characterization of *Streptomyces* spp collected from Bangladeshi soils on the basis of morphological and biochemical studies

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K e y w o r d s

Identification, Isolation, *Streptomyces*

A B S T R A C T

Streptomyces, the largest genus of Actinobacteria, is a group of bacteria which is most commonly isolated from soil. In this study, total of 24 indigenous *Streptomyces* spp were isolated from soil samples collected from different parts of Bangladesh. Of them, two were studied in detail and characterized. Their provisional identification was done following the criteria of Bergey's Manual of Systematic Bacteriology. The morphological and biochemical characterization and tentative identification methods were described.

Introduction

Streptomyces is the well-known genus of actinomycetes which is represented in nature by the largest number of species and varieties. Soil is the main source of actinomycetes (Takahashi and Omura, 2003). Although this group of bacteria can be found in aquatic habitats, they are mainly transient in nature. From the beginning of the 20th century, the genus *Streptomyces* has become very impotent for the production of antibiotics in controlling human ailment as well as animal and plant diseases specially crop diseases. Almost all *Streptomyces* spp. have been proved to be antibiotic producers. From economic and medical view points, extensive researches have been carried out worldwide to screen antibiotic producer *Streptomyces*.

The intense screening of soil for *Streptomyces* spp from the 1940s onward led to the discovery of many new chemical compounds including antibiotics and other bioactive substances. As interest in a particular product intensified so did the interest in the identity of the producing microorganisms. Since the late 1980s the search for new antibiotics has been downsized in many companies to accommodate interests in noninfectious diseases. Nonetheless products from *Streptomyces* spp are being evaluated in new detection systems. However, researchers in this field assume that only 10% of the total microbial population of the earth has been isolated and characterized (http//:globalscience.com). So, still there is opportunity to isolate and identify new

Streptomyces species and new compounds from this genus.

In an attempt to do these different researchers considered different characters such as morphology, cultural criteria such as growth on different media, biochemical characters like utilization of carbon sources etc. However, there is a big gap between traditional approaches and modern methods for the classification and identification of Streptomycetes (Antonieta Taddei *et al.*, 2006).

So their classification is still an important issue for the taxonomists as well as for the researchers who are working with this genus. The aim of our study is to isolate and characterize new *Streptomyces* spp using traditional morphological and biochemical characteristics.

Materials and Methods

Purification, maintenance and preservation of the organisms

A total of twenty-four isolates of indigenous *Streptomyces* spp were isolated from soil samples. Of them, two were selected for detail study designated as isolate no S-31 and S-109. The *Streptomyces* spp were purified initially by streak plate technique (Williams and Cross, 1965) on Oatmeal agar medium (Fig. 2). After purification these isolates were maintained throughout the study on Oatmeal agar slants.

The tubes were wrapped with polythene bags & kept in a refrigerator at 4°C for the purpose of preservation for a short time. Periodic transfers of the species were done at 15 day intervals. For long term preservation, the isolates were kept at - 20°C in 20% glycerol broth (Waksman and Henrichi, 1948).

M**orphological and cultural characteristics determination**

Gram staining (Hucker and Conn's, 1923 modified method), Acid-fast staining (Ziehl Neelson's, 1883) and cover slip culture technique (Kawato and Shinobu, 1959) were used to determine morphological characteristics. . Some special media (ISP media) such as ISP-1, ISP-2, ISP-3, ISP-4, ISP-5, ISP-6 and ISP-7 formulated by the collaboration in the "International *Streptomyces* Project" (ISP) were used to determine the growth responses and chromogenesis of the selected isolates.

Biochemical screening

The bio-chemical activities of the selected species were determined by a series of biochemical tests such as IMVIC tests; Nitrate Reduction test; Test for gelatin hydrolysis; Test for starch hydrolysis; Lipid hydrolysis test; Test for esquilin hydrolysis; Catalase test; Fermentation tests; General fermentation test etc.The utilization of different carbon sources as well as production of melanin were also studied.

Physical properties

Temperature tolerance and pH requirement of the selected species were determined by growing them at different temperature such as 10, 20, 30, 37 and 45^0C and over a wide range of pH from 4.0 to 9.0 on Bennett agar (Fig. 1).

Results and Discussion

The microscopic studies and staining properties of selected *Streotomyces* Spp (S-31 and S-109) (Fig. 3) showed that both of them were Gram positive and non-acid fast and they had filamentous, branched and coenocytic mycelia (Table 1). The growth

patterns, amount of growth, aerial mass color, reverse color and soluble color of the selected species on different ISP media were observed and recorded.**(**Table 2 and 3). The biochemical test result showed that they were catalase positive and responded positively to nitrate reduction and both were esqulin and starch hydrolyzer .There was negative response in indole production and Voges-Proskauer tests in case of both species (Table 4).

In general fermentation tests, several carbohydrates such as lactose, mannitol, maltose, sucrose, glucose, and salicin were added to the nutrient broth in presence of phenyl red indicator to observe the fermenting capability of the *Streptomyces* spp. and the observations were summarized (Table 4). The tests for carbon utilization by the organisms were performed. The carbon sources employed were sucrose, fructose, mannitol, arabinose and xylose. Glucose was used as positive control and only basal agar medium without any carbohydrate was used as negative control.

The selected *Streptomyces* spp. were grown at different temperature; both were able to grow at 10°C and 45°C. They were mesophilic, growing best at 30° to 37°C.The selected species behaved as neutrophilic in culture, growing between p^H 4.0 and 9.0 with an optimum closeness to neutrality.

Twenty four indigenous *Streptomyces* spp. from the soil samples were isolated and two of them(S-31 and S-109) were selected and characterized based on their morphological, physical, cultural and biochemical properties with the help of Bergey's Manual of Systematic Bacteriology (Locci 1989).These selected species produce aerial mycelium of various colors such as gray, white, ash, brown which can be easily detected with naked eyes. They were found to be Gram positive and non-acid fast, which is one of the important criteria of the *Streptomyces* spp. Both of the species were studied morphologically and microscopically following cover slip culture on solid medium (Kowato and Shinobu, 1959). Microscopic observation revealed that the selected species showed better performance in the production of aerial or reproductive mycelia as well as sporulation on solid media.

The morphology of their substrate mycelium and aerial mycelium was studied. The vegetative mycelium was coenocytic. These species were found to produce filamentous, profusely branched mycelium with net like structure. Species of *Streptomyces* were characterized by the production of typical aerial mycelium super imposed upon the substrate growth. Aerial hyphae were found to vary considerably in length. The spore chain ornamentation of S-31 and S-109 was rectiflexible and retinocularaparti, respectively.

Biochemical characteristics of the selected S-31 and S-109 were analyzed (Table 4). Nitrate reduction and catalase tests were found to be positive in case of both species. They were found to be capable of hydrolyzing starch and esquilin but they could not hydrolyze lipid (Fig. 4). Both species were tested for their capability to ferment different types of carbohydrate such as lactose, mannitol, maltose, sucrose, glucose and salicin (Table 4). The results showed that all were capable of fermenting sugar without producing gas. S-31 produces no acid except lactose; S-109 produces no acid except mannitol (Fig. 5).

Growth characteristics of the S-31 and S-109 were studied on different ISP media. Results showed that ISP-3 (Oatmeal agar) was an excellent growth medium for the selected species.

Table.1 Microscopic studies and staining properties of the selected isolates

Isolate No	Vegetative Mycelium	Aerial mycelium	Gram reaction	Acid fast reaction
S-31	Filamentous, Branched Coenocytic	Rectiflexible	G(+ve)	Non acid fast
S-109	Filamentous, Profusely Branched, Coencocytic	Retinocularaparti	G(+ve)	Non acid fast

Table.2 Cultural characteristics of the selected isolates (S-31) on different media

Media name	Growth		Aerial mycelium color	Soluble pigment	Reverse color
	Range	Colony Properties			
Bennett Agar Plate	+++	Light brown, granular, spreading	Cream	_	Yellow
Yeast Extract - Malt Extract Plate	+++	Brown, Cottony, spreading	White	Brown	Brown
Oatmeal Agar Plate	+++	Gray, Cottony, spreading	Gray	_	White
Nutrient Agar Plate	+	Non-spreading	Cream	_	_
Bennett Broth	+++	Tobacco brown, Thick ring like pellicle, no sediment and turbidity	Gray	_	
Inorganic Salt starch Agar (ISP4) Plate	++++	Light Gray Spreading Powdery	Gray	_	Brown
Glycerol Asparagines (ISP5) Agar Plate	+++	Light Brown, Powdery, Non-spreading	Light Gray	Light Brown	Brown
Tryptone-Yeast Extract Broth (ISP1)	+++	Light Brown, Ring Like growth on the surface, turbid, no sediment	Gray	_	_

Highly growth (++++); good growth (+++); Weak growth (++); growth Rare/Trace growth (+); Not growth (-)

Table.3 Cultural Characteristics of the selected isolates (S-109) on different media

Media name	Growth		Aerial mycelium color	Soluble pigment	Reverse color
	Range	Colony Properties			
Bennett Agar Plate	+++	Brown , spreading, powdery	Gray	_	Brown
Yeast Extract - Malt Extract Plate	+++	Deep brown , spreading, powdery	Blackish brown	Brown	Blackish brown
Oatmeal Agar Plate	+++	Deep brown , spreading, powdery	Coffee	Light Brown	Brown
Nutrient Agar Plate	++++	Spreading	Cream	_	Butter Cream
Bennett Broth	++++	Yellow, Thick ring like pellicle, no sediment and turbidity	Gray	_	
Inorganic Salt starch Agar (ISP4) Plate	++++	Brown, Spreading, Powdery	Deep Gray	_	Brown
Glycerol-Asparagines (ISP5) Agar Plate	++	Brown, Non-Spreading, Granular	Brown	Light Brown	Brown
Tryptone-Yeast Extract Broth (ISP1)	++++	_	Thick ring like pellicle, sediment present	_	_

Highly growth (++++); good growth (+++); Weak growth (++); growth Rare/Trace growth (+); Not growth (-)

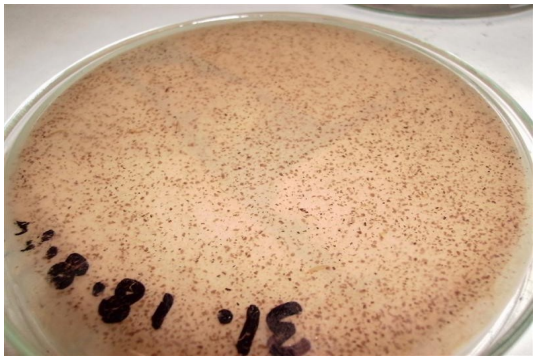

Figure.1 Growth isolate on Bennett agar plate

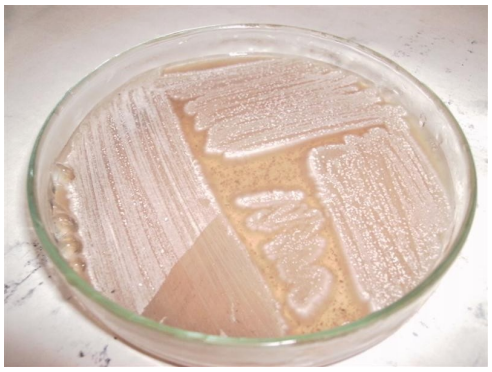

Figure.2 Growth isolates on oatmeal agar plate

Table.4 Biochemical properties of the selected isolate

Test parameter	Isolates name	
	S-31	S-109
Hydrolsis of starch	+	+
Hydrolsis of Lipid	-	-
Hydrolsis of Gelatin	+	-
Hydrolsis of Esqulin	+	+
Nitrate test	+	+
Catalase test	+	+
IMVIC test		
Indole test	-	-
MR test	-	-
VP test	-	-
Citrate test	-	-
Fermentation test		
Lactose	-	-
Manitol	-	-
Maltose	-	-
Sucrose	-	-
Glucose	-	-, g
Salicin	-	-
Carbon source		
Glucose	+++	+++
Sucrose	+	+++
Fructose	+++	+++
Mannitol	+++	-
Arabinose	-	-
Xylose	++	+++

Strongly positive utilization (++++); Positive utilization (+++); Weak Utilization (++); Utilization doubtful/Trace (+); Utilization negative (-); g= gas production.

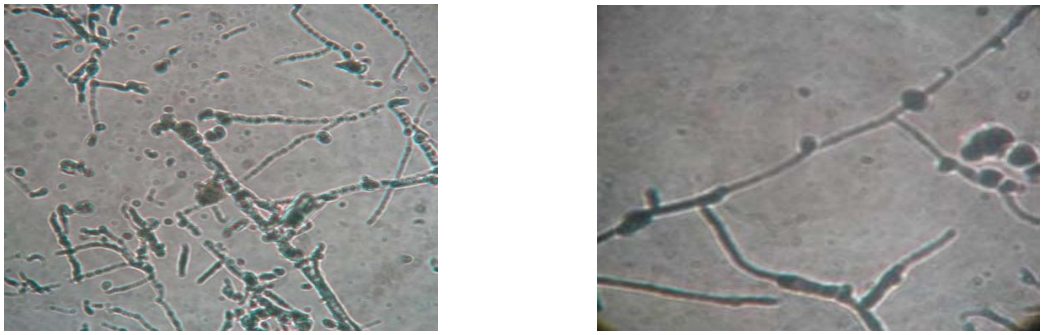

Figure.3 Microscopic observation of selected isolate (Left S-31 and Right S-109)

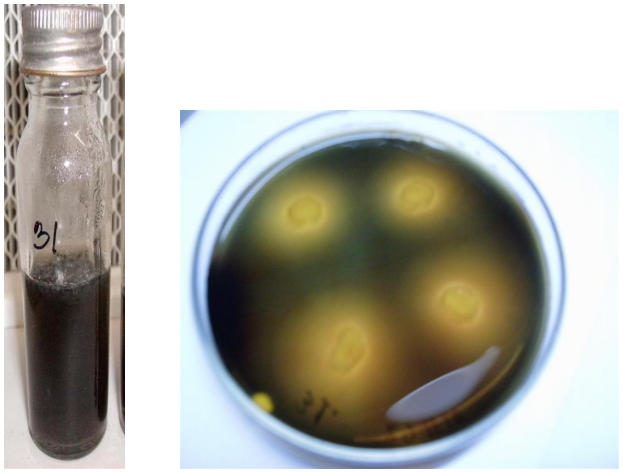

Figure.4 Esquilin test (Left: broth and Right: plate)

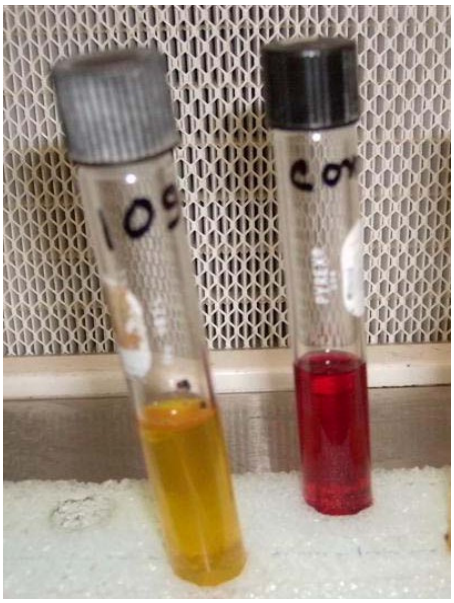

Figure.5 Utilization of sugar

After proper incubation, the isolates produced aerial mycelium. Results (Table 2 and 3) indicated that S-31 was in gray series while S-109 was in coffee series.

The color of the substrate growth is an important criterion for categorizing the *Streptomyces* spp. The reverse color of both species while growing on ISP media was studied (Table 2 and 3). Different *Streptomyces* spp. gave different substrate colors. The *Streptomyces* spp. was tested whether they can produce diffusible pigment on peptone-yeast extract iron agar (ISP-6) and tyrosine agar (ISP-7) or not. The results revealed that S-31 and S-109 produced diffusible pigment on ISP-6 medium; S-109 produced the darkest pigment on both media.

Temperature is one of the physical factors that governs the distribution and activities of *Streptomyces* spp. in natural habitats. Most species of them were mesophiles, growing at temperature between 10°C and 37°C. There were also thermotolerant and thermophilic species (Kutzner, 1981). A few species grew slowly at 4°C (William *et al.,* 1983). In our study, the selected *Streptomyces* spp. were grown at different temperature. Both were able to grow at 10°C and 45°C. Data revealed that they were mesophilic, growing best at 30° to 37°C

Most *Streptomyces* spp. behaved as neutrophilic in culture, growing between P^H 5.0 and 9.0 with an optimum closeness to neutrality. Acidophilic and acidoduric strains had been isolated from acidic soils and other materials (William and Davies, 1971; Khan and Williams, 1975; Hagedorn, 1976). In our study, the species were grown on Bennett agar adjusted at different P^H ranging from 4.0 to 9.0.

Overall the characteristics of the selected S-31 and S-109 species were similar to most of the reported *Streptomyces* spp. Further research is necessary for species identification as well as enzyme activity determination.

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