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Antagonistic Activity of Antibiotic Producing *Streptomyces* sp. against Fish and Human Pathogenic Bacteria

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ABSTRACT

In this study, attempts were made to isolate Streptomyces *sp. from soil samples of two different regions of Bangladesh and evaluate their antagonistic activity against fish and human pathogenic bacteria. A total of 10 isolates were identified as* Streptomyces *sp. based on several morphological, physiological and biochemical tests. Cross streak method was used to observe the antagonistic activity of the* Streptomyces *sp. isolates against different fish pathogens belonging to the genus* Aeromonas, Pseudomonas *and* Edwardsiella *and human clinical isolates belonging to the genus* Klebsiella, Salmonella *and* Streptococcus*. Seven* Streptomyces sp. *isolates showed antagonism against both fish and human pathogenic bacteria. Four isolates viz., N24, N26, N28 and N47 showed broad spectrum of antagonistic activity (80-100%) against all genera of fish and human pathogenic bacteria. The isolate N49 exhibited highest spectrum of antagonism against all fish pathogens (90-100%) but comparatively lower degree of antagonism against human pathogens (50-60%). Rest of the two isolates (N21 and N23) showed variability in their antagonism. Results showed that broad spectrum antibiotic(s) could be developed from the isolates N24, N26, N28 and N47against several human and fish pathogens. The isolate N49 could be a potential source of antibiotic, especially for fish pathogenic bacteria.*

Key words: *Streptomyces* sp., antagonistic activity, cross streak method, antibiotic

INTRODUCTION

Streptomyces are Gram-positive soil microbes represented as the largest number of species and varieties among the family Actinomycetaceae. *Streptomyces* species are the source of thousands of bioactive compounds and screening programs have shown that secondary metabolites can be isolated (Wendisch and Kutzner 1992; Santos 2012), which bind to active sites of enzyme organelles and receptors (William et al. 1983). *Streptomyces* sp. is responsible for the production 50% of clinically useful antibiotics (Miyadoh 1993).

Soil is a natural reservoir for the microorganisms with their antimicrobial products and provides an excellent resource for the isolation and identification of therapeutically important products (Dancer 2004). Among the soil microbes, *Streptomyces* sp. are the important group producing antibiotics of agricultural and medicinal importance and over 6,000 compounds have been reported to be produced by *Streptomyces* (Takahashi and Omura 2003; Kavitha et al. 2010). However, with the alarming increasing rate of drug resistance in pathogenic microorganisms throughout the world, the demand to discover newer and safer antibiotics with lesser side effects

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are increasing day-by-day (Gupte et al. 2002). But, the search for a new, safer, broad-spectrum antibiotic with greater potency has been progressing slowly. The present work was undertaken with an effort to isolate antibiotic producing *Streptomyces* from two different soil samples of Bangladesh and evaluate their antagonistic potentials against some fish and human pathogenic bacteria.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from two different regions of Bangladesh during June to August 2011. Samples were taken up from a depth of 10 cm form each area, placed in sterile plastic bags, transported to the laboratory under ambient condition and air-dried at room temperature. The samples were pretreated by drying at 70^{o}C for 1h in a hot air dryer.

Isolation of and enumeration of microbes from soil

For isolation of *Streptomyces* sp., serial dilution technique was followed using different aqueous dilutions (10^{-1} to 10^{-4}). Each diluted sample was inoculated by standard spread plate method on Starch-Casein Agar (SCA) medium containing (g/l) Glycerol 10, Casein 0.3, KNO_3 2.0, K2HPO_4 2.0, $MgSO_4$ 0.05, $CaCO_3$ 0.02, $FeSO_4$ 0.01, Agar 18 and distilled water 1L (pH 7.0± 0.1) (Kuster and Williams 1964). After incubation of the plates at 30^{o} C for seven days, typical *Streptomyces* sp. colonies were selected (Shirling and Gottlieb 1966) and total number of bacteria as well as total number *Streptomyces* colonies (presumptive colonies) were counted and expressed as colony forming unit per gram of soil sample (CFU/g). The selected colonies were re-streaked on *Streptomyces* Agar Medium (SAM) containing (g/l) Glucose 10, Beef extract 4.0, Peptone 4.0, NaCl 2.5, Yeast extract 1.0 and Agar 20 (Atlas 1997) and incubated at 30^{o} C to obtain the pure culture.

Identification of *Streptomyces* sp. isolates

Ten presumptive *Streptomyces* sp. isolates were characterized by morphological and physiological tests, which included colony characteristics (size, shape and color), Gram staining, presence of aerial mycelium, motility and spore formation, and biochemical tests, which included oxidase and catalase activity, H_2S production, Methyl Red test (MR), Voges-Proskauer Test (VP), acid production from carbohydrates (glucose, lactose and sucrose), Indole test, hydrolysis of casein and starch, utilization of different carbon and nitrogen sources. The bacterial isolates were identified following the Bergey's Manual of Determinative Bacteriology (Bergey and John 1994).

Test microorganisms

Both fish pathogenic bacteria (*viz.*, *Aeromonas* sp., *Pseudomonas* sp. and *Edwardsiella* sp.) and human clinical isolates (*viz.*, *Klebsiella* sp., *Salmonella* sp. and *Streptococcus* sp.) were used as test organism to determine the antagonistic activity of the *Streptomyces* sp. isolates. Ten isolates from each bacterial genus were used to evaluate the antagonistic activity of the *Streptomyces* sp. isolates. All of the test isolates were obtained from the USDA project laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh. The test organisms were cultured in Nutrient Broth (NB) at 27 and 37°C for 24h for fish and human pathogens, respectively.

Evaluation of antagonistic activity of *Streptomyces* sp. Isolates

The antagonistic activity of soil isolates was evaluated by Cross Streak method (Rahman et al. 2011). Each of the isolate was streaked on *Streptomyces* Agar Medium (SAM) as straight line and incubated at 30°C for six days. Then the plates were seeded with test organisms by a single streak at a 90° angle to the *Streptomyces* isolates and incubated at 37 and 27°C for 24 h for human and fish pathogenic bacteria, respectively. The antagonistic effect of *Streptomyces* sp. isolates on test organism was analyzed by the determination of size of inhibition zone. The percentage of antagonism of *Streptomyces* isolates to each genus of test organisms was estimated.

RESULTS AND DISCUSSION

Isolation and enumeration of microbes from soil

The colony forming unit (CFU) per gram of soil was estimated to know total bacterial load and load of presumptive *Streptomyces* sp. in different soil sample. The highest microbial count and *Streptomyces* count was 3.1×10^{6} CFU/g of soil and 2.8×10^{4} CFU/g of soil, respectively in the pond sediment sample of Sylhet region. The lowest

bacterial count was 0.8×10^6 CFU/g of soil in the soil sample collected from the hill of Sylhet (Table 1). In the present study, the highest load of *Streptomyces* sp. was obtained in the pond sediment sample of Sylhet region of Bangladesh. This pond was used for fish farming and was rich in organic compounds that might be the reason for highest count of *Streptomyces* sp.

Table 1 - Soil samples with their total bacterial count and *Streptomyces* count.

Origin of soil sample	Nature of soil sample	Total bacterial count (CFU/g of soil)	Total *Streptomyces* count (CFU/g of soil)
Agricultural land of Joypurhat	Water logged	1.8×10^6	0.7×10^4
Pond sediment of Joypurhat	Mud	2.3×10^6	1.9×10^4
Pond sediment of Sylhet	Mud	3.1×10^6	2.8×10^4
Hill of Sylhet	Sandy	0.8×10^6	1.4×10^4
Hill of Sylhet	Red- dry soil	2.1×10^6	1.1×10^4

Identification of *Streptomyces* sp. isolates

Baesd on the morphological, physiological and biochemical characteristics, all the isolates were identified as *Streptomyces* sp. All the isolates were Gram positive with aerial mycelium and most of them were filamentous with long chain of spores (Fig. 1A). The isolates were non-motile, catalase positive, oxidase positive, ureaese positive and positive for H_2S production, but negative for nitrate reduction, MR test and VP test. Detailed results of morphological, physiological and biochemical tests of the isolates are shown in Tables 2 and 3. Similar methods were followed by Shirling and Gottlieb (1966); Berd (1973) and Rahman et al. (2011) for the identification of *Streptomyces* sp. from soil.

Table 2 - Morphological and physiological properties of *Streptomyces* sp. isolates**.**

Isolates name	Colony characteristics			Gram staining	Aerial mycelium	Filament	Spore	Motility	Growth at 30^0-37^0 C
	Color	Size	Shape						
N21	Off-white	Small	Round	G+	+	+	+	-	+
N23	Off-white	Medium	Round	G+	+	+	+	-	+
N24	Brown	Medium	Round	G+	+	+	-	-	+
N26	Dark- brown	Small	Round	G+	+	+	+	-	+
N28	Off-white	Large	Round	G+	+	+	+	-	+
N32	Yellow	Small	Round	G+	+	+	+	-	+
N33	Brown	Medium	Round	G+	+	+	-	-	+
N36	Off-white	Medium	Round	G+	+	+	+	-	+
N47	Orange	Large	Round	G+	+	+	+	-	+
N49	Yellow	Medium	Round	G+	+	+	+	-	+

G+ = Gram positive, + = Positive/ Present, -= Negative/ Absent

Table 3 - Biochemical characteristics of *Streptomyces* sp. isolates.

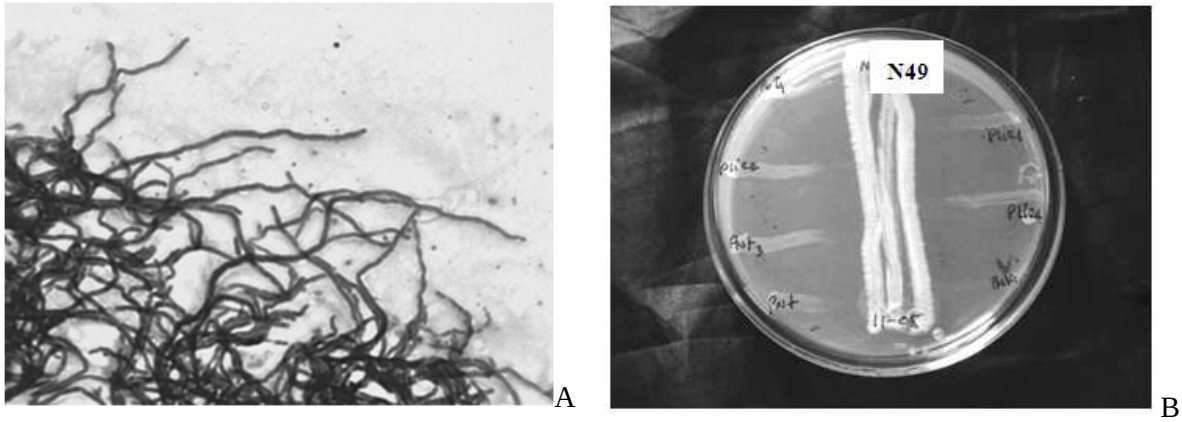
Tests Performed	Results
Melanin pigment	D
Catalase	+
Oxidase	+
Urease	+
H_2S production	+
Nitrate Reduction	-
Methyl Red (MR)	-
Voges-Proskaur (VP)	-
Citrate utilization	+
Hydrolysis of	
Casein	+
Starch	D
Lipid	+
Utilization of carbon source	
D-glucose	+
D-manitol	+
Fructose	+
Sucrose	D
Utilization of Nitrogen source	
D-alanine	+
L-arginine	+
L-tyrosine	+

+ = positive reaction, - = negative reaction, D = different isolates gave different reaction.

Antagonistic activity of *Streptomyces* sp. isolates

Among the ten *Streptomyces* sp. isolates, seven showed antagonistic activity against different fish pathogenic and human clinical isolates in cross streak method used in this study (Fig. 1B). Cross streak method is relatively easy and reliable method, especially for the screening program to test the antagonistic activity of antibiotic producing microbes, which is commonly used (Lemos et al. 1985; Ceylan et al. 2008; Arifuzzaman et al. 2010; Rahman et al. 2011; Valli et al. 2012). Determination of antagonism against different microorganisms is very important to determine the spectrum of antibiotic produced by an isolate. Here, the percentage of antagonism against different fish and human pathogenic bacteria were evaluated to measure the spectrum of antibiotic produced by the soil isolates. Similar type of study was also conducted by others

(George et al. 2010). In this study, four of the seven antagonistic isolates *viz.*, N24, N26, N28 and N47 showed broad spectrum of antagonistic activity (80-100%) against the test isolates belonging to all bacterial genera. The isolate N49 exhibited highest spectrum of antagonism against all fish pathogens (90-100%) but comparatively lower degree of antagonism against human pathogens (50-60%). Hence, the isolate N49 could be considered as a valuable source for the development of antibiotic against fish disease caused by *Aeromonas* sp., *Pseudomonas* sp. and *Edwardsiella* sp. The other two isolates N21 and N23 showed variability in their antagonism but maximum to fish pathogenic *Pseudomonas* sp. (80%) and *Edwardsiella* sp. (70%), respectively. The antagonistic activity of *Streptomyces* sp. isolates to different fish and human pathogenic bacteria has given in Table 4.



A B

Figure 1 - (A) Microscopic view of *Streptomyces* sp. isolate N41 (100x) showing long aerial hyphae, after Gram staining. (B) Antagonistic activity of *Streptomyces* sp. isolate N49 against different fish pathogenic *Aeromonas* sp. isolates.

Table 4 - Antagonistic activity of *Streptomyces* sp. isolates against fish and human pathogenic bacteria.

Isolates Name	Test microorganisms with their percentage (%) of growth inhibition					
	Fish pathogen			Human pathogen		
	Aeromonas	*Pseudomonas*	*Edwardsiella*	*Klebsiella*	*Salmonella*	*Streptococcus*
N21	60	80	70	70	50	60
N23	60	60	70	40	60	60
N24	80	80	100	90	90	80
N26	80	80	80	100	100	80
N28	80	80	90	80	90	90
N47	80	90	80	90	100	80
N49	100	90	90	50	60	60

Various microorganisms have survived for thousands of years by their ability to adapt against antimicrobial drugs and this process enables some bacteria to develop resistance to certain antibiotics, rendering the antibiotics ineffective. As a result, most of the bacteria resistant to multiple antibiotics, causing a crucial threat for the treatment of diseases of human and animals (Bennett 2008). The increasing frequency of multi-drug resistant pathogenic bacteria in recent years has created an urgent demand in the pharmaceutical industry for screening of new antibiotics (George 2010; Foysal et al. 2011; Sharmeen et al. 2012; Fuad et al. 2012). The results of present study showed that broad spectrum antibiotic could be developed from the isolates N24, N26, N28 and N47, which could be effective against several human and fish diseases. The isolate N49 could be used as a better source to reduce the prevalence of fish disease.

CONCLUSION

Soil samples from two different parts of Bangladesh were evaluated for isolating potent *Streptomyces* sp strains. Results showed that these soils could be good source for isolating antibiotic producing *Streptomyces* but more studies should

be carried out to find out valuable antibiotic producing bacteria. Further investigation is also necessary to purify the active novel metabolites from these isolates.

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