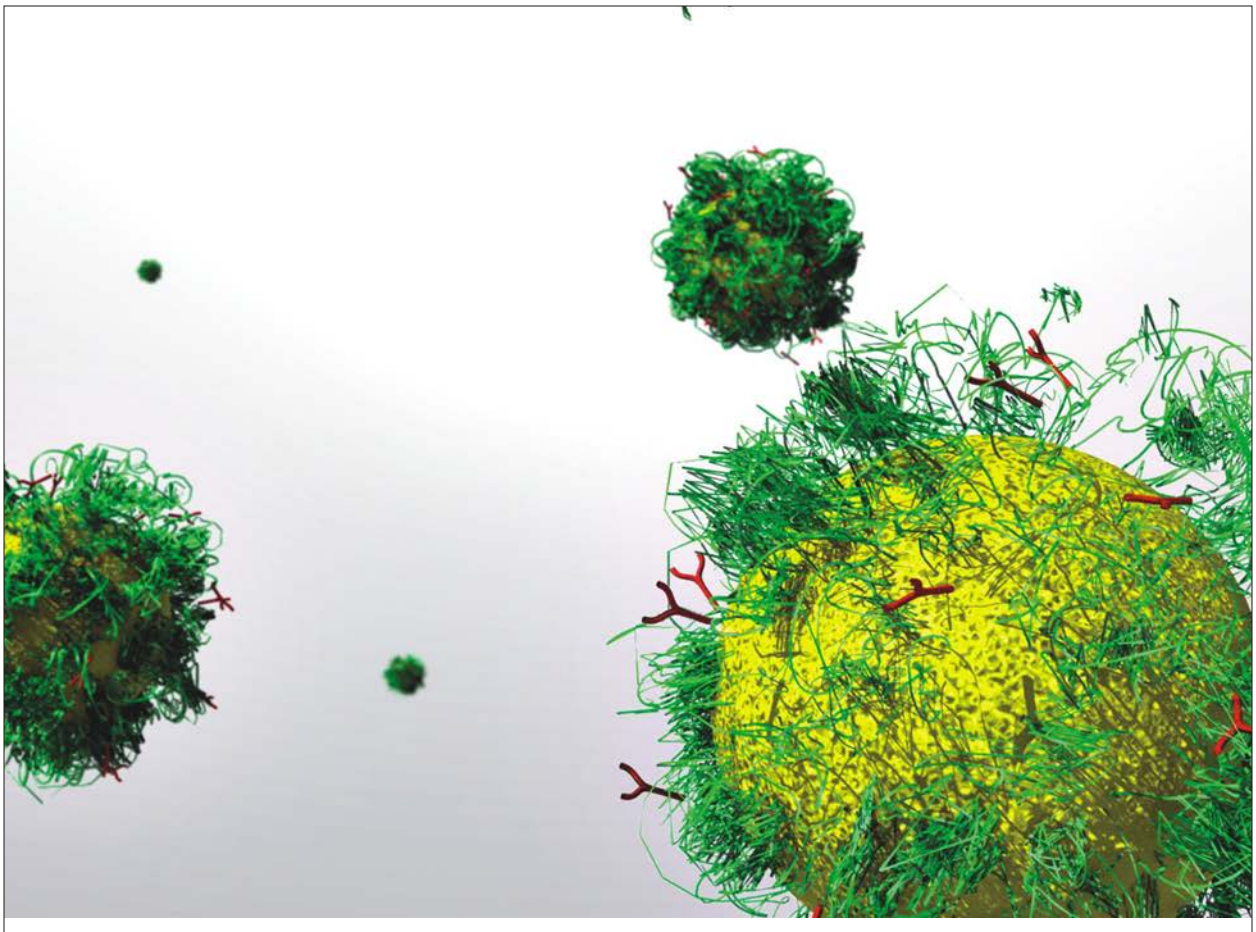

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 3 (2): 95-105, 2013
ISSN 2150-427x / DOI: 10.3923/ajdd.2013.95.105

Studies on Actinomycetes Collected from Pond Sediment Inhibiting Fish Pathogenic and Human Clinical Bacterial Isolates

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ABSTRACT

The present study was conducted with a view to isolate and identifies antibiotic producing microorganisms from pond sediments against fish pathogenic and human clinical bacterial isolates. During the course of study, a total of twenty actinomycetes isolates were recovered from 10 soil samples from different regions of Satkhira and Sylhet district using starch-casein-agar and glycerol-arginine-agar media supplemented with cyclohexamide (5 μg L^{-1}). Selected isolates from the isolation media was carried out by multiple streak method to obtain the pure cultures of selected isolates. All of the 20 isolates exhibited a range of colors including brown, creamy, yellowish and greenish in the isolation media. All of the isolates were then screened for their antagonistic activity against 4 genera of fish pathogenic and 2 genera of human clinical bacterial isolates using agar streak method. The fish pathogenic isolates belonged to *Pseudomonas* sp. *Aeromonas* sp. *Flavobacterium* sp. and *Edwardsiella* sp. whereas human clinical samples included *Klebsiella* sp. and *Salmonella* sp. isolates. All of the isolates showed remarkable antimicrobial activities against the test organisms and isolate No. N4/10 and N4/4 appeared to be the most promising ones. Several morphological, physiological and biochemical characterization tests were performed to identify the antibiotic producing soil isolates up to genus level. All the isolates were identified as actinomycetes.

Key words: Pond sediments, actinomycetes, isolation, antibacterial activity, USDA

INTRODUCTION

Antibiotics are one of the pillars of modern medicine (Ball *et al*., 2004) but the rate of loss of efficacy of old antibiotics is outstripping their replacement with new ones for many species of pathogenic bacteria (Hancock, 2007). The emergence of antibiotic resistant bacteria is a problem of growing significance in dermatological and surgical wound infections (Colsky *et al*., 1998; Giacometti *et al*., 2000). In general, the most important resistance problems in the management of wounds have been observed with *S. aureus* and coagulase-negative *Staphylococci* among the Gram-positive species and with *E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa* among the Gram-negative species (Filius and Gyssens, 2002).

The history of new drug discovery processes shows that skeletons have, in the majority of cases, come from natural sources (Bevan *et al*., 1995). Considerable research is being done in order to find new chemotherapeutic agents isolated from soil (Rondon *et al*., 2000; Crowe and Olsson, 2001;

Courtois *et al.*, 2003). Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere and they participate in various biological activities. Accordingly, they are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance (Hackl *et al.*, 2004).

Many groups of microorganisms like Gram-positive, Gram-negative bacteria and fungi have the ability of synthesizing antimicrobial agents and the top cultivable antimicrobial agent producers present in soils are the actinomycetes (Pandey *et al.*, 2002). On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics. The actinomycetes yielded about 70% of these and the remaining 30% are products of filamentous fungi and non-actinomycete bacteria (Nanjwade *et al.*, 2010). Actinomycetes are responsible for the production of about half of the discovered bioactive secondary metabolites (Berdy, 2005), notably antibiotics (Strohl, 2004).

Actinomycetes encompass a wide range of bacteria. They have universal occurrence and play an active part in the cycle of nature. They are a well defined group of Gram positive, free-living, saprophytic bacteria with high G+C content in their DNA (Lo *et al.*, 2002). They are predominant in dry alkaline soil. Many of the presently used antibiotics such as streptomycin, gentamicin, rifamycin and erythromycin are the product of actinomycetes. Keeping in mind the above mentioned ideas the present study was aimed to screen actinomycetes from pond sediments for antibacterial compounds against some fish pathogenic and human clinical bacterial isolates.

MATERIALS AND METHODS

Collection of soil samples: A total of 10 soil samples were collected from pond sediment of different localities of Satkhira and Sylhet district during February 2012 to April 2012. All the soil samples were collected using some clean, dry and new polythene bags along with sterile spatula and transported to the USDA (United States Department of Agriculture) Project Laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet for further studies.

Pretreatment of soil samples: All the soil samples were pretreated for inhibiting or eliminating unwanted microorganisms. Soil samples were dried at 70°C for 15 min to kill other competing bacteria and enabled the desired microbes to grow on the provided media.

Isolation of actinomycetes: Numerous media have been used for the isolation of actinomycetes from soil and other natural materials. In this investigation, starch-casein-agar (Kuster and Williams, 1964) and glycerol-arginine-agar (GAA) medium (Porter *et al.*, 1960) were used for isolation of actinomycetes. Cyclohexamide (50 μg L^{-1}) was added into the isolation media just before pouring into the plates.

The soil samples (1g) were added to a conical flask containing 100 mL of sterile water and few drops of Tween 80. The flasks were shaken for 30 min in an orbital shaker incubator at 27°C and their contents were designated as stock cultures. A series of culture tubes containing 9 mL of sterile water was taken. From the stock culture, 1 mL suspension was transferred aseptically to the 1st tube (10^{-1}) and mixed well. From the 1st tube, 1 mL of suspension was transferred into 2nd tube (10^{-2}), mixed well. Similarly, dilutions up to 10^{-5} were made (serial dilution technique). Suspension (0.1 mL) from each culture tube was spread on starch-casein-agar and glycerol-arginine-agar medium plates aseptically in a laminar-air low cabinet. The plates were incubated at 27±2°C for 84 h. The plates were observed intermittently during incubation. After 72 h, whitish pin-point colonies, characteristic of actinomycetes and with a clear zone of inhibition

around them were seen. The pinpoint colonies with inhibitory or clear zone of inhibition were selected and purified into nutrient agar plates by multiple streaking methods. The stock cultures of each selected isolate was prepared and maintained in nutrient agar slants at 4°C. The pinpoint colonies isolated were selected for further studies.

Study of antibacterial activity of soil bacterial isolates: All the soil bacterial isolates were tested for their antibacterial activity against four genera of fish pathogenic namely *pseudomonas* sp., *Aeromonas* sp., *Flavobacterium* sp., *Edwardsiella* sp. and two genera of human clinical (*Klebsiella* sp. and *Salmonella* sp.) bacterial isolates. The microbial sensitivity of the soil isolates was analyzed by agar streak method. Each of the isolate was streaked as a straight line on nutrient agar plate and incubated at 27°C for 6 days (144 h). On 6th day of incubation, test microorganisms were streaked at right angle but not touching each other and then incubated at 30°C for 24 h. If the test organism is susceptible to the antibiotic produced by the selected strains, then it would not grow near that strain. The zone of inhibition against each test organism was noted. Based on their antimicrobial properties, isolates were chosen for further morphological and biochemical characterization.

Characterization of the selected isolates: The selected soil bacterial isolates were characterized by morphological and biochemical methods. Morphological characteristics such as colony size, shape, colour etc. were observed. Recovered soil bacterial isolates with supporting growth characteristics of actinomycetes were subjected to various biochemical tests named melanoid pigment formation, catalase, oxidase, Hydrogen Sulphide Production (H_2S), Methyl Red (MR), Voges Proskauer (VP), casein hydrolysis, starch hydrolysis and indole reaction.

RESULTS

Isolation of actinomycetes: This study was undertaken with a view to screen actinomycetes having antimicrobial activity. In the present study, eighty one whitish pinpoint colonies bearing colony characteristics of actinomycetes and with a clear zone of inhibition were isolated using starch-casein agar and glycerol-arginine-agar media. Out of 81 actinomycetes isolates that were subjected for primary screening process, only 20 isolates showed inhibitory activities against test organisms. Pertinent details of the soil samples and the actinomycetes are shown in Table 1.

Table 1: Characteristics of soil samples and No. of isolated colonies

			No. of colonies on isolation media		
Soil sample No.	Dilution of soil sample	Heat treatment 70°C, 1 h	SCA	GAA	Nature of soil sample
1	10^{-4}	HA	--	--	Saline-soil
2	10^{-4}	HA	--	--	Saline-soil
3	10^{-3}	HA	--	--	Saline-soil
4	10^{-4}	AP	17	--	Organic matter rich soil
5	10^{-3}	AP	--	3	Loamy
6	10^{-3}	AP	15	--	Mud
7	10^{-4}	AP	17	--	Black-dry soil
8	10^{-2}	AP	--	--	Yellow-dry soil
9	10^{-5}	AP	8	6	Dry and hard soil
10	10^{-5}	AP	11	4	Organic matter rich soil

HA: Heat not applied, AP: Heat applied, AGA: Glycerol-arginine-agar, SCA: Starch-casein-agar, Dilution of soil samples was done by two-fold serial dilution technique

The isolates were designated as N2/1-N2/10 and N4/2-N4/11. Among 20 isolates 6 isolates exhibited significant antibacterial activity against test microorganisms (Table 2-7). The test organisms used in this study were collected from USDA project Laboratory, Shahjalal University of Science and Technology, Sylhet and MAG Osmani Medical College, Sylhet, Bangladesh. Antimicrobial tests conducted showed that isolate N4/10, N2/9 and N4/5 were the most promising for *Pseudomonas* sp. isolates (Table 2 and Fig. 1, 2) whereas N4/4 and N4/10 for *Aeromonas* sp.

Table 2: Antibacterial activity of soil bacterial isolates against fish pathogenic *Pseudomonas* sp. isolates

	Pseudomonas sp. isolates name					
Antibiotic producing isolates	P_2F_4	$Cla_2$87	Cla_1B/8	PFN_3	Cla_2B	$Pukl_2$
N2/1	-	-	-	+	+	++
N2/2	+++	++	-	-	++	+
N2/3	+++	+	+	-	+	-
N2/4	+	++	-	+++	++	+
N2/5	++	-	+	+	-	-
N2/6	++	+	+	+	-	+
N2/7	++	-	+	+	-	-
N2/8	++	++	+	++	-	-
N2/9	+++	+++	++	+	++	+
N2/10	+	-	+	+++	++	++
N4/2	+	+	-	++	+	-
N4/3	+	-	-	-	+	++
N4/4	+++	++	+	+	++	-
N4/5	+	+++	++	+	+	+
N4/6	+	+	-	-	+	++
N4/7	+	-	+	+	+	++
N4/8	+	+	-	+	+	++
N4/9	+	-	+	-	+	++
N4/10	+++	+++	+++	+++	++	++
N4/11	+	-	-	-	+	++

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

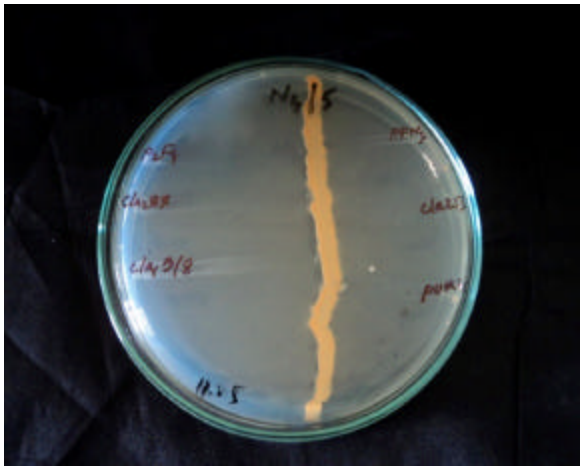

Fig. 1: Antibacterial activity of isolate N4/5 against *Pseudomonas* sp. isolates

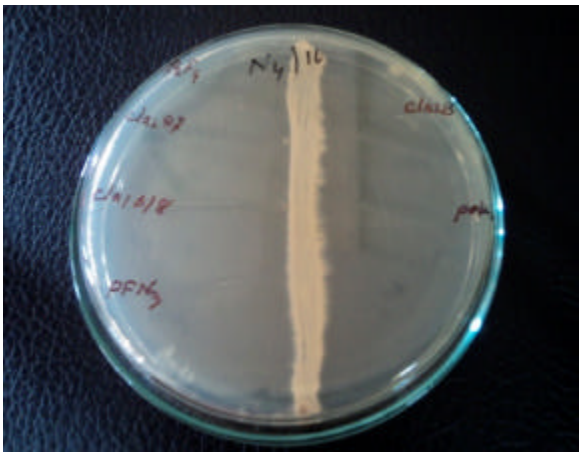

Fig. 2: Antibacterial activity of isolate N4/10 against *Pseudomonas* sp. isolates

Table 3: Antibacterial activity of soil bacterial isolates against fish pathogenic *Aeromonas* sp. isolates

Antibiotic producing isolates	*Aeromonas* sp. isolates														
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
N2/1	+	-	-	+++	+	-	++	+	+	+	-	++	+	+	-
N2/2	++	+	++	+	++	+	-	++	+	-	+	+	-	-	++
N2/3	-	+	+	+	-	-	++	++	-	++	-	+	+	-	+
N2/4	+	-	++	+	+	-	+	+	-	+	-	-	+	+	-
N2/5	-	++	+++	+	++	+	+	-	++	-	+	+	-	+	++
N2/6	-	+	-	+	+	-	-	++	-	-	-	+	+	-	-
N2/7	+	+	+	++	+	-	-	++	-	+	-	+	+	++	-
N2/8	-	+	-	+	+	-	+	-	-	-	+	+	++	+	++
N2/9	-	+++	+++	+++	++	-	++	+	-	+	-	-	+	+	+
N2/10	+++	++	-	++	++	+	++	++	++	-	++	++	-	++	-
N4/2	++	++	-	-	++	+	-	+	-	++	+	-	-	-	+
N4/3	-	-	-	+	++	-	-	-	-	-	-	+	+	+	-
N4/4	+	+++	+++	+++	++	+	+++	+	+	++	++	+	+	+	+
N4/5	++	++	++	++	-	+	++	+	-	++	+++	++	-	+	++
N4/6	-	+	-	+	++	+	-	-	++	-	-	+	+	+	-
N4/7	+	-	+	+	+	-	-	+	-	+	-	+	+	-	-
N4/8	-	+	-	+	++	+	+	-	+	-	+	+	-	+	++
N4/9	-	+	-	+	+	-	-	++	-	-	-	+	+	-	-
N4/10	+	+++	++	++	+++	+	+++	++	+	++	+	+	+++	+	+
N4/11	+	++	-	-	-	+	-	+	+	+	-	+	-	-	-

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

isolates (Table 3). Besides this, isolate N2/9, N2/10 and N4/5 were found to be most effective against *Salmonella* (Table 7), *Klebsiella* (Table 6) and *Edwardsiella* sp. (Table 5).

Identification of soil bacterial isolates: Identification of the isolates was carried out using morphological and biochemical properties. Morphological studies were carried out by microscopic

Table 4: Antibacterial activity of soil bacterial isolates against fish pathogenic *Flavobacterium* sp. isolates

	Flavobacterium sp. isolates name									
Antibiotic producing isolates	FXRH1	FXRH2	FXRH3	FXRH4	FXKHT	Tila1	Koit1	Koit2	Koit3	FXS
N2/1	-	-	+	-	-	-	+	+	++	-
N2/2	+	+	-	+	++	-	-	-	+	-
N2/3	++	-	-	-	+++	-	-	+	-	-
N2/4	-	-	+	++	-	+	+	-	+	-
N2/5	+	+	-	+	+	-	-	-	-	-
N2/6	-	-	+	++	-	+	+	+	+	-
N2/7	+	-	+	-	++	-	-	+++	+	-
N2/8	+	++	-	-	-	-	-	++	+	-
N2/9	-	-	+	++	+	+++	-	+	+	+
N2/10	-	++	-	+	++	++	-	++	-	+
N4/2	-	++	+	+++	-	+	++	++	++	-
N4/3	+	-	-	+	-	++	-	++	-	-
N4/4	+	+	+	+	+	++	+	+	-	++
N4/5	-	-	+	+	+	+++	+	+	-	-
N4/6	+++	++	-	+	-	-	-	+	-	-
N4/7	-	-	+	+	+	+	-	-	-	-
N4/8	+	+++	+	-	-	-	++	+	+	+
N4/9	-	-	-	+	+	+	-	-	++	++
N4/10	+++	+++	+	+	-	++	++	++	+	+
N4/11	+	++	-	-	-	-	+	+	-	-

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

Table 5: Antibacterial activity of soil bacterial isolates against fish pathogenic *Edwardsiella* sp. isolates

	Edwardsiella sp. isolates name					
Antibiotic producing isolates	Eds32	Eds33	Eds34	Eds35	Eds36	Eds37
N2/1	-	-	-	++	-	+
N2/2	-	+	-	+	+	-
N2/3	-	-	-	+	+	-
N2/4	-	+	-	-	++	-
N2/5	+	-	+	-	+	-
N2/6	+	-	-	+	-	-
N2/7	+	+	-	-	-	+
N2/8	-	+	-	-	+	+
N2/9	++	-	-	+	+	+
N2/10	+	+	+	+	+	-
N4/2	-	-	-	+++	++	+
N4/3	-	+	++	-	+	-
N4/4	-	-	-	+	+	+
N4/5	+	+++	++	+	+	-
N4/6	+	+	-	-	-	-
N4/7	-	-	+	+	-	-
N4/8	-	-	-	+	+	+
N4/9	+	-	+	+	-	-
N4/10	+	+	-	+++	+	++
N4/11	-	+	-	-	+	+

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

Table 6: Antibacterial activity of soil bacterial isolates against human clinical *Klebsiella* sp. isolates

Antibiotic producing isolates	*Klebsiella* sp. isolates name														
	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15
N2/1	-	++	+	-	+	-	-	-	-	+	-	-	++	-	-
N2/2	+	++	-	-	-	+	+	+	-	+	-	-	-	+	+
N2/3	-	-	-	-	+	++	+++	-	-	-	+	+	+	-	-
N2/4	++	+	+	-	-	-	++	-	-	+	+	-	+	-	+
N2/5	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-
N2/6	++	+	+	-	+	-	-	-	-	-	+	++	-	+	+
N2/7	-	+	+	-	-	++	-	+	+	+	-	-	-	++	+
N2/8	-	-	++	-	+	++	-	-	-	-	+	-	+	-	-
N2/9	++	+	+++	+	++	-	-	+	++	-	+	+	-	+	+
N2/10	+++	++	+	+	+	-	++	-	+	+	+	+	-	-	+
N4/2	-	-	-	+	-	+++	+	++	-	-	-	+	++	++	++
N4/3	+	-	-	+	+	+	-	-	-	-	+++	++	-	-	-
N4/4	-	+	+++	+	+	-	+	-	-	-	+	-	-	+	+
N4/5	+	-	++	-	+	+	-	++	+++	-	++	++	+	-	+
N4/6	-	++	-	-	+	-	-	-	+	-	-	+	+	-	-
N4/7	++	+	-	-	+	-	+	-	-	++	-	-	+	-	+
N4/8	-	+	-	-	+	+++	++	-	-	-	+	-	+	-	-
N4/9	-	-	-	-	-	++	++	-	-	-	-	-	-	-	-
N4/10	++	+	+	+	+	-	-	-	+	-	++	+	-	+	-
N4/11	++	-	-	-	-	++	-	+	+	-	-	-	+	+	-

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

Table 7: Antibacterial activity of soil bacterial isolates against human clinical *Salmonella* sp. isolates

Antibiotic producing isolates	*Salmonella* sp. isolates		
	ST_1	ST_2	ST_3
N2/1	+	+	-
N2/2	-	+	-
N2/3	+	-	+
N2/4	-	++	-
N2/5	+	+	-
N2/6	-	++	-
N2/7	+	-	-
N2/8	-	-	+
N2/9	++	+++	+
N2/10	+	+	++
N4/2	++	+	+
N4/3	-	-	+
N4/4	+	+	+
N4/5	+++	+	+
N4/6	-	++	-
N4/7	+	-	-
N4/8	-	-	-
N4/9	-	+	+
N4/10	+	-	+
N4/11	-	-	-

+++: Very good inhibition, ++: Good inhibition, +: Moderate inhibition, -: No inhibition

Table 8: Biochemical characterization of soil bacterial isolates

	Tests performed									
Antibiotic producing isolates	G	Melanoid formation	C	O	H_2S	MR	VP	CH	SH	I
N2/1	+	Creamy	+	+	+	-	-	+	+	-
N2/2	+	Yellowish	+	+	+	-	-	+	+	-
N2/3	+	LBP	+	+	+	-	-	+	+	-
N2/4	+	Creamy	+	+	+	-	-	+	+	-
N2/5	+	Creamy	+	+	+	-	-	+	+	-
N2/6	+	Creamy	+	+	+	-	-	+	+	-
N2/7	+	Creamy	+	+	+	-	-	+	+	-
N2/8	+	LBP	+	+	+	-	-	+	+	-
N2/9	+	Creamy	+	+	+	-	-	+	+	-
N2/10	+	Yellowish	+	+	+	-	-	+	+	-
N4/2	+	Creamy	+	+	+	-	-	+	+	-
N4/3	+	Creamy	+	+	+	-	-	+	+	-
N4/4	+	Yellowish	+	+	+	-	-	+	+	-
N4/5	+	Yellowish	+	+	+	-	-	+	+	-
N4/6	+	Creamy	+	+	+	-	-	+	+	-
N4/7	+	LBP	+	+	+	-	-	+	+	-
N4/8	+	LBP	+	+	+	-	-	+	+	-
N4/9	+	Yellowish	+	+	+	-	-	+	+	-
N4/10	+	Creamy white	+	+	+	-	-	+	+	-
N4/11	+	Brownish	+	+	+	-	-	+	+	-

G: Gram staining, C: Catalase, O: Oxidase, H_2S: Hydrogen sulfide, MR: Methyl Red, VP: Voges-proskauer, CH: Casein hydrolysis, SH: Starch hydrolysis, I: Indole, LBP: Light brown pigmentation (+): Positive result, (-): Negative result

observation and studies on growth characteristics in Petri dishes. On the basis of morphological and biochemical characteristics (Table 8) all the isolates were belonged to the actinomycetes group.

DISCUSSION

The increase in the frequency of multi-resistant pathogenic bacteria is created an urgent demand in the pharmaceutical industry for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity, which resist the inactivation processes exploited by microbial enzymes (Saadoun and Gharaibeh, 2003; Motta *et al*., 2004).

Screening and isolation of promising actinomycetes with potential antibiotics is still a thrust area of research and it is suggested that the exploration of materials from different areas and habitats have a vital role to play in the search for new microbes and novel metabolites and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance (Saadoun and Gharaibeh, 2003). The present work was conducted with a view to isolate and identifies actinomycetes from pond sediments of Sylhet and Satkhira district and to screen their potential to generate antimicrobial activities. Several researchers have also screened soil samples collected from different parts all over the world for antimicrobial agent producing microorganisms (Huddleston *et al*., 1997; Ouhdouch *et al*., 2001; Haque *et al*., 1992; Thakur *et al*., 2007; Yadav *et al*., 2009; El-Naggar *et al*., 2006).

In the present study, the soil samples were heated at 70°C for 15 min for the recovery of actinomycetes and it is found that, the temperature at 70°C for 15 min was suitable treatment method to isolate more actinomycetes colonies from soil samples. Seong *et al*. (2001) also reported

that when the soil samples were cultured without pretreatment (heat at 70°C) the number of colonies recovered was in the order of other bacteria, *Streptomyces* fungi and non-streptomycete actinomycetes. During the present study, four soil samples were cultured without pretreatment and it was apperared that no actinomycetes colonies were present on the isolation media. The results of the present study gives clear picture about the significance of pre-treatment method for the isolation of actinomycetes. This type of pre-treatment methods for isolation of actinomycetes has also been suggested by several researchers (Hayakawa and Nonomura, 1987; Hayakawa *et al.*, 1991; Jensen *et al.*, 1991; Kim *et al.*, 1994).

The isolation media used in this study was supplemented with cyclohexamide (50 μg L^{-1}) to inhibit fungal growth because fungi often contaminate the isolation media. Saadoun *et al.* (2007) also supplemented the isolation medium with cyclohexamide (50 μg L^{-1}) in succeeding experiments. The present study was carried out using Starch-casein-agar (SCA) and glycerol-arginine-agar media for the isolation of actinomycetes. In this study starch-casein-agar media was found more effective than glycerol-arginine-agar media for the isolation of actinomycetes. Similar studies were also carried out by Raja *et al.* (2010). Different types of bacterial colonies were developed on isolation medium. Colonies having characteristic features such as powdery appearance with convex, concave or flat surface and color ranging from white, gray to pinkish and yellowish were selected. Isolates were initially screened based on zone of clearance and they were then subjected to preliminary screening. From the preliminary screening, twenty bacterial isolates were collected having antibacterial activities. However, out of these twenty bacterial isolates six isolates showed remarkable antibacterial activities against test organisms.

All of the 20 isolates showed antibacterial activity with at least one test bacteria. The test microorganisms included 6 Gram negative fish pathogenic and human clinical bacteria as indicated earlier. Then the antibiotic producing soil bacterial isolates were screened against the laboratory isolates belonged to six genera viz. *Pseudomonas* sp., *Aeromonas* sp., *Flavobacterium* sp., *Edwardsiella* sp., *Klebsiella* sp., *Salmonella* sp., Isolates N4/5, N4/10 and N2/9 showed antibacterial activities against all six isolates of *Pseudomonas* sp., having N4/10 was the most promising (Table 2). Isolate No. N4/10 showed very good inhibition against *Pseudomonas* sp., isolates P_2F_4, $Cla_2$87, Cla_1B/8 and PFN_3 (Table 2). Besides thzs, isolate no. N4/4 and N4/10 showed 100% antibacterial activities against all fifteen isolates of *Aeromonas* sp. and N4/10 was the most promising (Table 3). None of the antibiotic producing isolates showed 100% antibacterial activity against all of the isolates of fish pathogenic *Flavobacterium* sp. However, the isolates N4/4 and N4/10 showed 90% antibacterial activity against *Flavobacterium* sp. isolates (Table 4). None of the soil bacterial isolates showed promising result against the fish pathogenic *Edwardsiella* sp. isolates except N4/10 (Table 5). On the other hand isolates N2/9 and N2/10 exhibited promising result against all human clinical *Klebsiella* sp. isolates (Table 6). Above all isolate no. N4/10 appeared to be the most potent antibiotic producing isolate.

The soil bacterial isolates that were selected through microbial sensitivity test were further taken for physico-biochemical characterization. All the isolates were gram positive, catalase positive, oxidase positive, H_2S positive and negative for MR, VP and indole test. Besides they were able to hydrolyze casein and starch. Based on the biochemical and morphological characteristics the isolates were identified as actinomycetes. It is expected that the current attempt for the isolation, characterization and the study on actinomycetes from pond sediments will be useful for the identification of new antibiotics effective against challenging pathogens.

ACKNOWLEDGMENT

The authors gratefully acknowledged to the authority of USDA Project Laboratory of the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh for providing facilities to carry out this research work.

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