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Antimicrobial potentialities of *Streptomyces* species isolated from mangrove soil samples of Sundarbans

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ABSTRACT
To combat against recurrently emerging multi drug resistant pathogen new broad spectrum antimicrobial agents are instantly required. The most capable group of microorganisms isolated from unexplored regions of the world may be the eventual solution to this problem. Thus this study was designed to isolate several bioactive Streptomyces species capable of producing antimicrobial secondary metabolite from soil of Sundarbans, the only mangrove tiger land of the world. In this study total 6 strains of Streptomyces were isolated. In preliminarily screening of antimicrobial activity, 5 isolates showed better mild to moderate antimicrobial activity against 2 Gram-positive, 2 Gram-negative, 1 fungal strains. The most potent ten isolates were subjected to dichloromethane extraction. The secondary antimicrobial activity was performed by well diffusion method where the crude extract of A(1), B(1), B(2)1, B(2)3, E(2)1 exhibited antimicrobial activity against selected five microbes and other crude extracts didn't display significant antimicrobial effect.
KEYWORDS: *Streptomyces, Secondary metabolites, Antimicrobial resistance, Sundarbans.*

INTRODUCTION

Antibiotic resistance (ABR) is a global health threat. Bacteria, not humans or animals, become antibiotic-resistant. In recent years, clinically relevant bacteria strains with multiple drug resistant (MDR) are increasing at alarming rate all over the world (Andersson et al., 1999). These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases.

Natural products are the main sources of new drugs and lead structures. Now-a-days, more than 50% of the drugs prescribed in the USA are natural products or semisynthetic derivatives (Veeresham, 2012). Microorganisms are generally a rich source of structurally diverse, biologically active secondary metabolites. Their ability to produce a diverse array of bioactive molecules has made them important in the search for novel therapeutic or preventive agents. The phylum Actinobacteria produces approximately 10,000 clinically important compounds, of which about 75% are isolated from the genus *Streptomyces* (Berdy, 2005). They are well known for their prolific abilities to produce antibiotics. Through this experiment, the mangrove habitat of Sundarbans is going to be studied to raise new compounds for the treatment of human antimicrobial infections.

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METHODOLOGY

The following steps will be carried out for the proposed study:

a. Soil samples collection and isolation of *Streptomyces*

Soil samples was collected from different part of mangrove forest Sundarbans. Samples were collected as aseptically as possible. Then soil samples was dried in hot air oven at 60-65˚C for about 3h to reduce the number of bacteria other than Actinomycetes. Starch-casein media was used as isolation media. Only which organisms capable of degrading these complex polymers (mostly molds and *Streptomyces*) can grow. Isolation of *Streptomyces* species was done by using spread plate technique (Bernard, 2007). After a preliminary antibacterial activity testing, potent *Streptomyces* were selected and stored.

b. Optimization of cultural condition and extraction of secondary metabolites

Suitable media, optimum growth temperature, incubation period, pH of media and other physical condition were developed by trial and error basis to get maximum secondary metabolites. After optimization of culture conditions, small scale liquid fermentation was carried out and extraction of the metabolites was done using dichloromethane, CH_2Cl_2 (DCM). DCM is used as solvent of extraction due to its solubility in lots of organic solvents. The concentrated extract was used for pharmacological evaluation.

c. Antimicrobial activity test

Antibacterial and antifungal activity of the extract was determined by disc diffusion method (Zaidan et al., 2005) against different types of bacteria and fungi. Nutrient agar media was prepared and filter paper discs (6 mm in diameter) was impregnated with different concentration of crude extract. Standard kanamycin of 30µg/disc was used against bacteria and as standard Nystatin 50µg/disc against fungas. Then the petridishes were kept in incubator at 37°C for 16 h to allow the growth of the microorganisms. After proper incubation, the antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a calibrated scale. Minimum inhibitory concentration (MIC) & Minimum bactericidal concentration (MBC) was also determined using standard protocol (Hassan et al., 2009).

RESULT

Preliminary Screening of Antimicrobial Activity

The resulting 13 pure cultures were tested for antimicrobial properties through the use of streak plating technique. This test is based on the fact that when the plates containing growth media are streaked with Streptomyces and incubated for four or five days, certain ones will produce antibiotic substances in the media. When the test bacteria were streaked perpendicularly to the streak line, some of them gave clear zones of inhibition around the line, which indicated the production of antimicrobials by the isolates. The extent of clearing indicates the potency of the metabolite produced. Preliminary cross steaks was performed on 13 steaks (Table 1-See Appendix) with five different microbes (gram negative- *Escherichia coli, Salmonella enterica*; gram positive- *Staphylococcus aureus, Bacillus subtilis*; fungal strain- *Candida albicans*). Among them 10 strains found showing more or less antimicrobial activity (Figure 1- See Appendix).

Evaluation of Antimicrobial Activity of Secondary Metabolites

Both gram positive and gram-negative bacterial strains and antifungal stains were taken for the test. These organisms were collected from the Microbiology Lab. of Pharmacy Discipline, Khulna University Khulna. Five crude extracts showed better antimicrobial activity against both Gram-positive and Gram-negative strains compared to control than the rest other stains. B(2)2 extract indicated the highest zone of inhibition. Result is compiled in table 2- (See Appendix). The value of MBC was higher than the values of MIC which indicate the bacteriostatic characteristics of extract.

DISCUSSION

The number of drug-resistant pathogens are increasing now days, particularly the acquired multi-drug resistant strains, cause serious public health problem throughout the world. Therefore, the need for antimicrobial discovery and better treatments of these infections, particularly in hospitals where antibiotic resistance is immediately life threatening, is becoming a rapidly growing concern. The study of different environments throughout the world has yielded a lot of antimicrobial agents that are of great value for the treatment of many infectious diseases. Therefore, the present study shows that isolation and purification of economically important secondary metabolites from actinomycetes of Sundarban mangrove forest and characterization of the bioactive compounds is a challenging solution for exploring antimicrobial compounds from natural sources. The ultimate goal of this study was to search antibacterial metabolite producing novel strain. In this study, 13 microbial strains were isolated. Preliminary cross streak of isolates showed strong antimicrobial property. Preliminarily it was observed 10 strains giving antimicrobial activity, where after extraction only 5 strains showed antimicrobial potential by disc diffusion method. Among them one extract showed highest antimicrobial potentiality. The solvent DCM used during extraction was polar in nature, if the metabolites were non-polar, they could not grow or

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show effectiveness. Recent study of Suhaidi et al., 2012 had shown that, crude extract of ethyl acetate shown microbial activity but hexane extract does not show any activity. So it is possible to get varieties of antimicrobial compounds through different solvent system. The result of this investigation revealed that the mangrove *Streptomyces* of Sundarban area is a potent source of novel antibiotics and bioactive compounds.

CONCLUSION

The dearth of new antibiotics in the face of widespread antimicrobial resistance makes for discovering new antibiotics critical for the future management of infectious disease. Purpose of the research was to search for novel bioactive secondary metabolites from *Streptomyces* species which could be helpful in the development of new antibiotic to face the challenges of resistances. Findings do proper justice with the purpose of this research. Further study on pure compound isolation from these bacterial crude extracts will help to develop better antimicrobials.

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APPENDIX
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Figure 1: Cross steaks for isolated colonies with different microbes

B(2)2 with *Bacillus subtilis*

B(2)2 with *Escherichia coli*

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E(2)1with *Staphylococcus aureus*

A(3) with *Salmonella enterica*

B(1) with *Salmonella enterica*

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Table 1: Isolated strains from the soil samples of Sundarban

Soil Collection point	Soil sample as per serial dilution	Number of isolated colony	Pure colony labeled
Karamjal (A)	10^{-1} g/ml	1	**A(1)**
	10^{-2} g/ml	-	-
	10^{-3} g/ml	2	A(3)1, **A(3)2**
	10^{-4} g/ml	-	-
Hiron point (B)	10^{-1} g/ml	1	**B(1)**
	10^{-2} g/ml	2	**B(2)1, B(2)2**
	10^{-3} g/ml	-	-
	10^{-4} g/ml	-	-
Dublar Char (C)	10^{-1} g/ml	-	-
	10^{-2} g/ml	-	-
	10^{-3} g/ml	1	**C(3)**
	10^{-4} g/ml	1	**C(4)**
Kotka Forest (D)	10^{-1} g/ml	1	**D(1)**
	10^{-2} g/ml	-	-
	10^{-3} g/ml	-	-
	10^{-4} g/ml	-	-
Kotka beach (E)	10^{-1} g/ml	1	E(1)
	10^{-2} g/ml	2	**E(2)1**, E(2)2
	10^{-3} g/ml	1	**E(3)**
	10^{-4} g/ml	-	-

Pure colonies indicated in bold were subjected to extraction based on cross steaks technique

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Table 2: Antimicrobial activity of the crude extract (100 µg/disc)

	Diameter of zone of inhibition in mm				
	Bacterial strain				Fungal strain
	Gram positive bacteria		Gram negative bacteria		
	S. aureus	*B. subtilis*	*E. coli*	*S. enterica*	*C. albicans*
Blank	-	-	-	-	-
Standard	28	33	22	24	14
A(1)	13	9	9	15	-
B(1)	9	11	17	7	6
B(2)1	12	12	9	7	-
B(2)2	19	15	14	21	5
E(2)1	9	9	11	8	-
MIC of B(2)2 (µg/ml)	66.5	125	150.5	150	N/A
MBC of B(2)2 (µg/ml)	125.5	250	260	300	N/A

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration