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Susceptibility of Crude Metabolites and Two Isolated Compounds of Soil *Streptomyces* Species to Pathogenic Organisms

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This investigation was an attempt to determine the primary selection of the compounds as therapeutic agents, isolated from an antagonistic *Streptomyces* species. The antimicrobial metabolites were extracted by $CHCl_3$ from the fermentation broth of organism. The antimicrobial activity profile of the crude metabolites (M) as well as two isolated compounds (BM-3 and BM-5) from the crude metabolites, was interesting against some pathogenic bacteria. The minimum inhibitory concentration (MIC) of the crude metabolite (M) and compounds against six pathogenic bacteria was found to be between 16 and 64 μg ml^{-1} respectively.

Key words: *Streptomyces* species, antimicrobial activity, pathogens, crude metabolite

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Introduction

The modern era of antimicrobial chemotherapy began in 1929 with Fleming's discovery of the powerful bactericidal substance penicillin. Antibiotics first became widely available in the 1940s when they were hailed as "magic bullets", able to cure everything including the common cold. Since that time, the pharmaceutical industry has developed more than 100 varieties of these drugs and it is estimated that more than 150 million prescriptions are written for antibiotics each year in the United States alone (Emanuel *et al.*, 2001). This growth in antibiotic usage globally has however, been paralleled by the ability of bacteria to resist being killed by these agents and has resulted in a steady decline in the number of effective antibiotics each year. Spontaneous mutations of bacterial DNA, which could confer resistance to a particular class of antibiotic, are estimated to occur in every 10^5-10^{10} generations (Emanuel *et al.*, 2001). These novel genes can then be shared with other bacteria following transfer of plasmids by conjugation, transformation or transduction. At its most extreme, the acquisition of antibiotic resistance genes has resulted in at least four species of bacteria for which there are no effective forms of conventional therapy available. Methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus faecalis* (VRE), *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* have been dubbed "superbugs" by the media and between them account for the deaths of many people in the world (Emanuel *et al.*, 2001). In order to combat these infections, new antibiotics with high potent will need to be developed.

Many currently available antibiotics (tetracycline, chloramphenicol, kanamycin etc.) are either the original products or modifications of agents produced by soil *Streptomyces* species. As a part of such efforts on the microbial metabolites from soil sample of northern parts of Bangladesh, we isolated an antagonistic strain of *Streptomyces* species (Holt *et al.*, 1994). In the present study, we have evaluated the antimicrobial spectra of compounds (BM-3 and BM-5) isolated from the culture filtrate of the *Streptomyces* species.

Materials and Methods

Isolation and identification of the organism: The organism was isolated from soil sample, collected from a cultivated land of Northern part of Bangladesh, at a depth of 1 m; by using crowded plate technique (Hammond and Lambert, 1978). The organism was identified on the basis of its morphological, biochemical and some cultural characteristics in milk, potato-agar, cellulose, gelatin and starch etc. (Bytul, 2002) according to the Bergeys Manual of Determinative Bacteriology, 9th edition.

Production of antimicrobial metabolites: The organism was cultured in Czapek-dox broth alkaline (pH 8) media containing 0.5% NaCl, utilizing sucrose as carbon sources and after 10 days of incubation at 37°C, the culture filtrate was extracted with $CHCl_3$. The $CHCl_3$ fraction thus obtained was evaporated under reduced pressure to give a brown semisolid mass.

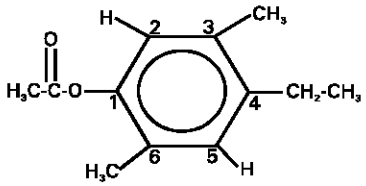
Isolation of the compounds: The compound BM-3 and BM-5 were isolated by PTLC technique from crude $CHCl_3$ extract using the solvent system $CHCl_3$:CH_3OH (15:1) and (12:1) respectively having R_f value 0.49 and 0.61 respectively.

Antibacterial screening: The antibacterial activities of crude metabolite (M) and compounds (BM-3 and BM-5) were determined against 14 selected pathogenic test organisms by disc diffusion method, (Barry, 1980; Beur *et al.*, 1966) at concentrations of 30 and 100 μg disc^{-1} of each sample and compared the zone of inhibition with that produced by the standard antibiotic kanamycin at 30 μg disc^{-1}. The MIC values of the compounds were determined against six test organisms by serial tube dilution technique (Reiner, 1982).

The standard test organisms were collected from the Department of Microbiology, University of Dhaka, Bangladesh.

Results and Discussion

The isolated organism was identified as *Streptomyces* species (Bytul, 2002; Holt *et al.*, 1994) was subjected to the production of antimicrobial metabolites using Czapek-dox broth alkaline media. The crude $CHCl_3$ extract of the culture filtrate of *Streptomyces* species under chromatographic analysis yielded two compounds designated as BM-3, whose structural elucidation is in progress and BM-5 was characterized as 3,6- dimethyl-4- ethyl-O-acetyl benzene on the basis of its spectral data (Bytul, 2002).



3,6- dimethyl- 4-ethyl- O-acetyl benzene

The crude metabolites and both compounds (BM-3, BM-5) showed

Table 1: Antibacterial activity of the crude metabolites (M), compounds (BM-3 and BM-5) and Kanamycin

	Diameter of zone of inhibition (in mm)						
	M		BM-3		BM-5		K
Test organisms	30 μg/disc	100 μg/disc	30 μg/disc	100 μg/disc	30 μg/disc	100 μg/disc	30 μg/disc
Gram positive							
Sarcina lutea	12	20	9	16	7	14	17
Bacillus megaterium	12	15	10	16	9	17	18
Bacillus subtilis	13	16	10	17	7	12	18
Streptococcus-β-haemolyticus	12	16	12	21	7	14	24
Staphylococcus aureus	15	25	11	20	9	17	32
Gram negative							
Salmonella typhi	11	17	9	16	7	13	21
Shigella dysenteriae	12	18	10	18	10	26	27
Shigella boydii	16	25	10	18	8	14	35
Shigella sonnei	12	18	9	14	8	13	18
Shigella flexneri	11	16	8	15	7	13	18
Shigella shiga	10	15	10	17	8	15	24
Escherichia coli	11	15	10	19	9	18	20
Pseudomonas aeruginosa	10	15	8	14	8	15	28
Klebsiella sp.	10	14	10	16	7	13	20

Table 2: The MIC values of crude metabolites (M) and two isolated compounds BM-3 and BM-5

	MIC values (μg mL^{-1})		
Test organism	M	BM-3	BM-5
Escherichia coli	64	32	32
Salmonella typhi	64	64	64
Shigella dysenteriae	64	32	32
Bacillus megaterium	64	32	32
Streptococcus-β-haemolyticus	64	16	32
Bacillus subtilis	64	32	64

significant antibacterial activity against the selected test pathogens in comparison with that of standard kanamycin (Table 1). However, the activity profile of these two compounds were interesting against *Streptococcus-β-haemolyticus*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Escherichia coli* in comparison with other pathogens.

The minimum inhibitory concentrations (MIC) values (Table 2) of BM-3 against *E. coli*, *Salmonella typhi*, *Shigella dysentiriae*, *Bacillus megaterium*, *Streptococcus-β-haemolyticus* and *Bacillus subtilis* were found to be 32, 64, 32, 32, 16 and 32 μg ml^{-1} respectively and that for compound BM-5 were 32, 64, 32, 32, 32 and 64 μg ml^{-1} respectively. The MIC of the crude metabolite (M) against all of the above pathogenic bacteria was observed as 64 μg ml^{-1}).

The antibacterial activity suspects the susceptibility or resistance of compounds to pathogenic organism. On the basis of the result further study is warranted to establish their identity, safety and efficacy as antibacterial agents.

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