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Antimicrobial Screening of Two Serine Analogues Isolated from *Streptomyces* Species

M.A. Mojid Mondol
School of Science and Technology, Bangladesh Open University, Gazipur, 1705, Bangladesh

Abstract: This investigation was an attempt to determine the primary selection of the compounds as therapeutic agents, isolated from an antagonistic *Streptomyces* species. Two antimicrobials metabolites were extracted by ethylacetate from the fermentation liquid broth of *Streptomyces* species. The antimicrobial activity profile of the two isolated compounds (MM-2 and MM-4) from the crude metabolites was interesting against some pathogenic bacteria. The Minimum Inhibitory Concentration (MIC) of the two isolated compounds against six pathogenic bacteria were found between 16 and 128 μg mL^{-1} respectively.

Key words: *Streptomyces* species, antimicrobial activity, pathogens, susceptibility.

INTRODUCTION

From the inception of civilization, human being struggles for existence against the affliction of three Ds-Disease, Decay and Death. It is the eternal want of human to remain healthy and cure from his surroundings. But in the ancient era, millions of people died of various infectious diseases like plague, cholera, diarrhoea, tuberculosis in the epidemic form. Man tried to find out remedy of these diseases. In 1928, Sir Alexander Fleming, discovered penicillin, a powerful bactericidal antibiotic from *Penicillium notatum*. Antibiotics first became widely available in the 1940s when they were hailed as magic bullets able to cure everything including common cold. The work of Chain, Falk and Florey in 1941 during 2nd World War penicillin was the most potent anti-infective agent, which was called the Golden Age of antibiotics.

Microorganisms have historically provided a rich sources of structurally diverse and biologically active secondary metabolites. Approximately 85% of the antibiotics are produced by Acetinomycetes, 11% by fungi and 4% by bacteria. Among the Antinomycetes, most of the research work carrying out on the *Streptomyces* species since this species has been proved to potential source of antibiotics.

A large number of antibiotics are used to treat numerous infectious diseases caused by the pathogenic microorganisms like viruses, bacteria, fungi, protozoa and worms. Researchers have revealed many thousands of natural and semisynthetic antibiotics so far. Among them only about 100 have been in medical practice and it is estimated that more the 150 million prescriptions are written for antibiotics each year in the United State[1].

In recent years, indiscriminate and careless use of antibiotic and other unknown reasons, the pathogenic organisms are gaining resistance to the existing antimicrobial agents resulting in a steady decline in the number of effective antibiotics. Hence, the search for newer, safer and more potent antibiotics against these organisms is pressing need. As a part of such efforts, the antimicrobial spectra of two compounds (MM-2 and MM-4) isolated from the liquid culture broth of *Streptomyces* species have been evaluated in August 2002.

MATERIALS AND METHODS

Isolation and identification of the organism: The organism was isolated from soil sample collected from a cultivated land of Naogaon district of Bangladesh at a depth of 0.25 m by using crowded plate technique[2]. The organism was identified on the basis of its morphological, biochemical and some cultural characteristics in milk, potato-agar, cellulose, gelatin, starch, etc.[3] according to the Borgey's Manual of Determinative Bacteriology.

Production of antimicrobial metabolites: The organism was cultured in Czapek-Dox broth (alkaline, pH 8) medium containing 0.75% NaCl utilizing maltose as carbon sources. After 8 days of incubation at 37.5°C, the culture filtrate was extracted with ethylacetate. The ethylaceted fraction thus obtained was evaporated under reduced pressure to give reddish brown semisolid mass.

Isolation of the compounds: The compounds MM-2 and MM-4 were isolated by PTLC technique from crude ethylacetate extract using the solvent system ethylacetate: petroleum ether (6:1) having R_f value 0.311 and 0.482, respectively.

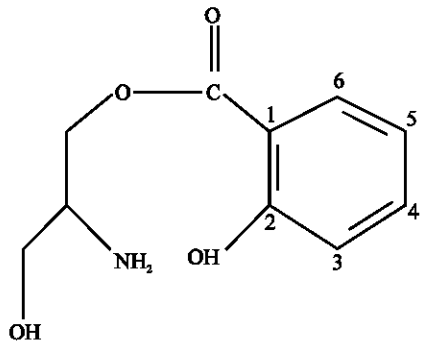

Fig. 1A: 2-hydroxybenzoate-deoxoserine (MM-2)

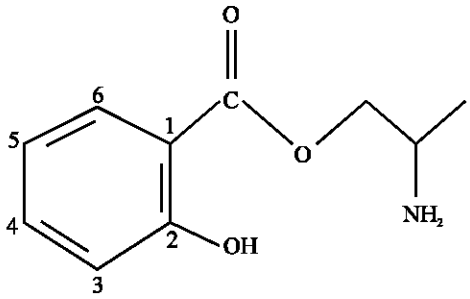

Fig. 1B: 2-hydroxybenzoate-deoxo-dehydroxyserine (MM-4)

Antimicrobial activity: The antimicrobial activities of the compounds (MM-2 and MM-4) (Fig. 1A and B) were determined against 14 selected pathogenic test organisms by disc diffusion assay method[7] at concentration of 100 and 200 μg/disc of each sample and compared the zone of inhibition with that produced by the standard kanamycin antibiotic at 30 μg/disc. The MIC values of the compounds were determined against 6 pathogenic test organisms (Table 2) by serial tube dilution technique[5].

RESULTS AND DISCUSSION

The isolated organism was identified as *Streptomyccs* species[3,4] was subjected to the production of antimicrobial metabolites using Czapek-Dox alkaline broth medium. The crude ethylacetate extract of the culture filtrate of *Streptomyces* species under chromatographic analysis yielded two compounds MM-2 and MM-4 were characterized as 2-hydroxybenzote-deoxoserine and 2-hydroxybenzoate-deoxo-dehydroxyserine, respectively on the basis of its spectral data[3]. Both compounds (MM-2 and MM-4) showed clear antimicrobial 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 *Bacillus subtilis, Salmonella typhi, Shigella flexneri* and *Shigella shiga* in comparison with other pathogens. The Minimum Inhibitory Concentrations (MIC) of MM-2 against *Sarcina lutea, Straphylococcus aureus, Bacillus subtilis, Shigella boydii, Escherichia coli* and *Salmonella typhi* were found to be 16, 32, 64, 32, 32 and 64 μg mL^{-1} and that for compound MM-4 were 128, 64, 64, 128, 32 and 128 μg mL^{-1}, respectively. On the basis of the result further study is warranted to establish their identity, safety and efficacy as antimicrobial agents.

Table 1: Antimicrobial activity of the compound MM-2, MM-4 and standard Kanamycin (K)

	Diameter of zone of inhibition (mm)				
	MM-2 (μg/disc)		MM-4 (μg/disc)		K (μg/disc)
Test organisms	100	200	100	200	30
Gram positive					
Sarcina lutea	17	19	17	22	24
Bacillus meraterium	20	24	15	20	25
Bacillus subtilis	20	22	13	20	26
Streptococcus-β-haemolyticus	16	20	12	18	22
Staphylococcus aureus	19	21	15	21	24
Gram negative					
Salmonella typhi	19	22	16	20	25
Shigella dysenteriae	16	20	17	21	28
Shigella boydii	18	21	16	20	26
Shigella sonnei	14	20	17	21	27
Shigella flexneri	20	26	15	22	29
Shigella shiga	18	22	20	26	30
Escherichia coli	14	19	18	22	27
Pseudomonas aeruginosa	18	20	13	19	26
Klebsiella sp.	15	19	18	21	25

Table 2: The MIC values of the compounds MM-2 and MM-4

	MIC values (μg mL^{-1})	
Test organisms	MM-2	MM-4
Sarcina lutea	16	128
Staphylococcus aureus	32	64
Bacillus subtilis	64	64
Shigella boydii	32	128
Escherichia coli	32	32
Salmonella typhi	64	128

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