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Characterization and *In vitro* Antimicrobial Activity of the Two Novel Compounds of *Streptomyces* Species

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The objective of this investigation is to determine the *in vitro* antimicrobial activity of the two novel compounds from *Streptomyces*. This antagonistic micro-organism was isolated and identified on the basis of morphological and biochemical study from the soil of the Northern district of Pabna, Bangladesh. The organism yielded maximum antimicrobial principle when grown at Czapek-Dox broth medium of pH 8 for 8 days at temperature 37.5°C having rhamnose as carbon source. The antimicrobial principles were extracted from the fermentation broth by choloroform. The extract on chromatographic resolution yielded two antimicrobial compounds: Streptomysone-A (I) and Streptomysone-B (II). Both the compounds were screened for antimicrobial activity against eighteen pathogenic organisms which showed moderate to strong antimicrobial activity. The minimum inhibitory concentration (MIC) of the compounds against five pathogenic organisms were found to be between 32 to 128μg ml^{-1}.

Key words: Streptomyces sp., streptomysone-A, streptomysone-B, antimicrobial activity

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Introduction

In the old age, millions of people were died in the epidemic form of infectious diseases like plague, cholera etc. But after the introduction of newer antibiotics and tremendous advancement of medical sciences and technology, these infectious diseases could be managed successfully (Carlson *et al.*,1983). Micro-organisms have historically provided a rich source of structurally diverse, biologically active metabolites (Jan *et al.*, 1979).

In recent years owing to indiscriminate use of antibiotics the pathogenic organisms are gaining resistance to the existing antimicrobial and chemotherapeutic agents (Julia *et al.*, 1992). Hence the search for newer antimicrobial drugs active against those strains is a pressing need. As a result, scientists have engaged themselves to isolate the newer and effective antibiotics from microbes.

As a part of our continuing search of metabolites from micro-organisms, soil samples were collected throughout Bangladesh. In this investigation, we report the antimicrobial spectra of the two compounds isolated from the culture filtrate of the *Streptomyces* species.

Materials and Methods

Collection of soil samples and identification of the organism: For screening purposes, soil samples of various depth up to 1m, were collected from various places like construction sites, road sides, grave yards, food wastage, agriculture waste, drains and sewage of Bangladesh. The organism was identified on the basis of morphology and biochemical study (Holt *et al.*,1994).

Selection of suitable broth medium for antibiotic production: A number of different media such as Czapek-Dox broth (acidic), Czapek-Dox broth (alkaline), Glucose broth, Yeast extract glucose broth, Jenson medium modified, Nutrient broth etc., were tried for the maximum antibiotic production from the organism. The antibiotic activity of the liquid cultures was tested against *Bacillus subtilis*, *Pseudomonas aureginosa*, *Staphylococcus aureus*, *Shigella dysenteriae* by disc diffusion method (Bauer *et al.*, 1966).

Selection of the suitable culture conditions: The effect of different carbon sources (e.g. Sucrose, D-Glucose, D-Fructose, D-Galactose, Maltose, D-Mannose, lactose, D-mannitol, L-arabinose, D-xylose and rhamnose); incubation period (up to 14 days); temperature (30, 32.5, 37.5, 30, 40, 42.5 and 45°C); pH values (3, 4, 5, 6, 7, 8 and 9) and NaCl concentration (0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 9 % respectively), on antibiotic production by the organism were studied by disc diffusion method (Bauer *et al.*, 1966) using *Shigella dysenteriae* as test organism.

Production, isolation and characterization of the compounds: The organism was allowed to grow in a number of culture flasks of 500ml capacity containing Czapek-Dox broth alkaline medium (100ml in each flask) at 37.5°C. After 8 days (due to maximum yield of antibiotic) the broth was separated from the mycelial mat. The culture filtrate then subjected to repeated chloroform ($CHCl_3$) extraction (3 × 30 ml) and the extract was evaporated under reduced pressure. The crude antibiotic fraction was resolved by thin layer chromatography (TLC), preparative TLC (PTLC) and obtained on large scale by column chromatography (CC) (Beckett *et al.*, 1986). The isolated antibiotics were characterized as streptomysone-A (I) and streptomysone-B (II) on the basis of their UV, IR and NMR data.

Antimicrobial screening: The antimicrobial activity of the compounds I and II (25 and 50μg disc^{-1} respectively) were determined against six gram positive bacteria, eight gram negative bacteria and four pathogenic fungus by the standard disc diffusion method (Bauer *et al.*, 1966). Amoxycillin disc (25μg disc^{-1}) for bacterial and Grisofulvin (20μg disc^{-1}) for fungal test were used as standard for the comparison of antimicrobial activity. The test organisms were collected from the Department of Microbiology, Universtiy of Dhaka, Bangladesh. The MIC values of the compounds were determined against *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* by serial dilution technique (Hammond *et al.*, 1978).

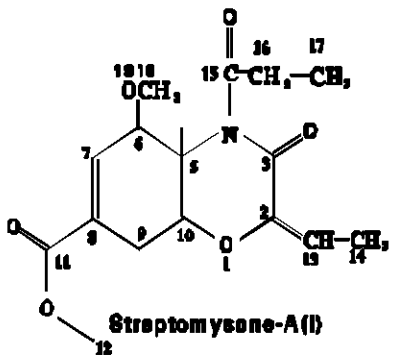
Result and Discussion

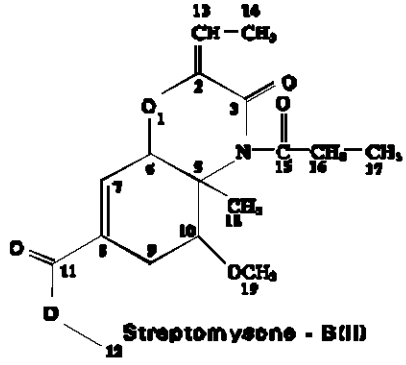
Identification of the organism: The organism was identified as *Streptomyces* species, on the basis of the following characteristics (Holt *et al.*,1994):

Spore chain morphology.
The spores were ornamented.
The mature spores were colored.
It was able to produce melanoied pigment.
It was able to utilize under standardized conditions particular carbon containing compounds for growth.
It was able to produce antibacterial factors.
Vegetative mycelia were colored.

Suitable culture condition: The effect of different physical parameters on antibiotic production was observed. It was found that the production of antibiotic from the organism in Czapek-Dox broth alkaline medium after 8 days of incubation (Fig. 1), at pH 8 (Fig. 2) at temperature 37.5°C and rhamnose as carbon sources (Fig. 3) was found to be the most suitable.

Isolation and characterization of the compounds: The $CHCl_3$ extract of the culture filtrate of *Streptomyces* sp. on chromatographic analysis yielded two novel antimicrobial compounds designated as streptomysone-A (I) and Streptomysone-B (II) on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR (Anisuzzaman 2000). The structure of the compounds are as follows:





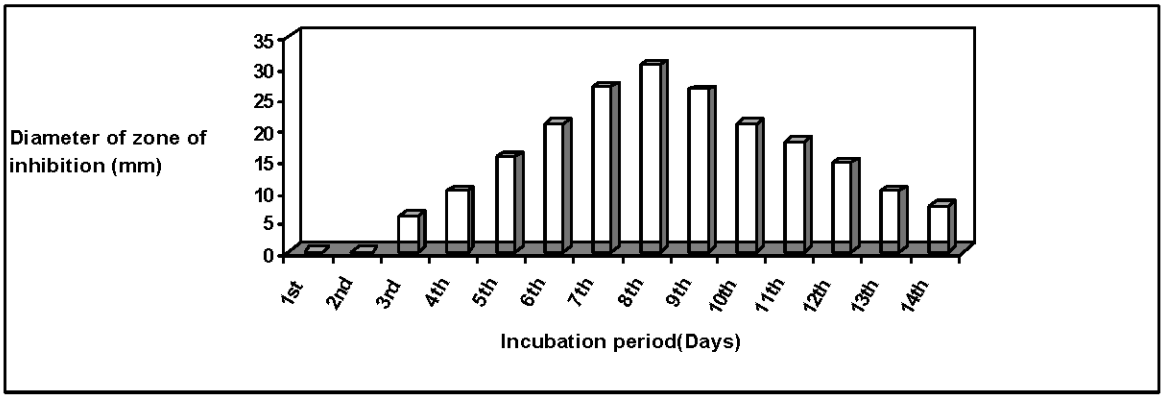


Fig. 1: Effect of incubation period on the production of antibiotic

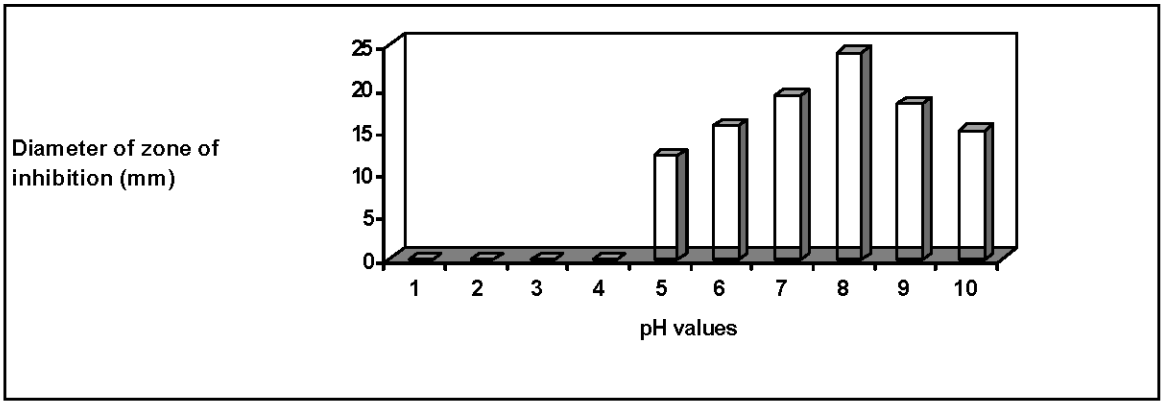


Fig. 2: Effect of pH on the production of antibiotic

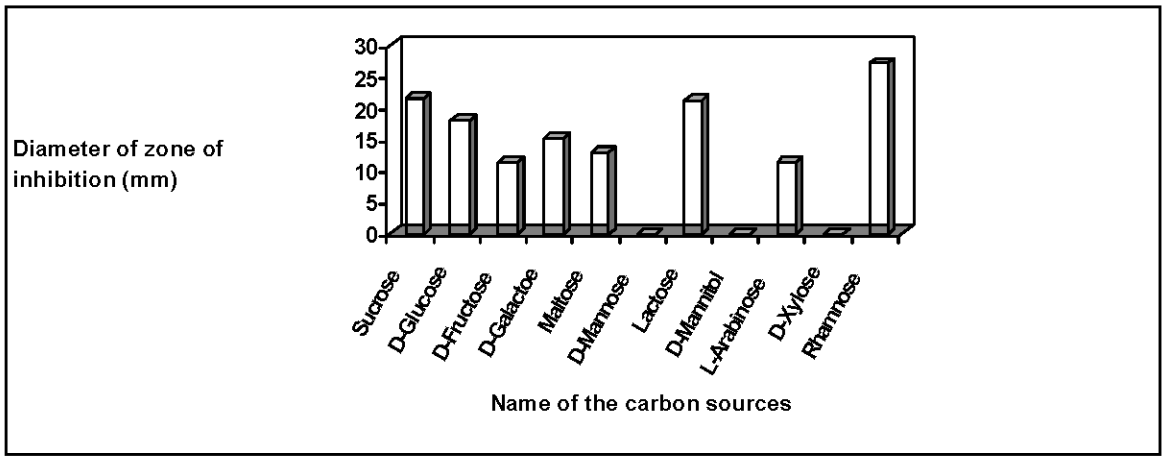


Fig. 3: Effect of Carbon sources on the production of antibiotic

Antimicrobial activity of the compounds: Both the compounds showed significant antimicrobial activity against the test pathogens. The results are shown in the Table 1 and Table 2. However, the compound (I) exhibited the strong activity against *Bacillus subtilis, Bacillus megaterium, Escherichia coli* and *Klebselia* species and comparatively weak activity was observed against *Pseudomonas aureginosa. Salmonella typhi-A. Shigella dysenteriae.* While the compound (II) exhibited strong activity against gram negative bacteria than gram positive bacteria. The compounds were also active

Table 1: Antibacterial activity of the compound I and II.

	Zone of inhibition in mm.				
	Compound-I		Amoxycillin-Trihydrate	Compound-II	
Test bacteria	25 μg disc^{-1}	50μg disc^{-1}	25 μg disc^{-1}	25 μg disc^{-1}	50μg disc^{-1}
Gram positive					
Bacillus cereus	15	24	30	11	14
Bacillus subtilis	17	25	29	15	20
Bacillus megaterium	18	25	27	12	16
Sarcina lutea	12	18	25	13	18
Staphylococcus aureus	11	16	26	09	14
Streptococcus-β-hemolyticus	12	16	27	10	14
Gram negative					
Shigella dysenteriae	16	24	33	22	28
Shigella flexneri	15	18	31	21	25
Shigella boydii	10	14	30	19	24
Shigella shiga	11	14	29	17	21
Escherichia Coli.	20	28	33	25	21
Psuedomonas aureginosa	12	17	28	18	25
Klebsiellas sp.	14	22	25	17	26
Salmonella typhi-A	11	19	29	20	29

Table 2: Antifungal activity of the compound I and II.

	Diameter of zone of inhibition (mm)		
Test fungus	Compound- I 25μg disc^{-1}	Griseufulvin 20μg disc^{-1}	Compound- II 100μg disc^{-1}
Tinea pedis	21	16	11
Tinea corporis	16	17	19
Candida albicans	15	15	17
Rhizoctonia solani	14	19	09

Table 3: MIC value of the compound (I).

	Concentration of the compound (μg ml^{-1})									
Test organism	512	256	128	64	32	16	8	4	2	1
Bacillus subtilis	-	-	-	-	-	+	+	+	+	+
Streptococas-β-hemolyticus	-	-	-	-	+	+	+	+	+	+
Escherichia coli	-	-	-	-	-	+	+	+	+	+
Pseudomonus aureginosae	-	-	-	-	+	+	+	+	+	+
Salmonella typhi-A	-	-	+	+	+	+	+	+	+	+

Table 4: MIC value of the compound (II).

	Concentration of the compound (μg ml^{-1})									
Test organism	512	256	128	64	32	16	8	4	2	1
Bacillus subtilis	-	-	-	-	-	+	+	+	+	+
Streptococas-β-hemolyticus	-	-	-	-	+	+	+	+	+	+
Escherichia coli	-	-	-	-	-	+	+	+	+	+
Pseudomonus aureginosae	-	-	-	-	+	+	+	+	+	+
Salmonella typhi-A	-	-	+	+	+	+	+	+	+	+

+ = Growth, - = No growth

against pathogenic fungus i.e. *Tinea pedis, Tinea corporis, Candida albicans* and *Rhizoctonia solani.*

Minimum Inhibitory concentrations of the compounds: The minimum inhibitory concentration of the compound (I) and (II) were shown in the Table 3 and Table 4, respectively. The MIC values of the compound (I) against *Bacillus subtilis, Streptococcus-β-hemolyticus, Escherichia Coli, Psuedomons aureginosa* and *Salmonella typhi* - A were 16, 32, 16, 32 and 128μg ml^{-1} respectively, and that for compound (II) were 16, 32, 16, 32 and 128μg ml^{-1} respectively. From the MIC values, it was found that both the compounds were more potent against *Bacillus subtilis* and *Escherichia Coli.*

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