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In vitro Antibacterial Activity of an Active Metabolite Isolated from *Streptomyces* Species

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Abstract: The ethyl acetate extract of yeast extract-glucose broth medium of an *Actinomycetes* strain, *Streptomyces* yielded a reddish yellow antibiotic metabolite by chromatographic technique and was identified as 2 N-butanamide 3-methyl 4-methoxy 5-β-L-arabinosyl propanophenone (MZ-4) on the basis of spectral data. The compound exhibited significant antibacterial activity against five gram positive and nine gram negative bacteria when compared with standard Kanamycin antibiotic. The zone of inhibition were observed between 17 to 20 mm. The minimum inhibitory concentration (MIC) of the compound was determined against four gram positive and gram negative bacteria and the values were found between 32 and 64 μg ml^{-1}.

Key words: Antibacterial activity, *Streptomyces* species, 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone

Introduction

From the inception of civilization, human being struggles for existence against the affliction of 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 from various infectious diseases like plague, cholera, diarrhea, tuberculosis etc. in epidemic form, which has instigated the man to endeavor for remedy from their sufferings (Pelczar *et al.*,1993). In fact in the way of long struggle for relieving from such infectious diseases as well as illness, physical discomforts, injuries, pain and wounds, man would achieve the mastery over the diseases specially infectious diseases when Alexander Fleming accidentally discovered penicillin from a microorganism.

The first systematic search for antibiotics, resulted in the discovery of Actinomycetin from *Actinomycete* bacteria. In United States and Japan between 1953 to 1970 approximately 85% of the antibiotics were produced by *Actinomycetes*, 11% by fungi and 4% by bacteria (Reiner, 1982). Although antibiotics are life saving drugs but now a days, in order to careless and promiscuous use of antibiotics, various pathogenic microbes are gaining resistance. To overcome this problem and to treat many serious infectious diseases like AIDS, cancer, viral fever continuous search of new more potent antibiotic is going on. As a part of our continuous search, a *Streptomyces* species was isolated from the soil sample collected from Bogra, a northern district of Bangladesh, which under optimum culture conditions yielded the antibacterial

compound.
In the present study, we report the antibacterial spectra of a compound isolated from the ethyl acetate extract of yeast extract-glucose broth medium of the organism.

Materials and Methods

Isolation and identification of antagonistic organisms: The experiment was carried out in Microbiology research laboratory, Department of Pharmacy, Rajshahi University, Bangladesh. For screening purpose, dry warm soil samples were collected ranging from 0.25 to 1.5 m in depth from different places like roadside, construction site, grave yards, river bank, ploughed field, food wastage, sewage, play ground and under medicinal plants of different parts of Bangladesh. An antagonistic strain was isolated and its antimicrobial activity was tested by crowded plate technique (Hammond and Lambert, 1978). The organism was identified on the basis of its morphological and biochemical study according to John *et al.* (1994).

Selection of suitable broth medium and optimum culture conditions for the production of antibiotic: For maximum production of antimicrobial compound from the selected organisms, several number of broth culture media such as Czapek-Dox broth (acidic), Czapek-Dox broth (alkaline), yeast extract glucose broth, Bonner Addicot broth and Oat broth were tried. Activity of the liquid culture was tested against *Bacillus subtilis* and *E. coli* by disc diffusion assay method (Beaur *et al.*, 1966).
On the production of antibiotic various physical parameters such as effect of different carbon sources (Sucrose, D-glucose, D-fructose, D-galactose, maltose, D-mannose, lactose, D-mannitol, L-arabinose, D-xylose and rhamnose) incubation period (up to 16 days), temperature (30, 32.5, 35, 37.5, 40 and 45°C), pH values (3, 4, 5, 6, 7, 8, 9 and 10) and salt concentration (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0% w/v) were studied against *Bacillus subtilis* by disc diffusion assay method (Beaur *et al.*, 1966).

Extraction, isolation and characterization of the compound: To yield maximum metabolites, organism was grown on yeast extract glucose broth media at 37.5°C. The liquid broth was separated through filtration. Then the filtrate was extracted with ethyl acetate and concentrated. By using thin layer chromatographic technique (Egon and Stahl, 1969), a pure antimicrobial compound (MZ-4) was isolated from the ethyl acetate extract and identified as 2 N-butanamide 3-methyl 4-methoxy 5-β-L-arabinosyl propanophenone on the basis of its spectral data (Sultan, 2002).

Antibacterial screening: The antibacterial activity of MZ-4 was tested at a concentration of 250 µg/disc against fourteen test pathogenic organisms by the standard disc diffusion method (Beaur *et al.*, 1966). Standard Kanamycin disc (K-30, 30 µg/disc) was used for comparison of the antibacterial activity. Nutrient agar was used as a bacteriological media. The minimum inhibitory concentration (MIC) was determined against *Bacillus subtilis*, *Bacillus megaterium*, *Streptococcus-β-hemolyticus*, *Staphylococcus aureus*, *Shigella dyscenteriae*, *E.coli*, *Klebsiella* species and *Pseudomonas aeruginosa* by serial dilution

technique (Hammond and Lambert, 1978).

Results and Discussion

Identification of the organism: The organism was identified as *Streptomyces* species on the basis of its following morphological characteristics observed under microscope from 2nd day of incubation (John *et al.*, 1994):

1) Growth occurred from a spore or fragment of mycelium 2) Hyphae were initially white and then changed to ash colour when it was being mature 3) Colony appeared velvety 4) Mycelial filaments tend to remain intact 5) Spore chains were abundant 6) Non motile aerial mycellia were present.

Suitable broth medium and correct culture conditions: Different broth media, carbon sources and different physical parameters were tested for maximum production of antibiotics. It was found that yeast extract glucose broth media after 10 days incubation (Fig. 2) at 2% salt (NaCl) concentration (Fig. 3), at pH 8 (Fig. 4), using maltose as a carbon source (Fig. 5) and at 37.5°C temperature (Fig. 6) was the most suitable for large scale production of antibiotic.

Isolation of the compound: From the crude ethyl acetate extract of the culture filtrate of *Streptomyces* species a pure antibacterial compound was isolated and purified by preparative thin layer chromatographic technique and was characterized as 2 N-butanamide 3-methyl 4-methoxy 5-β-L-arabinosyl propanophenone (Fig. 1) on the basis of its spectral data (Sultan, 2002).

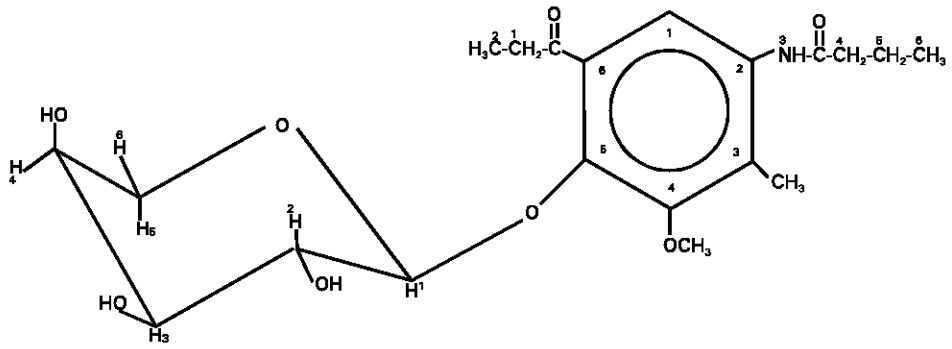

Fig. 1: 2-N-butanamide-3-methyl 4-methoxy-5-β-L-arabinosyl- propanophenone

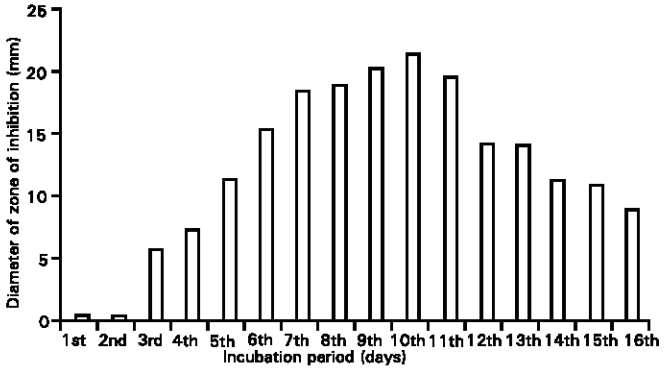

Fig 2: Effect of incubation period on the production of antibiotic

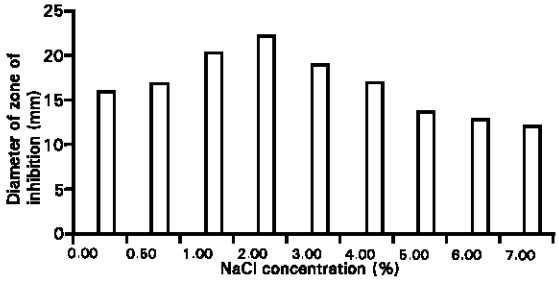

Fig. 3: Effect of NACl concentration on the production of antibiotic

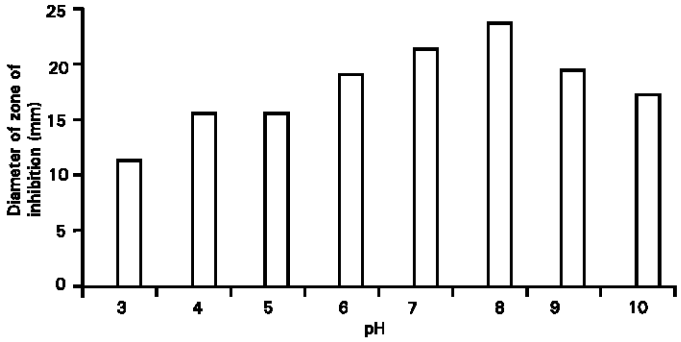

Fig. 4: Production of antibiotic at different pH

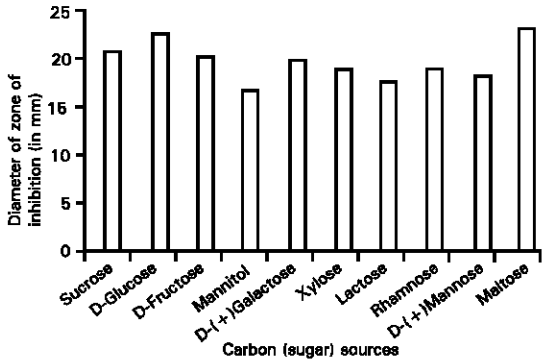

Fig. 5: Effect of different carbon sources on the production of antibiotic

Table 1: Antibacterial activity of the antibiotic MZ-4

	Zone of inhibition (mm)	
Test bacteria	MZ-4 (250 µg/disc)	Kanamicin K-30 (30 µg/disc)
Gram positive		
Bacillus subtilis	20	29
Bacillus megaterium	17	27
Sarcina lutea	19	28
Staphylococcus aureus	19	28
Streptococcus-β- haemolyticus	20	29
Gram negative		
Salmonella typhi	20	29
Shigella flexneri	19	27
Shigella boydii	19	28
Shigella sonnei	18	28
Shigella dysenteriae	20	28
Shigella shiga	19	29
Escherichia coli	20	29
Pseudomonas aeruginosa	19	30
Klebsiella sp	18	26

Antibacterial activity of the compound: The compound showed significant antibacterial activity against 14 pathogenic organisms (Table 1). From the results, it is evident that the compound showed strong antishigella activity against all *Shigella* strains such as *Shigella dysenteriae* (20 mm), *Shigella boydii* (19 mm), *Shigella shiga* (19 mm), *Shigella sonnei* (18 mm) and *Shigella flexneriae* (19 mm) at a concentration of 250 µg/disc and the range of the zone of inhibition was between 18-20 mm. The extract also showed strong activity against *Salmonella typhi* (20 mm), *Pseudomonas aeruginosa* (19 mm), *Bacillus subtilis* (20 mm), *Sarcina lutea* (19 mm) and *Strep-β- hemolyticus* (20 mm) and moderate activity against other tested bacteria at a concentration of 250 µg/disc.

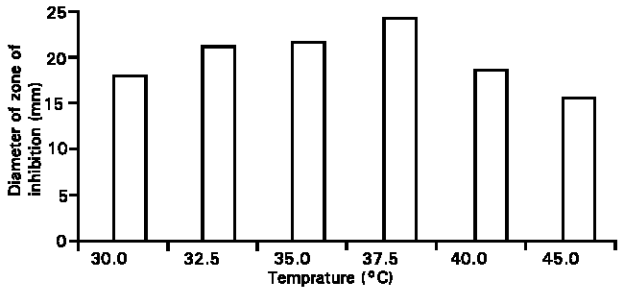

Fig. 6: Effect of temperature on production of antibiotic

Table 2: The MIC of the antibiotic MZ-4 against six test organisms

Test organisms	Minimum inhibitory concentration (µgm/ml)
Gram positive	
Bacillus subtilis	64
Bacillus megaterium	64
Streptococcus-β-haemolyticus	64
Staphylococcus aureus	32
Gram negative	
Escherichia coli	64
Shigella dysenteriae	64
Klebsiellia sp.	64
Pseudomonas aeruginosa	64

Minimum inhibitory concentration: The MIC of the compound was 64 µg ml^{-1} against *Bacillus subtilis*, *Bacillus megaterium*, *Streptococcus-β-hemolyticus*, *Shigella dysenteriae*, *E.coli*, *Klebsiella* sp. and *Pseudomonas aeruginosa* and was 32µg ml^{-1} against *Staphylococcus aureus*.

From the antibacterial experimental results, it is evident that the active metabolite 2 N-butanamide 3-methyl 4-methoxy 5-β-L arabinosyl propanophenone showed significant antibacterial activity especially against *Shigella* species but were less potent than that of standard kanamycin. The results of this study strongly support that the isolated metabolite may be used in the management of microbial infection. In recent years the pathogenic organisms are gaining resistance to existing antimicrobial agents hence the search for new, safe and more effective antimicrobial agents is a pressing need. Thus the findings of this investigation and previous investigation on other microbes and plants (Rahman *et al.*, 2000; Sathi *et al.*, 2001) would give valuable support to make clinical trial as well as toxicity studies of the isolated antibacterial metabolite to get a more potent antimicrobial agent.

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