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Antibacterial and Cytotoxic Activities of the Metabolites Isolated from a *Penicillium* Strain

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Abstract: Three antibiotics (RA-1, RA-2 and RA-3) were isolated from the chloroform extract of the fermentation broth of a *Penicillium* strain of an unidentified species. These compounds exhibited a significant *in vitro* antibacterial activity against both gram-positive and gram-negative organisms. The MIC values for RA-1, RA-2 and RA-3 were also determined against *Bacillus subtilis, Shigella sonnei* and *Escherichia coli*. The chloroform extract also showed strong cytotoxicity in brine shrimp lethality assay.

Key words: Antibacterial activity, cytotoxicity, *Penicillium*

Introduction

Although scientists are discovering newer and more potent anti-microbial agents, infectious diseases are still a leading health problem with high morbidity and mortality in the developing countries and cease about 17 million lives annually (Black *et al*., 1982; UNDP, 1994). The pathogenic microorganisms with their determination to survive are gaining resistance by curious mechanisms. Moreover, most of the effective antibiotics are not free from untoward effects and the organisms are becoming resistant to the available antibiotics due to their indiscriminate uses. So, there still remains rooms for a large number of antibiotics with various properties and the scientists are paying more and more attention globally for the development of newer and better anti-microbial agents.

The genus *Penicillium* is already well known for the production of antibiotics (Julia *et al*., 1992; Kill *et al*., 1988). As a part of continuing studies on the microbes from soil samples collected from different part of Bangladesh, the authors isolated a *Penicillium* strain which when grown in Czapek-Dox agar acidic medium produced three antibiotics (RA-1, RA-2 and RA-3). The isolation and *in vitro* antibacterial activity of the three antibiotics are reported here. The preliminary cytotoxic effect of the chloroform extract of the culture filtrate of the *Penicillium* strain is also discussed.

Materials and Methods

Isolation of the *Penicillium* strain: The organism was isolated from a soil sample collected from Savar, Bangladesh by crowed plate technique (Hamand and Lambert, 1978) and was identified as a *Penicillium* strain (Amin, 1995).

Production, isolation and purification of the antibiotics: The *Penicillium* strain was cultured in Czapek- Dox broth acidic medium of pH 4 and after 5 days of incubation at 37°C, the temperature at which maximum growth of the organism and the highest production of antibiotics were observed, the culture filtrate (2000 ml) was extracted with chloroform (3X600 ml). The red mass (210 mg) obtained after evaporation of the solvent was subjected to column chromatography using water saturated chloroform-ethyl acetate- acetic acid -methanol (15:5:1:0.5) as the developing solvent to yield 90 fractions (1 ml each). These column fractions were analysed by TLC and identical fractions were bulked together to yield 4 fractions (A-1, A-2, A-3 and A-4). The column fractions A-1, A-3 and A-4 were again subjected to preparative TLC and the antibiotics RA-1, RA-2 and RA-3, respectively were collected in slightly impure form. The plates were developed with the same solvent system used in column chromatography. Finally, the antibiotics (RA-1 and RA-2) were purified by HPLC (equipped with a UV detector) using methanol-water (7:3) on a C_{18} bonded silica column to yield pure RA-1 (3 mg) and RA-2 (2 mg). The chromatograms are shown in Fig. 1.

Antibacterial screening of the fungal metabolites: The antibacterial activity of the isolated antibiotics was determined against 6 gram-positive and 9 gram-negative bacteria by the standardized disc diffusion method (Barry, 1980, Bauer *et al*., 1966). Standard Kanamycin disc (Kanamycin K-30, 30 μg $disc^{-1}$) was used for comparison of the antibacterial activity.

Determination of MIC values: The minimum inhibitory concentration values of the antibiotics were determined against *Bacillus subtilis, Shigella sonnei* and *Escherichia coli* by serial dilution method (Reiner, 1982).

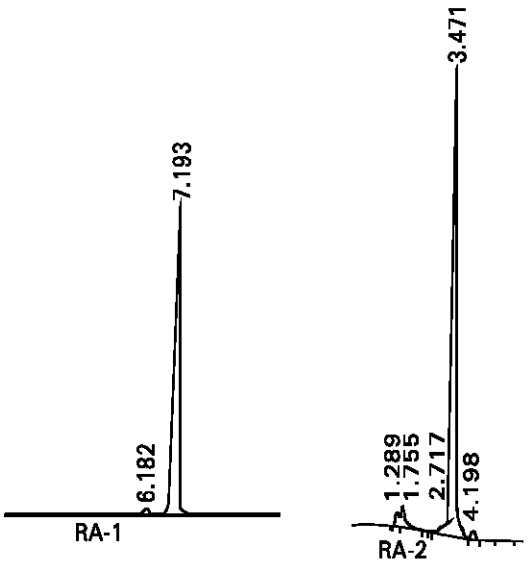

Fig. 1: HPLC traves of antibiotics RA-1 and RA-2, the retention times being expressed in minutes

Determination of cytotoxic activity of the chloroform extract: The cytotoxic activity of the fungal metabolites was studied by the brine shrimp lethality bioassay technique (Meyer *et al*., 1982; Persoone, 1980). In brief, the eggs of brine shrimp, *Artenia salina*, were hatched in seawater. Ten mature larvae (nauplii) were kept in glass vials containing 10 ml of seawater. The test compound dissolved in DMSO (10 mg/ml) was applied to the nauplii in each vial. However, not more than 50 µl of DMSO was added to the vials containing the shrimps. For each concentration, vials containing the same volume of DMSO plus seawater and shrimps was used as control. After 24 h, the vials were observed for mortality, if any. The number of survived nauplii in each vial was counted and from this data the percentage of lethality of the brine shrimp nauplii was calculated. From this value the LC_{50} of the sample was determined (Goldstein *et al*., 1974).

Results and Discussion

The chloroform extract of the culture filtrate of a *Penicillium* strain afforded three antibiotics designated by RA-1, RA-2 and RA-3. All of these compounds exhibited a significant antibacterial activity when tested against both gram-positive and gram-negative bacteria at a concentration of 100 and 200 µ gm $disc^{-1}$ (Table 1). The inhibitory activities produced by the test compounds were compared with that demonstrated by a broad-spectrum antibiotic, Kanamycin at 30 µ gm $disc^{-1}$. The zone of inhibition produced by RA-1, RA-2 and RA-3 were found to be 10-21, 12-23 and 10-22 mm at 100 µgm $disc^{-1}$ and 13-26, 16-27 and 14-26 mm at 200 µ gm $disc^{-1}$, respectively.

Table 1: Antibacterial activities of the chloroform extract, isolated antibiotics and kanamycin

	Diameter of Zone of Inhibition (mm)							
		RA-1 µg $disc^{-1}$		RA-2 µg $disc^{-1}$		RA-3 µg $disc^{-1}$		
Test bacteria	Chloroform Extract (1mg)	100	200	100	200	100	200	Kanamycin 30 µg $disc^{-1}$
Gram positive								
Bacillus cereus (QL-29)	15	17	21	19	23	10	14	16
Bacillus subtilis (QL-40)	19	21	26	17	21	13	19	21
Bacillus polymyxa	15	10	14	15	21	20	24	23
Bacillus megaterium (QL-38)	18	20	23	14	19	18	21	20
Staphylococcus aureus (ATCC-25923)	17	12	17	13	16	11	14	28
Streptococcus-β-haemolyticus (CRL)	15	19	21	20	24	19	23	25
Gram negative								
Shigella dysenteriae (AL-35587)	21	12	18	23	27	21	25	20
Shigella shiga (ATCC-26107)	15	15	17	21	25	20	22	24
Shigella sonnei (AJ-8992)	19	19	23	22	24	22	26	21
Shigella boydii (AL-17313)	16	15	21	19	21	18	23	20
Shigella flexneri (AL-30372)	21	12	17	20	23	21	25	26
E. coli (FPFC-281)	18	16	20	18	23	18	21	27
Pseudomonas aeruginosa (CRL)	16	14	19	12	19	15	18	30
Salmonella typhi A	14	13	18	21	25	20	24	23
Salmonella tyrhi B-56	12	10	13	18	23	17	20	20

*Strain Nos are included in the parentheses

Table 2: Minimum inhibitory concentrations of the isolated antibiotics

	Minimum inhibitory concentration (μg/ml)		
Test bacteria	RA-1	RA-2	RA-3
Bacillus subtilis	128	128	64
Shigella sonnei	128	64	128
E.coli	256	128	64

Table 3: Results of brine shrimp lethality bioassay of the shloroform extract

Concentration of sample (μg/ml)	Log concentration	% mortality	LC 50 value (μg/ml)
0	0	0	
10	1.0	36	
20	1.3	53	17.78
50	1.7	73	
100	2.0	100	
200	2.3	100	

On the other hand, Kanamycin produced 16-30 mm of zone of inhibition. It appears from Table 1 that the activities of RA-1, RA-2 and RA-3 were comparable to that exhibited by Kanamycin. However, the later was almost 3 fold more potent than the isolated compounds.

The MIC values of RA-1, RA-2 and RA-3 were also determined against *B. subtilis, S. sonnei* and *E. coli* and were found to be 64-256 μ gm ml^{-1} (Table 2).

The chloroform soluble fraction of the fermentation broth of the *Penicillium* strain was also screened by the brine shrimp lethality bioassay for probable cytotoxicity. The crude extract demonstrated a strong cytotoxic activity with a LC_{50} value (concentration for 50% lethality) of 17.78 μ gm ml^{-1} (Table 3).

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References

Amin, A.R.M. Ruhul, 1995. Studies on a *Penicillium* species. M. Pharm. degree Thesis, Dhaka University, Dakha, Bangladesh.

Barry, A.L., 1980. Procedures for testing antimicrobial agents in agar media. In: Antibiotic in Laboratory Medicine (V. Lorian, Ed.), Williams and Wilkins Company, Baltimore, USA, pp: 1-23.

Bauer, A.W., W.M.M. Kirby, J.C. Sherries and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 492-493.

Black, R.E., K.D. Brown, S. Becker and M. Yunus, 1982. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. Am. J. Epidemiol., 115: 305-315.

Goldstein, A., L. Aronow and S.M. Kalkan, 1974. Priciples of Drug Action, 2nd ed., Willey Biomedical Health Publication, pp: 376-381.

Hamand, M.S. and A.P. Lambert, 1978. Antibiotics and antimicrobial action. Edward Arnold Limited, London, pp: 12-15.

Julia, A., F. Enrique and P. Jaime, 1992. Isolation and identification of ethisolide as an antibiotic product from *Penicillium* capsulatum. Appl. Microbiol. Biotechnol., 37: 279-300.

Kill, P.B., M. Nakagawa, A. Hirota and M. Nakayama, 1988. Methylenolactocin, a novel antitumour antibiotic from a *Penicillium* species. J. Antibiot., 41: 751-758.

Meyer, B.B., N.R. Ferringi, J.F. Futnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: a convenient general bioassay for active plant constituents.Planta Medica, 45: 31-34.

Persoone, G., 1980. Proceedings of the international symposium on brine shrimp, Artemia salina. Universa Press, Witteeren, Belgium, pp: 1-3.

Reiner, R., 1982. Detection of antibiotic activity. In: Antibiotics, an introduction. Roche Scientific Services, Switzerland, pp: 21-25.

UNDP publication, 1994. A new Dimensions of Human Security. Human Development Report, pp: 27-28.