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ANTIMICROBIAL EFFECT OF PROTEIN(S) ISOLATED FROM A MARINE MOLLUSC *TELESCOPIUM TELESCOPIUM*

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Abstract : Ammonium sulfate precipitated protein (SF–50) isolated from the spermatheca gland of *Telescopium telescopium,* an invertebrate marine snail, showed antimicrobial effect on *Escherichia coli.* The antimicrobial effect varied with the concentration of "SF–50" used and the effect was found to be comparable to antibiotics like amikacin, contrimoxazole and gentamycin in disc diffusion test. The "SF–50" was devoid of erythrocyte haemolysis property.

Key words: "SF–50" *Escherichia coli*

INTRODUCTION

A highly positive correlation between bacterial agglutination and bactericidal effect has been observed by lectins obtained from lobster, *Homarus americanus.* Other species possessing bacterial agglutinins in their body fluids include *P. bicarinatus, A. californica, M. mercenerias* and the mollusc *Otala lactea.* It has also been reported that a purified lectin from chinook salmon ova possesses antibacterial activity (1) A few drugs have already been found effective like the antibiotic cephalotin from the marine fungus *Cephalosporium acremonium* (2).

We also observed that "SF–50", the proteins from the spermatheca gland of a marine mollusc *Telescopium telescopium*, have agglutinating and spermicidal effect may be due to lysis of the sperm membrane as observed under scanning electron microscope (unpublished data).

"SF–50" being surface active (unpublished data), it was thought desirable to ascertain its antimicrobial property, if any.

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METHODS

Isolation of "SF-50"

The adult snails, *Telescopium telescopium*, were collected from the Sagar Island in West Bengal. Snails were narcotized in isotonic (7.2%) $MgCl_2$ solution for 15 min. (3). The outer shell wall was broken and the whole body removed carefully. Organ spermatheca was dissected from the body and washed thoroughly with 0.15 m phosphate buffer saline (pH 7.2). The collected organs were minced and homogenized at 4°C in 0.15M PBS. Grinded tissue was further fragmented by ultrasonic rays at 6 μ amplitude. Sonicated material was ultracentrifuged (114,000 r.p.m.) for 45 min. at 4°C. The supernatant was collected, lyophilized and kept at –20°C (Crude extract; CSPT). The crude extract that obtained was dissolved in 9.5 ml of 0.5 M PBS to get the partially purified protein by 50% ammonium sulfate precipitation (4). The dialized solution was lyophilized and aliquoted at –20°C (SF–50). A stock solution of 2 mg/ml of "SF–50" in sterilized 0.5 M PBS (pH 7.20) was prepared for the experimental purpose.

Antimicrobial effect of "SF-50"

Antimicrobial effect of "SF–50" was studied by standard disc diffusion method (5).

Preparation of discs

Discs of 4 mm diameter were made from whatman No.3 filter paper with the help of a paper puncher. The discs were placed in a petridish and sterilized in a hot–air oven for two hrs at 121°C. "SF–50" solution of different concentration (100 μl, 60 μl, 50 μl, 40 μl, 30μl and 20 μl) were taken from the stock solution and carefully placed on to different discs by seperate automatic pipette to moisten the discs and dried in a laminar flow chamber. The control test was run side by side with the discs moistened in sterilized 0.5 M phosphate buffer saline, pH 7.4. For other antibiotics discs (DIPCO) were used. All discs were stored in a refrigerator at 4°C.

Preparation of inoculum

Pathogenic strain of E. coli from cultured human urine was used for the study. Five identical colonies were isolated and subcultured in 2 ml of nutrient broth for 4 hrs at 37°C. Standarization of the inoculum was made by preparing a McFarland standard and was compared with the turbidity of the inoculum. The test organism was adjusted to 10^4 and 10^8 CFO/ml.

Preparation of standard

0.048 M hydrated barium chloride was mixed with 0.36 n sulphuric acid. The resultant suspension of barium sulfate precipitate is equivalent to 1×10^8 organisms/ml.

Inoculation of test plate

Muller Hinton agar plates were used for susceptibility testing by the disc diffusion technique. The culture plates were incubated with the lid agar until its surface was free from visible moisture. The bacterial inoculum was poured on to the culture media. The plates swayed in all directions to wet the whole of its surface. All excess

fluid was removed with a sterile pasteur pipette and the plates were made dry. Discs prepared with different concentration of "SF–50", antibiotics and with PBS were applied at adequate spacing (2 cm apart) to the surface of the plates with sterile fine pointed forceps and by pressing gently to ensure full contact with the medium and the discs. The plates were then incubated for 24 hrs at 37°C.

Reading and interpretation of result

The diameters of the zones of inhibition of growth (including 4 mm diameter of the disc itself) were measured with the help of a scale. Where more than 12 mm is sensitive zones between 4 to 12 mm is moderately sensitive and zones less than 4 mm is resistant.

Erythrocyte haemolysis assay :

Borosilicate test tubes (75 × 12 mm), two in each of 4 sets, were used. Tubes of each set were filled with 100 µl, 200 µl and 300 µl of SF–50 solution respectively from the stock solution. The last two tubes (4th set) served as control with 300 µl of triton–x 100 (0.1% solution in 0.15 M PBS; pH 7.6) solution.

Diluted human erythrocyte suspension (2.5 ml; 10%, v/v) in isotonic PBS; pH (7.2) was added to each tube and mixed thoroughly by gentle shaking. All the tubes were incubated for 30 min. at 37°C in an incubator and centrifuged individually at 3000 × g for 10 min. The supernatants were collected individually by separate Pasteur pipettes and measured at OD_{350} (6).

RESULT

In plate culture, "SF–50" showed significant antimicrobial effects comparable to the antibiotics like amikacin, cotrimoxazole, gentamycin. The diamater of the inhibitory zone varied from 11–14 mm (Fig. 1) with 60 µg. 50 µg. 40 µg. 30 µg. 20 µg. of SF-50 treated disc. The antibiotic treated inhibitory zones varied from 7–14 mm with other antibiotics like ciprofloxacin, gentamycin, mandelamine, norfloxacin, pefloxacin, chloramphenicol and cotrimoxazole (Fig. 2). The erythrocyte haemolytic assay showed normal change, > 0.52 (OD) reading) as against 3.1 (OD) reading) of triton-x 100 treated control.

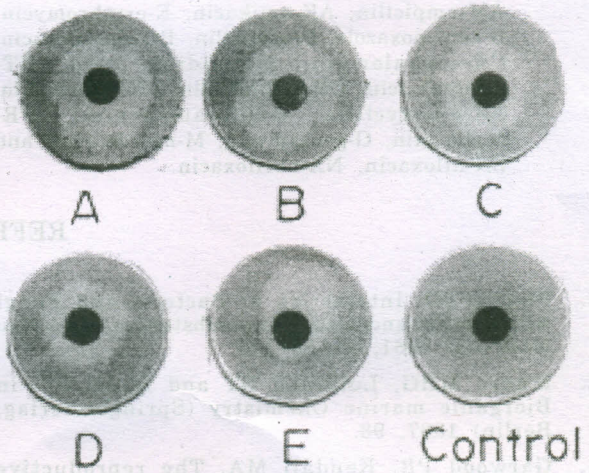

Fig. 1 : Showing the dose dependent antimicrobial effect on *E. coli.* SF-50 from A ----- E (60, 50, 40, 30, 20) µg respectively and control (only vehicle).

The protein, therefore, showed an insignificant effect on blood haemolysis.

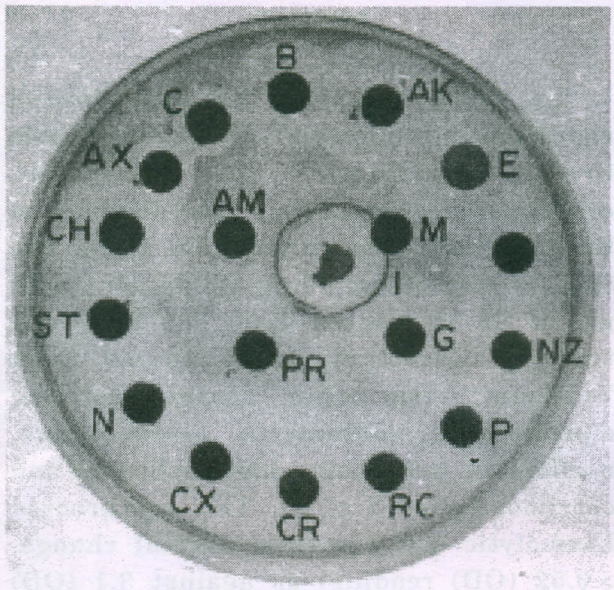

Fig 2: Showing the antimicrobial effect of SF-50 (100 µg) with other antibiotics e.g. AM-ampicilin, AK-amikacin, E-erythromycin, B-cotrimoxazole, P-penicillin, RC-ciprofloxacin, CR-cephaloridin, N-nalidixic acid, ST-streptomycin, CN-carbencillin, CX- cloxacillin, AX-amoxycillin, c-chloramphenicol, PR-cephalexin, G-gentamycin, M-mandelamin and ZN-afloxacin, NX-norfloxacin.

DISCUSSION

Sialic acid residues occur in the memberanes of almost all members of the animal kingdom phylogenetically above echynoderm (7) also on the human sperm cell memberane (8) and on the surface coat of *E. coli* (9).

In our previous experiment it was found that the "SF-50" is specific to sialic acid and both the agglutination and immobilization was found with normal human sperm cell which was dose dependant. The cidal effect of "SF-50 on human sperm cells was studied by Scanning electron microscope (SEM) which revealed the disruption of sperm plasma membrane (unpublished data).

The antimicrobial effect of "SF-50" on *E. coli* indicates that the binding of *E. coli* with "SF-50" is possibly due to formation of a sialoconjugate and thereby lysis of the bacterial cell membrane.

All the above evidences from *in-vitro* experiment suggest that the protein/s "SF-50" could be effective as an antimicrobial agent specially on *E. coli*.

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