Bull. Pharm. Sci., Assiut University, Vol. 21, Part 1, June 1998, pp. 37-40.

ANTIMICROBIAL ACTIVITY OF PYOVERDIN PIGMENT PRODUCED BY PSEUDOMONAS

A. Al-Shibib and A. Ebu-Talb

University of Philadelphia College of Pharmacy

أستهدفت هذه الدراسة معرفة التثبيط الميكروبى لصبغة البايوفردين من بكتيريا الزوائف الزرقاء. وقد تم جمع مائة عينة من مختلف المستشفيات المحلية وتم العزل من وحدات الحروق والتهاب الأذن المزمن وقيست القدرة التثبيطية بإستعمال الأنابيب الزراعية المخففة (Microdilution Plate Method) ووجد أن تركيز المادة المانعة هو ٨٥-١٠٠ مايكروجرام/سم٣ ومن ناحية أخرى درست قدرة وثبوتية هذه الصبغة اتجاه تراكيز الرقم الهيدروجينى ودرجة الحرارة والمعاملة الملحية خلال فترات زمنية مختلفة وتبين قدرة هذه الصبغة فى الثبات تجاه جميع هذه المتغيرات.

أستعملت طريقة Linear gradient technique وبإستعمال تركيز ٥٠٪ من الماء مع الميثانول وأمرار المستخلص على عامود الكروماتوجرافى لمدة ١٥ دقيقة. بلغت القمة الإمتصاصية للبايوفردين (٤٠٠) نانوميتر وهى أعلى قمة واضحة مقارنة بأنواع أخرى من صبغة البايوسيانين تنتجها البكتيريا.

The main purpose of this paper is to focus light on the possibilities of presence antimicrobail activity in pyoverdin pigment produced by pseudomonas, aeruginosa. The pyoverdin pigments were prepared from (1000) strains isolated from burns and chronic otitis media sources (Govermental and special Microbiological laboratories).

The inhibitory action were determined by both microdilution assay and minimum Inhibitory concentration. Values were between 85-105 ml.

The stability test of pyoverdin after extraction revealed that it is stable against different treatment of heat, pH and salt treatment.

INTRODUCTION

Siderophores are bacterial products which bind iron and increase the rate of bacterial iron transport. The results of siderophore function are best observed in low-iron media.

Several types of pigment were isolated by many studies of Al-Shibib.[1,2,4,5] Several forms of pyoverdin were isolated and characterized.[10] The function which is found by Cox *et al.*[12] revealed that pyoverdin promote bacterial growth in human plasma by stimulating their iron transport, also pyoverdin binds iron and function as a cellular iron carrier or siderophore. Some physical and chemical properties were investigated.[7] The purified pigments were tested for its antibacterial activity against enteric groups, and as in our previous paper of comparing pyocin and pyocyanin with antibiotics we compared pyoverdin with various types of antibiotics.

Materials and culture conditions

A total of one thousand strains subjected to this investigation all diagnostic method used previously by all papers Al-shibib[1,2,3,6] were applied. An 18 hr culture of 16 strains belonging to serotype O:11 were purified. The culture medium which used was:-

50% Kings media
50% Pseudomonas
1% (Casein hydrolyzed medium)

Purification of pyoverdin

Growth promotion assays were conducted in glucose minimal medium which contained the same ingredients as growth medium.[1,2] Siderophore production was measured in

Received in 7/4/1997 & Accepted in 21/2/1998

succinate minimal medium 20 M EDTA and after incubation for 20 h at 37°C, the culture was centrifuged and the sediment was washed three times by sterile, distilled water.

Viable bacteria were measured in dilutions of media as colony forming units appearing on tryptic soya agar after 24 h in water bath at 37°C.

Chromatography and purification technique

Bacteria were removed by centrifgation at 10000 rpm. The culture was filtered (seitz filter) using 0.45 M. The filtered culture medium was extracted with ethyl acetate 1:5 Vol/Vol, and rotary evaporator was used. The concentration was applied in polyacrylmide slab gel electrophoresis. Alternative chromatographic system equilibrates with 199% acetonitrile. A linear gradient to 50% water was conducted over 15 min, chromatography of crude culture concentrates making methonal extracts of dried pyoverdin was found soluble in methanol.

The chromatography to determine molecular weight of pyoverdin was performed by thin layer gel filtration.

Absorption spectra were obtained by using spectrophotometer and had calibrated as in our previous paper.[7]

Pyoverdin assay

Pyoverdin activity were assayed by addition of pyoverdin samples of each fraction to 20 ml of 2% tryptic soya agar, and the indicator strains, were spread over the agar surface by the use of abent glass rod afterwards the plates were incubated for 3 hr at 37°C and one drop (0.05 ml) of each serially diluted pyoverdin.

The pyoverdin activity was expressed as 20 time the reciprocal of the highest dilution which gave completely clear zone of inhibition, The method of MIC determination was used.

The turbidity of Initial inoculum of the tested strains were evaluated by measuring the corresponding absorbancy at 660 nm. After removal of pyocyanin by chloroform extraction, the absorbance of pyoverdin at 420 nm were determined and the peak of absorbance was determined.

RESULTS AND DISCUSSION

The activity of pyoverdin after extraction was estimated and compared with previous studies.[7,8] The recovery of pyoverdin was 90%. Data in Table (1) represented stability of each spectra against heating, pH, storage for four years. The pH-stability tests indicate a range from 6-9 for pyoverdin but average of 5-8 or for both pyocyanin and pyorubin.

The absorption spectra which was shown in Table (2) indicated that food origin isolates gave the range of 400 n m., while other sources were produced also pyocyanin, pyorubrin.

The activity of pyoverdin represented in Table (3) was 0.5 ug/ml similar of the activity of pyocyanine[10] but with wide range of activity against Neisseria, S aureus.

The comparative studies between pyoverdin and other antibiotics (Table 4) represented the similar activity with pyocin as in our paper of *in vitro* activity of pyocin activity agaist several indicator strains. The clinical effect on enteric group in comparison with other antibiotic revealed dramatic response and a good value of MIC Like pyomelanin.[5]

Table 1: Stability of pyoverdin

Treatment	No. of strains	% Activity remained
1- Heating at 80°C for 30, 1 hr, 1.30	1000	60%
2- Storage at 4°C for 4 years.	1000	60%
3- Ph 8-9	1000	70%
4- NaCl 10, 20, 30%	1000	30%

Table 2: Antibacterial activity of pyoverdin from different origin

Ps. aeruginosa	Origin	No. of strains	Properties of pyoverdin
I	Animal	182	256, 425, 329
II	Food	146	425, 465
III	Environment	150	256, 425, 465
IV	Human	522	256, 363, 425, 465

Table 3: Antibacterial spectra of pyoverdin antibacterial spectra

Pyoverdin	*P. mirabilis*	*E. coli*	*Neisseria*	*S. aureus*	*S. typhi*
I	+	+	+	+	+
II	+	+	-	-	+
III	+	+	-	+	+
IV	+	+	+	-	+

Table 4: Comparison between pyoverdin and other antibiotic

Group No.	Pyoverdin ug/ml	Tetracyclin ug/ml	Gentamicin ug/ml	Carbenicillin ug/ml	Ampicillin ug/ml
I	0.5	0.25	0.18	0.19	0.31
II	0.8	0.21	0.16	0.20	0.32
III	0.9	0.31	0.17	0.21	0.35
IV	1.2	0.27	0.13	0.22	0.32

REFERENCES

1- A. S. Al-Shibib, Pyocin and Pseudomonas aeruginosa Microbiological, Biochemical and genetical studies, Ph.D. thesis. University of Baghdad (1982).

2- A. S. Al-Shibib, S. Al-Mudaffar, F. Y. Al-ani and K. H. Hassan, Purification of pyocins produced by *Pseudomonas aeruginosa* 30, 25, 29 (1984).

3- N. S. Ayoub, Epidemiological markers for *Ps. aeruginosa* in general hospitals. M.SC thesis. College of Medicine university of Baghdad, Baghdad (1981).

4- A. S. Al-Shibib and S. A. Kandela, production of proticine by *Ps. aeruginosa* isolated from clinical Sources Arab, J. Phy. 9. (1991).

5- A. S. Al-Shibib and S. A. Kandela, A study of purified pyocyanine produced by *Ps. aeruginosa* properties and grouping, Acta Micrbiologica, Polonica 42, 314, 275, 352 (1993).

6- M. Ismail, Effect of gentamicin resistance on *P. aeruginosa* and purified pyocin, M.SC. thesis university of Baghdad (1983).

7- A. Al-Shibib and S. Kandela, Astudy of purified pyroverdin pigment produced by

Pseudomonas aeruginosa under publication (1996).

8- T. Shinomiya, and S. Shiga, S., Bactericidal activity of the tail of *Pseudomonas aeruginosa* bacteriophage PS17, J. Virology Vol 32. No. 3. 958-967 (1979).

9- B. W. Senior, B. W. and D. L. Leslie, Rare occurrence of *Proteus vulgaris*, J. Med. Microbiol. 16, 130-144(1986).

10- F. Y. Al-Ani, A. S. Al-Shibib, K. M. Khammas and R. Taher, Pyocyanin preparation from *Ps. aeruginosa* isolated from heterogenous clinical materials Folia, Microbiol. 31. 215-219 (1986).

11- S. A. Kandela and A. S. Al-Shibib, A.S., Spectroscopic study of pigments produced by Pseudomonas aeruginosa isolated from local clinical sources, Arab J. Phys 9,33 42,1 (1988).

12- C. Cox, C. Adams, P. Siderophore activity of pyoverdin *Ps. aeruginosa* infection and Immunity 48 (1) 130-138 (1985).