ANTIMICROBIAL ACTIVITY OF PYOVERDIN PIGMENT PRODUCED BY PSEUDOMONAS

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The main purpose of this paper is to focus light on the possibilities of presence antimicrobial activity in pyoverdin pigment produced by pseudomonas, aeruginosa. The pyoverdin pigments were prepared from (1000) strains isolated from burns and chronic otitis media sources (Governmental 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-Shbib.1,2,4,5 Several forms of pyoverdin were isolated and characterized.10 The function which is found by Cox et al.17 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-shhib1,2,3,6 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...
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 centrifugation 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 polyacrylimide 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 methanol 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 bent 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 pyorubrin.

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

The activity of pyoverdin represented in Table (3) was 0.5 µg/ml similar of the activity of pyocyanine10 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 against 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 2: Antibacterial activity of pyoverdin from different origin

<table>
<thead>
<tr>
<th>Ps. aeruginosa</th>
<th>Origin</th>
<th>No. of strains</th>
<th>Properties of pyoverdin</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Animal</td>
<td>182</td>
<td>256, 425, 329</td>
</tr>
<tr>
<td>II</td>
<td>Food</td>
<td>146</td>
<td>425, 465</td>
</tr>
<tr>
<td>III</td>
<td>Environment</td>
<td>150</td>
<td>256, 425, 465</td>
</tr>
<tr>
<td>IV</td>
<td>Human</td>
<td>522</td>
<td>256, 363, 425, 465</td>
</tr>
</tbody>
</table>

Table 3: Antibacterial spectra of pyoverdin antibacterial spectra

<table>
<thead>
<tr>
<th>Pyoverdin</th>
<th>P. mirabilis</th>
<th>E. coli</th>
<th>Neisseria</th>
<th>S. aureus</th>
<th>S. typhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>IV</td>
<td>+</td>
<td>+</td>
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</table>

Table 4: Comparison between pyoverdin and other antibiotic

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

REFERENCES


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