A NEW ANTIBIOTIC, PLATENOVIDIN

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A new antibiotic named platenocidin was isolated from the culture broth of Streptomyces H 273 N-SY2 and the producing microbe was classified as Streptomyces platensis. Platenocidin gives 5-hydroxymethyluracil by acid or alkaline hydrolysis and can be considered to belong to one of the nucleoside antibiotics. The antibiotic inhibits the growth of certain species of yeasts, but not that of bacteria.

The culture broth of Streptomyces H 273 N-SY2 was shown to produce a non-polyene type antiyeast antibiotic by the screening method for anticholesterol substances produced by microbes1). Streptomyces H 273 N-SY2 was isolated from a soil sample collected at Ichinomiya, Kumamoto Prefecture and classified as belonging to Streptomyces platensis2). The antibiotic was named as platenocidin after the producing microbe.

Fermentation, isolation, purification, and physicochemical and biological properties of platenocidin are described in this paper.

Fermentation

Streptomyces H 273 N-SY2 was cultured to prepare an inoculum seed in shaking flasks each containing 100 ml of an inoculation medium composed of 1.0 % soluble starch and 0.2 % yeast extract (pH 7.2) at 27°C for 48 hours on a reciprocal shaker (amplitude 7 cm, 130 strokes per minute). The inoculum was used to inoculate shaking flasks each containing 100 ml of a production medium composed of 1.5 % soluble starch, 1.0 % glucose, 2.0 % soyameal, 0.5 % Ebios (dried yeast distributed by Tanabe Pharmaceutical Co. Ltd.), 0.25 % NaCl and 0.3 % CaCO₃ (pH 7.6 before sterilization) and the culture was grown at 27°C for 96 hours on the shaker.

Isolation and Purification

The antibiotic can not be extracted with butanol nor can it be adsorbed on carbon from the broth filtrate. The antibiotic can be adsorbed on Amberlite IR 120 (H-form) and eluted with 1 N NH₃OH. The procedures of isolation and purification are respectively shown in Chart 1 and Chart 2.

Physicochemical Properties

Purified platenocidin was obtained as white

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Physicochemical Properties

Purified platenocidin was obtained as white
amorphous powder decomposing at 118–122°C. The elemental microanalysis gave C, 43.03; H, 4.73 and N, 15.62%, but neither halogen nor sulfur was observed. The antibiotic shows ultraviolet absorption maximum at 255 nm (E$_{1%}$/cm = 219) in H$_2$O, at 260 nm (E$_{1%}$/cm = 257) in 0.1 N HCl and at 257 nm (E$_{1%}$/cm = 230) in 0.1 N NaOH as shown in Fig. 1. The infrared absorption spectrum of platenocidin is shown in Fig. 2. The anti-

Chart 2. Purification of platenocidin

Crude powder of platenocidin (500 mg) dissolved in 0.1 M pyridine-acetate buffer (1 ml; pH 4.8)

DEAE-Sephadex A-25 column (18 × 1 cm, equilibrated with the same buffer)
eluted with the same buffer and collected in 10-ml fractions each

Fractions No. 5–8 (40 ml; total activity, 38.8%)
concentrated in vacuo bubbling with N$_2$ gas and again dissolved in 0.05 M pyridine-acetate buffer (1 ml; pH 4.8)

Sephadex G-10 column (90 × 1.5 cm, equilibrated with 0.05 M pyridine-acetate buffer of pH 4.8)
eluted with 0.05 M pyridine-acetate buffer (pH 4.8) and collected in 10-ml fractions each

Fractions No. 10–13 (40 ml; total activity, 33.9%)
concentrated in vacuo bubbling with N$_2$ gas and redissolved in 0.02 M pyridine-formate buffer (0.5 ml; pH 4.0)

SP-Sephadex C-25 column (15 × 1 cm, equilibrated with 0.02 M pyridine-formate buffer (pH 4.0))
eluted with 0.02 M pyridine-formate buffer (pH 4.0) and collected in 10-ml fractions each

Fractions No. 2–4 (30 ml; total activity, 30.1%)
concentrated in vacuo bubbling with N$_2$ gas and redissolved in 0.05 M pyridine-acetate buffer (0.5 ml; pH 4.8)

DEAE-Sephadex A-25 column (15 × 1 cm, equilibrated with 0.05 M pyridine-acetate buffer of pH 4.8)
eluted with 0.05 M pyridine-acetate buffer (pH 4.8) and collected in 7-ml fractions each

Fractions No. 13–16
concentrated in vacuo bubbling with N$_2$ gas and lyophilized

Purified platenocidin (23.1 mg; total activity, 24.0%)

Fig. 1. Ultraviolet absorption spectra of platenocidin.

Fig. 2. Infrared absorption spectrum of platenocidin in KBr
biotic is of an amphoteric nature. It is soluble in water, but insoluble in organic solvents such as butanol, acetone, ethyl acetate and ether. Plate
nocidin gives positive ninhydrin, KMnO$_4$, α-naphthol-phosphate and EHRLICH reactions, while the SAKAGUCHI reaction is negative.

The antibiotic was detected as a single spot on filter papers (Toyo Filter Paper Co. No. 52) developed with the three kinds of solvent systems by bioautogram, by ninhydrin reaction or under ultraviolet light. The Rf values are as follows; 0.26 (iso-BuOH - MeOH - H$_2$O, 2:2:1), 0.43 (75% aqueous phenol) and 0.34 (BuOH - AcOH - H$_2$O, 4:1:2).

Platenocidin was stable in a buffer solution of pH 4 - 6 when heated at 100°C for 5 minutes, but 20% of the activity at pH 2 or 75% of the activity at pH 8 was lost under the same condition.

Physicochemical properties of platenocidin are very similar to those of polyoxins A, B, C and I which are known as nucleoside antibiotics containing 5-hydroxymethyluracil.$^{3-6}$ Polyoxin A and

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**Table 1. Antimicrobial spectrum of platenocidin**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus FDA 209P</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Staphylococcus aureus Smith</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Micrococcus flavus FDA 16</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Sarcina lutea PCI 1001</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Bacillus subtilis PCI 219</td>
<td>&gt;100</td>
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<tr>
<td>Corynebacterium bovis 1810</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Escherichia coli K-12 ML 1629</td>
<td>&gt;100</td>
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<tr>
<td>Shigella sonnei 191-66</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>&gt;100</td>
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<tr>
<td>Klebsiella pneumoniae PCI 602</td>
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<td>Salmonella typhosa T-63</td>
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<tr>
<td>Mycobacterium smegmatis ATCC 607</td>
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<td>Candida tropicalis NI 7495</td>
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<tr>
<td>Candida albicans Yu 1200</td>
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<tr>
<td>Candida kru sei NI 7492</td>
<td>&gt;100</td>
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<tr>
<td>Saccharomyces cerevisiae</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa Ishii 14</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pseudomonas phaseolicola</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Paper chromatogram of polyoxin A, C, platenocidin and their acid hydrolyzate developed with BuOH - AcOH - H$_2$O (4:1:2).

**Fig. 4.** Paper chromatogram of polyoxins A and C, platenocidin and their alkaline hydrolyzate (A) was developed with BuOH - AcOH - H$_2$O (4:1:2) and (B) was developed with BuOH - MeOH - H$_2$O (3:1:1).
platenocidin were respectively hydrolyzed with 3 N HCl at 110°C for 1 hour followed by paper chromatography as shown in Fig. 3. Further, the results of alkaline hydrolysis of polyoxins A and C, and platenocidin with 0.5 N NaOH at 65°C for 4 hours are shown in Fig. 4. Thus, the existence of 5-hydroxymethyluracil moiety (probably the existence of polyoxin C moiety) is suggested in platenocidin.

Biological Properties

The antimicrobial spectrum of platenocidin is shown in Table 1. The antibiotic shows inhibitory activity only against a restricted number of yeasts, but not against bacteria. No toxic symptoms were observed when platenocidin was administrated intraperitoneally to mice at concentrations of 10 mg/mouse and 5 mg/mouse.

Discussion

Streptomyces platensis is known to produce three nucleosides, namely antibiotic U-44590 (5-aza-5,6-dihydropyrimidine nucleoside), 1-methylpseudouridine and pseudouridine7). Physicochemical and biological properties of platenocidin are differentiated from those of the above metabolites. Among known antibiotics, polyoxins A, B, C and I show quite similar ultraviolet absorption spectra with that of platenocidin. Nevertheless, polyoxins A and B have been reported to inhibit phytopathogenic fungi but not yeast, and polyoxins C and I are bio-inactive4-6). Thus, platenocidin can be differentiated from the polyoxins. Structural studies of platenocidin will be reported later.

Acknowledgement

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References