LUSTROMYCIN, A NEW ANTIBIOTIC PRODUCED BY STREPTOMYCES SP.

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A new antibiotic, lustromycin, was isolated from the cultured broth of Streptomyces sp. SK-1071. It exhibits selective antibacterial activity against anaerobic bacteria including Clostridium sp. The molecular formula C_{32}H_{38}O_{13} as determined by high resolution mass spectrometry, and elemental analysis and the NMR spectrum suggest structural resemblance of this antibiotic to luminamicin, an anti-anaerobic antibiotic reported previously.

In the course of screening for anti-anaerobic antibiotics of actinomycetes origin, we have found thiotetromycin\(^1\), clostomicin\(^2\) and luminamicin\(^3\). The continuing search led to the discovery of a new antibiotic, lustromycin, which showed antibacterial activity against anaerobic bacteria including Clostridium sp. It is produced by Streptomyces sp. SK-1071 isolated from a soil sample collected at Kiyose-shi, Tokyo. The structural and biological properties of lustromycin are similar to those of luminamicin.

The present paper deals with the producing organism, the production, isolation, physico-chemical and biological properties of lustromycin.

Taxonomy of the Producing Strain

Morphology

The vegetative mycelia of strain SK-1071 grow abundantly on both synthetic and complex agar media, and do not show fragmentation into coccosidal on bacillary elements. Abundant aerial mycelia are formed on yeast extract-malt extract agar and inorganic salts starch agar.

The spore chains are of the Spirales type and have more than twenty spores per chain (Plate 1). The spores are cylindrical in shape, 1.2×0.7 μm in size and have a hairy surface (Plate 1). Sporangia, sclerotial granules and zoospores were not observed.

Chemical Composition

LL-2,4-Diaminopimelic acid (A_{2}pm) was detected in the cell wall of the strain SK-1071 by the method of Lechevalier and Lechevalier\(^4\).

Cultured and Physiological Characteristics

The International Streptomyces Project (ISP) media recommended by Shirling and Gottlieb\(^5\) and media recommended by Waksman\(^6\) were used. Cultures were observed after incubation at 27°C for two weeks. Color names and hue numbers indicated in Table 1 are those of the Color Harmony Manual (4th Ed.) published by Container Corporation of America. The utilization of carbon sources was tested by growth on Pridham and Gottlieb's medium containing 1% of each carbon source at 27°C. The cultural and physiological characteristics, and the utilization of carbon sources of strain SK-1071 are shown in Tables 1, 2 and 3, respectively.

Strain SK-1071 exhibits the following properties. Spore chain, Spirales; spore, cylindrical and
Table 1. Cultural characteristics of strain SK-1071.

<table>
<thead>
<tr>
<th>Agar Type</th>
<th>G:</th>
<th>R:</th>
<th>AM:</th>
<th>SP:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract - malt extract agar*</td>
<td>Good, bamboo (2gc)</td>
<td>Mustard brown (2ni)</td>
<td>Abundant, velvety, covert gray (2fe)</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal agar*</td>
<td>Good, penetrant, light ivory (2ca)</td>
<td>Olive gray (11/2ig)</td>
<td>Moderate, velvety, light mustard tan (2ie)</td>
<td>None</td>
</tr>
<tr>
<td>Inorganic salts - starch agar*</td>
<td>Good, bamboo (2gc)</td>
<td>Beige brown (3ig)</td>
<td>Abundant, powdery, beige brown (3ig)</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol - asparagine agar*</td>
<td>Good, light ivory (2ca)</td>
<td>Camel (3ge)</td>
<td>Abundant, powdery, beige brown (3ig)</td>
<td>None</td>
</tr>
<tr>
<td>Glucose - asparagine agar</td>
<td>Good, light ivory (2ca)</td>
<td>Oatmeal (2ec)</td>
<td>Abundant, powdery, beige brown (3ig)</td>
<td>None</td>
</tr>
<tr>
<td>Peptone - yeast extract - iron agar*</td>
<td>Good, light ivory (2ca)</td>
<td>Light wheat (2ea)</td>
<td>Moderate, velvety, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Tyrosine agar*</td>
<td>Good, penetrant, light ivory (2ca)</td>
<td>Silver gray (3fe)</td>
<td>Moderate, velvety, silver gray (3fe)</td>
<td>None</td>
</tr>
<tr>
<td>Sucrose - nitrate agar*</td>
<td>Good, mustard gold (2pg)</td>
<td>Mustard gold (2pg)</td>
<td>Moderate, velvety, light ivory (2ca) or light gray (c)</td>
<td>None</td>
</tr>
<tr>
<td>Glucose - nitrate agar**</td>
<td>Good, camel (3ie)</td>
<td>Camel (3ie)</td>
<td>Poor, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol - calcium malate agar**</td>
<td>Good, penetrant, light ivory (2ca)</td>
<td>Light ivory (2ca)</td>
<td>Moderate, velvety, white (a)</td>
<td>None</td>
</tr>
<tr>
<td>Glucose - peptone agar**</td>
<td>Good, penetrant, pearl (3ba) or bamboo (2gc)</td>
<td>Pearl (3ba) or bamboo (2gc)</td>
<td>Poor, white (a) or covert gray (2fe)</td>
<td>None</td>
</tr>
<tr>
<td>Nutrient agar**</td>
<td>Good, penetrant, pearl (3ba)</td>
<td>Silver gray (3fe)</td>
<td>Moderate, powdery, white (a) or beige brown (3ig)</td>
<td>None</td>
</tr>
</tbody>
</table>

* Medium recommended by ISP.
** Medium recommended by S. A. Waksman.

Abbreviations: G; Growth of vegetative mycelium, R; reverse, AM; aerial mycelium, SP; soluble pigment.
Based on the taxonomic properties described above, strain SK-1071 is considered to belong to the genus Streptomyces and to be a strain of the white series or gray series of the PRIDHAM and TRESNER grouping. Strain SK-1071 has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name Streptomyces sp. SK-1071 with the accession No. FERM P-8107.

### Fermentation

Spores and vegetative mycelia of strain SK-1071 were inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a sterile seed medium. The flask was shaken on a rotary shaker for 60~75 hours at 27°C. The seed medium (pH 7.0) was composed of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, and CaCO₃ 0.4%. Two hundred milliliters of the seed culture was transferred to 20 liters of production medium (pH 7.0) consisting of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, CaCO₃ 0.4%, and 1 ml/liter trace metal solution (at 1 g/liter; FeSO₄·7H₂O, MnCl₂·4H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O and CoCl₂·2H₂O) in a 30-liter jar fermentor. The fermentation was carried out at 27°C with aeration of 10 liters/minute and agitation of 250 rpm. The amount of the antibiotic produced was determined by a paper disk-agar diffusion method using Clostridium perfringens as the test organism.

A typical time course for the fermentation is shown in Fig. 1. The antibiotic production started 40 hours after inoculation, then gradually increased and reached a maximum (56 µg/ml) at 90 hours.
Isolation

The culture broth (20 liters) was centrifuged to separate a supernatant fluid from mycelia cake. The supernatant fluid (13 liters), adjusted to pH 4.0 with 12 N HCl, was passed through a column of non-ionic porous resin, Diaton HP-20 (Mitsubishi Chemical Industries, Ltd., Tokyo, 600 ml). After washing the column with 1.5 liters of 30% aqueous acetone, the active principle was eluted with 1.5 liters of 70% aqueous acetone. The active fractions (1 liter) were collected and concentrated in vacuo to 150 ml. The aqueous solution was adjusted to pH 4.0 with 6 N HCl and extracted twice with 100 ml of ethyl acetate. The extracts were pooled and concentrated to dryness in vacuo to yield a brown paste (640 mg). The paste, dissolved in a small volume of benzene, was applied to a silica gel column (E. Merck, Kieselgel 60, 20 g) packed in benzene; then the active principle was eluted with a solvent of benzene - acetone (4 : 1). The active fractions were concentrated in vacuo to give a yellowish powder (50 mg). The powder was finally purified by HPLC apparatus (Jasco Tri Rotar V, column: YMC-Pack A-324 ODS, 10 × 300 mm, 65% aqueous CH₃CN, flow rate: 3.0 ml/minute, detection: UV 210 nm). Active fractions (retention time, 9.8 minutes) were combined and concentrated in vacuo to give a white powder. Colorless needles (25 mg) were obtained by crystallization from acetonitrile.

Physico-chemical Properties

The physico-chemical properties of lustromycin are summarized in Table 4. It is soluble in methanol, acetone and ethyl acetate, slightly soluble in chloroform, diethyl ether and benzene, and insoluble in water and n-hexane.

The molecular formula was determined as C₃₂H₃₈O₁₃ by elemental analysis (the compound contains...
no nitrogen atom, Table 4), high resolution mass spectrometry (found $m/z$ 630.2315, calcd 630.2312, Table 4) and $^{13}$C NMR spectrum (Fig. 2).

The UV and IR spectra of lustromycin are shown in Figs. 3 and 4, respectively.

### Biological Properties

Antimicrobial activities were assayed by a conventional agar dilution method using Mueller-Hinton agar for aerobic, and GAM agar for anaerobic bacteria in an anaerobic chamber. Lustromycin shows selective in vitro activity against the clinically important anaerobic bacteria, *Clostridium* sp. but no activity against aerobic bacteria, except *Micrococcus luteus*. Lustromycin was less active against some anaerobes than vancomycin which is used clinically in therapy of pseudomembranous colitis (Table 5).

Intraperitoneal injection to mice at 100 mg/kg had no toxic effects.

### Discussion

Based on the above physico-chemical properties, lustromycin was differentiated from all previously
The physico-chemical and biological properties of lustromycin are similar to those of luminamicin. The difference in molecular formula between lustromycin (C_{32}H_{38}O_{13}) and luminamicin (C_{32}H_{38}O_{12}) is one oxygen atom. Judging from their NMR spectra, lustromycin has two methoxy and one methyl groups in the structure (Fig. 2) while luminamicin has one methoxy and two methyl groups. Further studies on structure and biosynthesis are in progress.

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References


