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 coccoid 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, sclerotic granules and zoospores were not observed.

Chemical Composition

LL-2,4-Diaminopimelic acid (A_2pm) 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.

Yeast extract - malt extract agar*	G:	Good, bamboo (2gc)
	R:	Mustard brown (2ni)
	AM:	Abundant, velvety, covert gray (2fe)
	SP:	None
Oatmeal agar*	G:	Good, penetrant, light ivory (2ca)
	R:	Olive gray (1½ig)
	AM:	Moderate, velvety, light mustard tan (2ie)
	SP:	None
Inorganic salts - starch agar*	G:	Good, bamboo (2gc)
	R:	Beige brown (3ig)
	AM:	Abundant, powdery, beige brown (3ig)
	SP:	None
Glycerol - asparagine agar*	G:	Good, light ivory (2ca)
	R:	Camel (3ge)
	AM:	Abundant, powdery, beige brown (3ig)
	SP:	None
Glucose - asparagine agar	G:	Good, light ivory (2ca)
	R:	Oatmeal (2ec)
	AM:	Abundant, powdery, beige brown (3ig)
	SP:	None
Peptone - yeast extract - iron agar*	G:	Good, light ivory (2ca)
	R:	Light wheat (2ea)
	AM:	Moderate, velvety, white (a)
	SP:	None
Tyrosine agar*	G:	Good, penetrant, light ivory (2ca)
	R:	Silver gray (3fe)
	AM:	Moderate, velvety, silver gray (3fe)
	SP:	None
Sucrose - nitrate agar*	G:	Good, mustard gold (2pg)
	R:	Mustard gold (2pg)
	AM:	Moderate, velvety, light ivory (2ca) or light gray (c)
	SP:	None
Glucose - nitrate agar**	G:	Good, camel (3ie)
	R:	Camel (3ie)
	AM:	Poor, white (a)
	SP:	None
Glycerol - calcium malate agar**	G:	Good, penetrant, light ivory (2ca)
	R:	Light ivory (2ca)
	AM:	Moderate, velvety, white (a)
	SP:	None
Glucose - peptone agar**	G:	Good, penetrant, light ivory (2ca)
	R:	Pearl (3ba) or bamboo (2gc)
	AM:	Poor, white (a) or covert gray (2fe)
	SP:	None
Nutrient agar**	G:	Good, penetrant, pearl (3ba)
	R:	Silver gray (3fe)
	AM:	Moderate, powdery, white (a) or beige brown (3ig)
	SP:	None

* Medium recommended by ISP.

** Medium recommended by S. A. WAKSMAN.

Abbreviations: G; Growth of vegetative mycelium, R; reverse, AM; aerial mycelium, SP; soluble pigment.

Plate 1. Electron microphotograph of spore chains of strain SK-1071 grown on inorganic salts-starch agar for 14 days.
Bar represents 1.0 μm.

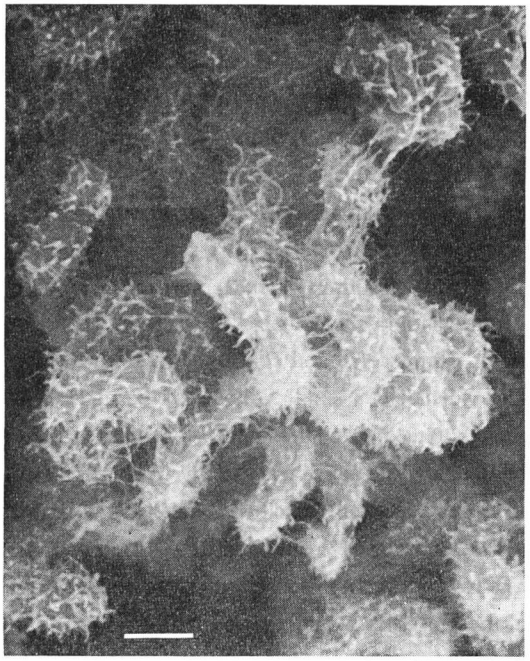


Table 2. Physiological properties of strain SK-1071.

Melanin formation	−
Tyrosinase reaction	−
H_2S production	−
Liquefaction of gelatin (21°C)	−
Peptonization of milk (37°C)	+
Coagulation of milk (37°C)	−
Cellulolytic activity	−
Hydrolysis of starch	+
Temperature range for growth	15~45°C

+: Active, −: not active.

Table 3. Utilization of carbon sources by strain SK-1071.

D-Glucose	+
D-Fructose	+
L-Rhamnose	+
D-Mannitol	+
L-Arabinose	+
i-Inositol	+
Raffinose	+
D-Xylose	+
Sucrose	+
Melibiose	+

+: Utilized.

hairy surface; color of vegetative mycelia, bamboo or light ivory; color of aerial mycelia, brownish gray; soluble pigment, none; A_2pm in cell wall, LL-type.

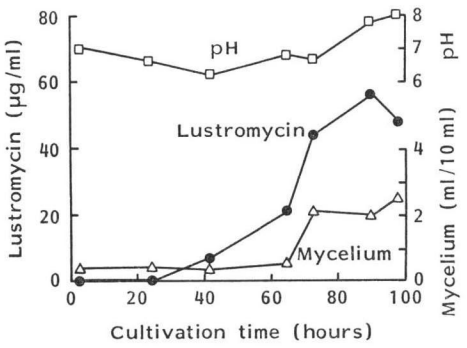
Based on the taxonomic properties described above, strain SK-1071 is considered to belong to the genus *Streptomyces* and to be a strain of the white series or gray series of the PRIDHAM and TRESNER grouping[7]. Strain SK-1071 has been deposited in the Fermentation Research Institute, Agency of Industrial 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 $CaCO_3$ 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%, $CaCO_3$ 0.4% and 1 ml/liter trace metal solution (at 1 g/liter; $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $CoCl_2 \cdot 2H_2O$) 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.

Fig. 1. Fermentation of lustromycin.



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, Diaion 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 CH_3CN, 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 $C_{32}H_{38}O_{13}$ by elemental analysis (the compound contains

Fig. 2. ^{13}C NMR spectrum of lustromycin (22.5 MHz, $CDCl_3$).

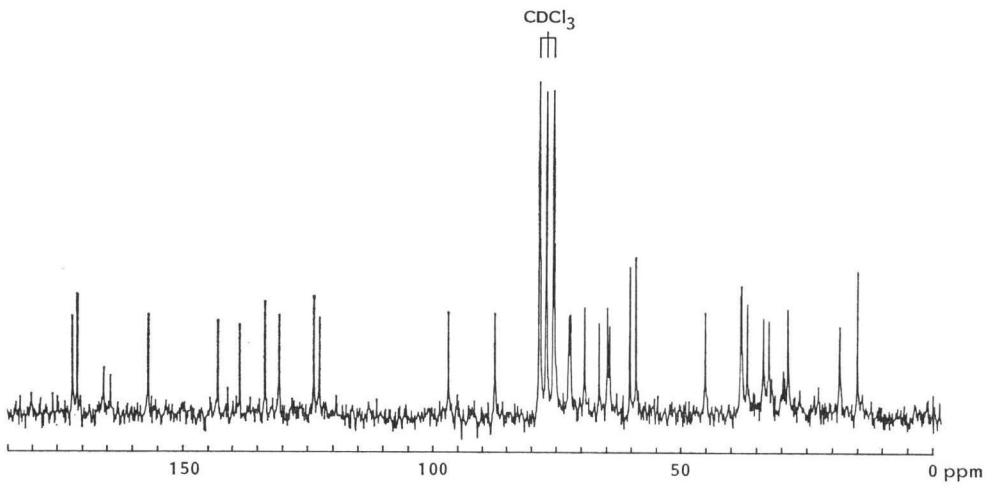
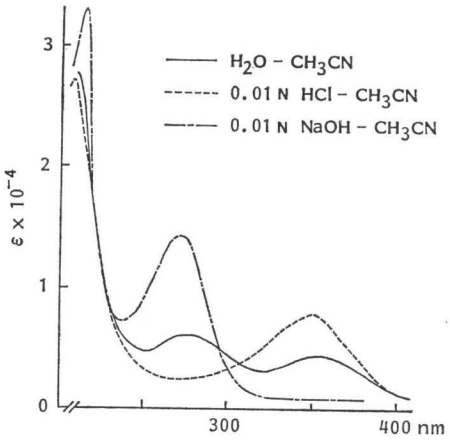


Table 4. Physico-chemical properties of lustromycin.

Nature	Acidic, colorless needles
MP	230~233°C
Formula	$C_{32}H_{38}O_{13}$
Anal	Found: C 60.50, H 6.07
EI-MS	Calcd for $C_{32}H_{38}O_{13}$: *m/z* 630.2312
	Found: *m/z* 630.2315
UV $\lambda_{max}^{CH_3CN}$ nm (ε)	277 (6,170), 350 (4,660)
IR ν_{max}^{KBr} cm^{-1}	3400, 1760, 1740, 1710, 1640, 1600

Fig. 3. UV spectrum of lustromycin.



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

Fig. 4. IR spectrum of lustromycin.

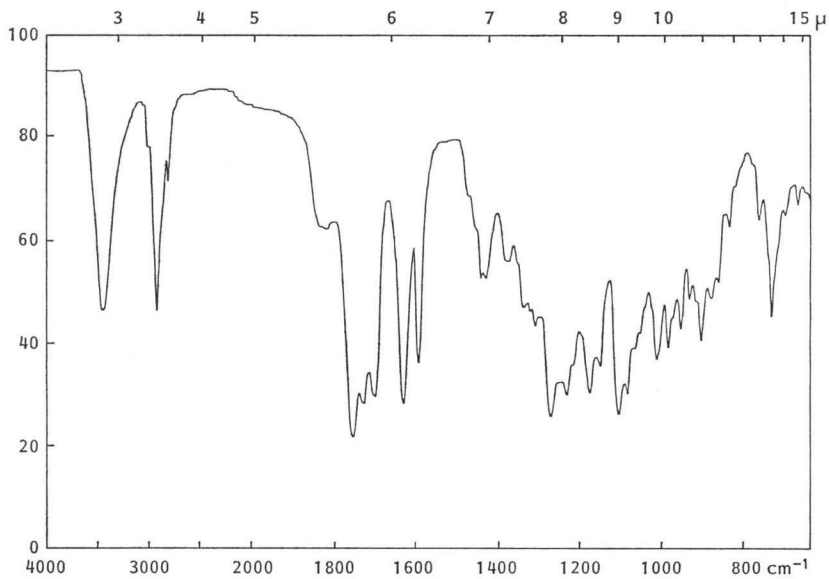


Table 5. Antimicrobial activities of lustromycin.

	MIC (μg/ml)	
	Lustromycin	Vancomycin
Staphylococcus aureus ATCC 6538P	>100	0.4
Bacillus subtilis ATCC 6633	>100	0.2
Micrococcus luteus ATCC 9341	25	0.4
Escherichia coli NIHJ	>100	50
Klebsiella pneumoniae ATCC 10031	>100	50
Salmonella typhimurium KB 20	>100	50
Proteus vulgaris IFO 3167	>100	50
Pseudomonas aeruginosa IFO 3080	>100	50
Clostridium perfringens ATCC 3624	6.25	1.56
C. kainantoi IFO 3353	25	1.56
C. difficile ATCC 9689	6.25	1.56
Bacteroides fragilis ATCC 23745	50	50
Fusobacterium varium ATCC 8501	100	100

reported antibiotics.

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.

Acknowledgment

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References

1) ŌMURA, S.; Y. IWAI, A. NAKAGAWA, R. IWATA, Y. TAKAHASHI, H. SHIMIZU & H. TANAKA: Thiotetromycin, a new antibiotic. Taxonomy, production, isolation, and physicochemical and biological properties. J. Antibiotics 36: 109~114, 1983
2) ŌMURA, S.; N. IMAMURA, R. ŌIWA, H. KUGA, R. IWATA, R. MASUMA & Y. IWAI: Clostomicins, new antibiotics produced by *Micromonospora echinospora* subsp. *armeniaca* subsp. nov. I. Production, isolation, and physico-chemical and biological properties. J. Antibiotics 39(10), 1986
3) ŌMURA, S.; R. IWATA, Y. IWAI, S. TAGA, Y. TANAKA & H. TOMODA: Luminamicin, a new antibiotic. Production, isolation and physico-chemical and biological properties. J. Antibiotics 38: 1322~1326, 1985
4) LECHEVALIER, M. P. & H. A. LECHEVALIER: The chemotaxonomy of actinomycetes. Proc. of Papers of Actinomycete Taxonomy Workshop., pp. 1~49, Soc. Ind. Microbiol., Texas, Aug. 13, 1978
5) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
6) WAKSMAN, S. A. (*Ed.*): The Actinomycetes. Vol. 2. Williams & Wilkins Co., Baltimore, 1961
7) PRIDHAM, T. G. & H. D. TRESNER: *Genus* I. *Streptomyces* Waksman and Henrici 1943, 339. *In* BERGEY's Manual of Determinative Bacteriology. 8th Ed., *Eds.*, R. E. BUCHANAN & N. E. GIBBONS, pp. 748~829, Williams & Wilkins Co., Baltimore, 1974