LYDICAMYCIN, A NEW ANTIBIOTIC OF A NOVEL SKELETAL TYPE

I. TAXONOMY, FERMENTATION, ISOLATION AND BIOLOGICAL ACTIVITY

YOICHI HAYAKAWA, NAOYUKI KANAMARU, AKIRA SHIMAZU and HARUO SETO

Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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A novel antibiotic, designated lydicamycin, was isolated from the fermentation broth of an actinomycete strain identified as *Streptomyces lydicus*. Lydicamycin was active against Gram-positive bacteria and a certain yeast, but inactive against Gram-negative bacteria.

In the course of our screening for new antibiotics, an actinomycete was isolated from a soil sample and was found to produce a novel antibiotic designated lydicamycin (Fig. 1). This paper describes the taxonomy of the producing organism, and the fermentation, isolation and biological activity of lydicamycin. The physico-chemical properties and structure elucidation are described in the accompanying paper[1].

Materials and Methods

Microorganism

A culture designated 2249-S3 was isolated from a soil sample collected at a beech-tree forest in Ohata-machi, Shimokita-gun, Aomori Prefecture, Japan. The culture was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, under the name *Streptomyces* sp. SANK 60390 with the accession number FERM P-11523.

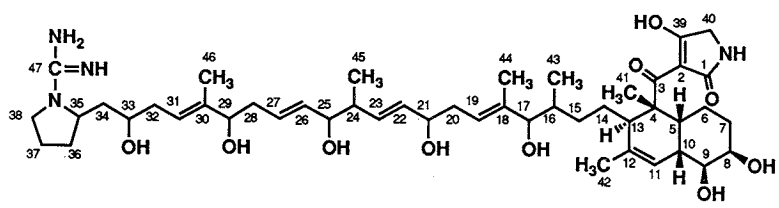
Taxonomic Studies

The characterization and identification of the culture were carried out mainly according to BERGEY's Manual[2], the International Streptomyces Project (ISP) report[3] and the methods described by WILLIAMS *et al.*[4,5]. For the evaluation of cultural characteristics, the strain was incubated for 14~28 days at 27°C. Cell wall composition was analyzed by the methods of BECKER *et al.*[6].

Antimicrobial Activity

The MICs for lydicamycin were determined by a 2-fold agar dilution method in nutrient agar against bacteria and in Sabouraud agar against yeasts or fungi.

Fig. 1. Structure of lydicamycin (relative stereochemistry).



Results

Taxonomy

The substrate mycelium of culture 2249-S3 neither fragmented nor formed spores. The aerial mycelium branched and formed spiral spore chains on its tip or the tip of a short branch. The spore chain contained 10 to 50 or more spores per chain with short or long coils in a small diameter ($2 \sim 3\ \mu m$) as shown in Fig. 2. The spore was non-motile and ellipsoidal, lenticular to allantoid ($0.4 \sim 0.6 \times 0.8 \sim 1.1\ \mu m$), and the spore surface was smooth or slightly irregular (Fig. 3). As a special morphology, the aerial mycelium often tangled and formed knots and nest-like structures, and the spore chain coalesced and formed amorphous hygroscopic masses of spores after maturity.

Whole cell analysis showed that the strain contained L,L-diaminopimelic acid, indicating cell-wall type I.

The cultural characteristics are summarized in Table 1. The color of the mature spore mass was in the gray color-series with sprinkling dark brown hygroscopic spots or patches. The colony reverse contained no distinctive pigments, and its color was slightly sensitive to pH, turning yellowish in acidic pH and reddish in basic pH.

The cultural, physiological and biochemical properties of 2249-S3 are summarized in Table 2. The culture did not produce melanoid pigments in the three media designated by the ISP, although producing a pinkish pigment in tyrosine agar. A pale yellowish brown soluble pigment was produced in several media and revealed similar pH sensitivity to the reverse color. The culture utilized D-glucose, D-xylose, D-fructose, sucrose, *meso*-inositol, mannitol and raffinose as carbon sources, but not L-arabinose or L-rhamnose.

From the morphological characteristics and cell wall type, this strain could be classified as the genus *Streptomyces*. On the basis of the above characteristics, *Streptomyces lydicus* and *Streptomyces violaceusniger* were selected as the similar strains to 2249-S3 among the streptomycete species described in BERGEY's Manual. The percentage positive probability matrix for the two related strains[4] presented in Table 2 was reduced to +, − and d (doubtful) forms, with entries ≥ 90 being set to +, ≤ 10 to − and the remainder to d. After deletion of common characters to the both species, a diagnostic table was prepared and compared with the properties of 2249-S3 (Table 3). The characters of the strain were in a good agreement with those of *S. lydicus*, but different in many cases from those of *S. violaceusniger*, especially with regard

Fig. 2. Spiral spore chains of strain 2249-S3 grown on inorganic salts-starch agar for 14 days.

Bar represents 5 μm.

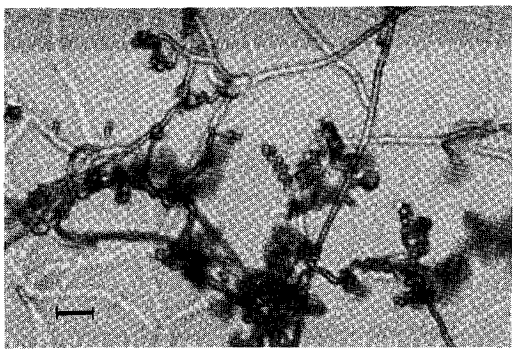


Fig. 3. Electron micrograph of spore chains with a smooth surface of strain 2249-S3 grown on inorganic salts-starch agar for 14 days.

Bar represents 1 μm.

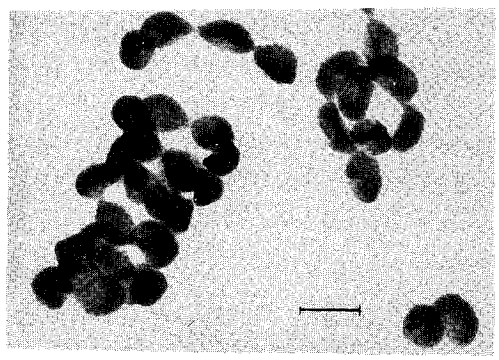


Table 1. Cultural properties of strain 2249-S3.

Sucrose-nitrate agar	AC:	White with grayish tinge
	RC:	Pale yellow to light yellow
	SP:	None or trace of yellowish
Glucose-asparagine agar	AC:	White with grayish tinge, sprinkled with dark brownish gray hygroscopic patches
	RC:	Pale yellow to light yellow; slightly pH sensitive, showing reddish tinge with 0.05 N NaOH or yellowish tinge with 0.05 N HCl
	SP:	Trace of yellowish
Glycerol-asparagine agar	AC:	White, sprinkled with brownish grayish tinge areas
	RC:	Pale yellow to light yellow
	SP:	Pale yellowish to brown
Inorganic salts-starch agar	AC:	Gray[a] (3fe-5fe), sprinkled with dark brownish gray hygroscopic patches
	RC:	Light yellow to pale yellowish brown or brownish gray
	SP:	Pale yellowish brown
Tyrosine agar	AC:	Whitish
	RC:	Light brownish gray, pH nonsensitive
	SP:	Pale pink at first, becoming light brownish gray
Nutrient agar	AC:	Whitish
	RC:	Pale yellow
	SP:	None
Yeast extract-malt extract agar	AC:	Gray[a] (3fe-5fe or 3ig), forming dark brownish gray to black hygroscopic patches at ages
	RC:	Light yellow to pale yellowish brown or dull yellow orange; slightly pH sensitive, showing reddish tinge with 0.05 N NaOH or yellowish tinge with 0.05 N HCl
	SP:	Pale yellowish brown, slightly pH sensitive as RC
Oatmeal agar	AC:	Gray[a] (2fe-5fe or 3ig), sprinkled with dark brownish gray hygroscopic small spots
	RC:	Pale olive or brownish gray
	SP:	Pale yellowish brown

AC: Aerial mass color, RC: reverse color of colony, SP: soluble pigments.
[a] Gray color-series.

to the character states which were most representative of and consistent within each species. These results clearly indicated that the strain was identified as *S. lydicus*, and therefore, strain 2249-S3 was designated as *Streptomyces lydicus* DE BOER, DIETZ, SILVER and SAVAGE.

Fermentation

The seed medium consisted of soluble starch 1.0%, molasses 1.0%, Polypepton 1.0% and meat extract 1.0%. The pH was adjusted to 7.2 before sterilization. Seed tubes containing 15 ml of the medium were inoculated with a stock culture of *S. lydicus* 2249-S3 maintained on a yeast extract-malt extract agar slant and were incubated by shaking at 27°C for 2 days. The seed culture at 2% was transferred to 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 2%, molasses 1%, casein 0.5%, Polypepton 0.1% and calcium carbonate 0.4% (pH 7.2). The fermentation was carried out at 27°C for 4 days on a rotary shaker at 200 rpm.

Isolation

The broth filtrate (2 liters) was applied to a Diaion HP-20 column (3.3 × 22 cm), which was washed with 1 liter of 50% methanol and then eluted with 50% acetone. After evaporation, the active eluate was

Table 2. Properties of strain 2249-S3 and percentage positive probability matrix for the related species.

	2249-S3	*Streptomyces lydicus*[a]	*Streptomyces violaceusniger*[a]
Presence of spores (AM)[b]	+	100	100
Spore chain *Spirales*[b]	+	100	100
Spore ornamentation[b]			
Smooth	+	64	0
Spiny	−	27	0
Hairy	−	9	0
Rugose	−	0	100*
Production of aerial spore mass[b]	+	100	100
Color of aerial spore mass[b]			
Red	−	9	0
Gray	+	91	100
Pigmentation of SM[c]	−	0	0
Production of diffusible pigments[c]	−	0	0
Melanin on PYI agar	−	0	0
Melanin on tyrosine agar	−	0	0
Fragmentation of mycelium[b]	−	0	0
Sclerotia formation[b]	−	0	0
Sporulation on SM[b]	−	0	0
Antimicrobial activity against			
Bacillus subtilis IAM 12118	+	73	67
Candida albicans IFO 1385	+	27	17
Streptomyces murinus ISP 5091	+	100	50
Lechitinase activity	−	64	0
Proteolysis on egg-yolk medium	+	73	83
Lipolysis on egg-yolk medium	−	18	100
Pectin hydrolysis	−	0	50
Chitin hydrolysis	−	9	83
Nitrate reduction	−	9	83
H_2S production	−	0*	100
Degradation of			
Hypoxanthine	+	100	83
Elastin	−	36	83
L-Tyrosine	+	100	100
Adenine	+	100	100
Xanthine	+	82	0*
Starch	+	100	100
Casein	+	91	100
Allantoin	+	18	50
Arbutin	+	100	100
Resistance to			
Neomycin (50 μg/ml)	−	18	0
Rifampicin (50 μg/ml)	−	9*	83
Oleandomycin (100 μg/ml)	−	9	50
Benzylpenicillin (10 i.u.)	+	91	17
Growth at			
45°C	−	0	50
pH 4.3	+	27	0
Growth with (% w/v)			
NaCl (7.0)	+	55	0
Sodium azide (0.01)	−	18	50
Phenol (0.1)	−	9	0
Potassium tellurite (0.001)	+	55	17
Growth on sole nitrogen source			
DL-α-Amino-*n*-butyric acid	−	9	100*

Table 2. (Continued)

	2249-S3	*Streptomyces lydicus*[a]	*Streptomyces violaceusniger*[a]
L-Valine	−	27	33
L-Phenylalanine	+	100	67
L-Histidine	+	36	100
L-Hydroxyproline	+	55	81
Growth on sole carbon source			
L-Arabinose	−	55	100
Sucrose	+	73	33
D-Xylose	+	27	67
meso-Inositol	+	91	67
Mannitol	+	91	100
D-Fructose	+	91	83
L-Rhamnose	−	18	83
Raffinose	+	82	83
Inulin	−	18	33
Adonitol	−	82	67
Salicin	−	64	83
D-Melibiose	+	82	83
D-Galactose	+	82	83
Cellobiose	−	73	100

AM: Aerial mycelium, SM: substrate mycelium, PYI: peptone-yeast-iron.
[a] Data from WILLIAMS *et al.*[4].
[b] In ISP medium No. 4 on day 14.
[c] On ISP medium No. 5 on day 14.
* Character states are most representative of and consistent within each character.

Table 3. Comparison of differential properties of *Streptomyces lydicus* and *S. violaceusniger* with those of strain 2249-S3.

	2249-S3	*S. lydicus*[a]	*S. violaceusniger*[a]		2249-S3	*S. lydicus*[a]	*S. violaceusniger*[a]
Spore smooth	+	d	−	Benzylpenicillin resistance	+	+	d
Spore rugose	−	−	+*	Growth at 45°C	−	−	d
Antibiosis against *S. murinus*	+	+	d	Growth at pH 4.3	+	d	−
Lecithinase activity	−	d	−	Aminobutylic acid utilization	−	−	+*
Lipolysis	−	d	+	L-Phenylalanine utilization	+	+	d
Pectin hydrolysis	−	−	d	L-Histidine utilization	+	d	+
Chitin hydrolysis	−	−	d	L-Arabinose utilization	−	d	+
Nitrate reduction	−	−	d	*meso*-Inositol utilization	+	+	d
H_2S production	−	−*	+	D-Fructose utilization	+	+	d
Hypoxanthine degradation	+	+	d	Cellobiose utilization	−	d	+
Xanthine degradation	+	d	−*				
Neomycin resistance	−	d	−				
Rifampicin resistance	−	−*	d				
Oleandomycin resistance	−	−	d				

+: 90% or more of strains are positive, −: 10% or less of strains are positive, d: 11~89% of strains are positive.
[a] Data from WILLIAMS *et al.*[4].
* Character states are most representative of and consistent within each species.

twice extracted with 100 ml of 1-butanol. The organic layer was concentrated and chromatographed on a silica gel column (1.5×35 cm) with chloroform-methanol-29% ammonia water (5:3:1). The active fraction was evaporated and subjected to Sephadex LH-20 column chromatography (2×45 cm). The active eluate with MeOH was concentrated to dryness to yield a colorless powder (28 mg) of lydicamycin.

Table 4. Antimicrobial activity of lydicamycin (MIC, μg/ml).

Bacillus subtilis ATCC 6633	<1.5
Staphylococcus aureus FDA 209P	3.1
S. aureus 56R	6.2
S. aureus 535 (MRSA)	6.2
Enterococcus faecalis 681	100
Escherichia coli NIHJ	>200
E. coli 609	>200
Klebsiella pneumoniae 806	>200
Proteus vulgalis 1420	>200
Pseudomonas aeruginosa 1001	>200
Cryptococcus neoformans 58063	25
Aspergillus fumigatus 10569	>50
Candida albicans SC	>50
Tricophyton mentagrophytes SC	>50

Biological Activity

The antimicrobial activity of lydicamycin is summarized in Table 4. Lydicamycin inhibited the growth of Gram-positive bacteria including methicilin-resistant *Staphylococcus aureus* (MRSA) and *Cryptococcus neoformans*. The MICs for Gram-positive bacteria were <1.5 to 100 μg/ml.

Acknowledgments

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