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Lomofungin, a New Antibiotic Produced by *Streptomyces lomondensis* sp. n.

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Lomofungin is a new antimicrobial agent obtained from the culture broth of *Streptomyces lomondensis* sp. n. UC-5022. Lomofungin is an acidic, olive-yellow, crystalline compound which inhibits, in vitro, a variety of pathogenic fungi, yeasts, and gram-positive and gram-negative bacteria.

Streptomyces lomondensis sp. n. produces a new antibiotic designated lomofungin [referred to in U.S. Patent 3,359,165 as lomondomycin (U-24,792)]. Bergy (*unpublished data*) characterized lomofungin as an acidic, olive-yellow, crystalline compound with the molecular formula $C_{15}H_{10}N_2O_6$ and a molecular weight of 314. The compound is soluble in dimethyl formamide, alkaline water, acidic acetone, and acidic methyl ethyl ketone. It is only slightly soluble in water, methanol, cyclohexane, acetone, ether, and ethyl acetate. Lomofungin does not melt below 320 C.

This paper describes the organism, the fermentation conditions, the paper chromatogram characterization, and some of the biological properties of the antibiotic.

MATERIALS AND METHODS

Culture. *S. lomondensis* was characterized by the methods cited by Dietz (2).

Medium. Seed flasks were inoculated with spore preparations of the culture which were maintained in soil. The culture was incubated at 28 C for 72 hr in a seed medium consisting of, per liter, 25 g of glucose monohydrate (Cerelose) and 25 g of Pharmamedia (Trader Oil Mill Co., Fort Worth, Tex.). The vegetative seed was used at a rate of 5% to inoculate a fermentation medium consisting of, per liter, 20 g of glucose monohydrate, 20 g of cottonseed meal (Trader Oil Mill Co.), 20 g of dextrin, 1 g of $(NH_4)_2SO_4$, 4 g of calcium carbonate, and tap water to 1 liter; the *p*H was adjusted to 7.2 before sterilization. Shaken-flask fermentations were run in 500-ml Erlenmeyer flasks containing 100 ml of medium, and for optimal yields were incubated at 28 C on a Gump rotary shaker operating at 250 rev/min with a 2.5-inch (6.4 cm) stroke.

Antibiotic assay procedure. The antibiotic concentrations were determined with a standard disc-plate agar diffusion assay. Samples (0.08 ml) and dilutions made in distilled water were placed on 12.7-mm paper discs and were assayed against *Penicillium oxalicum* (UC-1268). Spores of *P. oxalicum* were inoculated into an agar medium consisting of, per liter, 20 g of malt extract (Difco), 1 g of peptone (Difco), 20 g of dextrose, and 17.5 g of agar. The zones of inhibition of growth were measured after incubation for 18 hr at 28 C. The antibiotic activity was expressed in biounits, one biounit being the amount of antibiotic necessary to give a 20-mm zone of inhibition after 18 hr of incubation under these standard conditions.

The antibacterial in vitro spectrum was determined by twofold dilution end points in Brain Heart Infusion (Difco) broth. Readings were made after 24 hr of incubation at 37 C. The antifungal in vitro spectrum was determined by the agar dilution plate assay of Whiffen (11), with readings made after 72 hr at 28 C.

Lomofungin was differentiated from other antibiotics by its antibacterial and antifungal spectra, by its chemical properties, and by paper chromatography. The antibiotic was spotted on Whatman no. 1 filter paper and was developed without prior equilibration by use of the descending method and the solvent systems listed in Fig. 3. Antibiotic activity was located by plating the developed strips on trays of agar seeded with *P. oxalicum* or *Saccharomyces pastorianus* subspecies *arbingnensis* (ATCC 2366).

RESULTS AND DISCUSSION

Taxonomy. *Streptomyces lomondensis* Dietz, sp. n.

Color characteristics. Blue aerial mycelium. Melanin-positive. Appearance on Ektacolor is given in Fig. 1. Reference color characteristics on three agar media are given in Table 1. The culture may be placed in the Red (R) and Blue (B) color series of Tresner and Backus (10).

Microscopic characteristics. Warty to spiny spores (Fig. 2) borne on straight to open spiral to spiral sporophores (RF, RA, S) in the sense of Pridham et al. (8). Spores are poorly differentiated by carbon repligraphy.

Cultural and biochemical characteristics. See Table 2.

Carbon utilization. Growth of the culture on

carbon compounds in a synthetic medium was determined according to the procedure of Pridham and Gottlieb (7). Growth was good on D-xylose, L-arabinose, rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, sucrose, lactose, cellobiose, raffinose, dextrin, inulin, soluble starch, glycerol, D-mannitol, inositol, sodium acetate, and sodium succinate; slight on

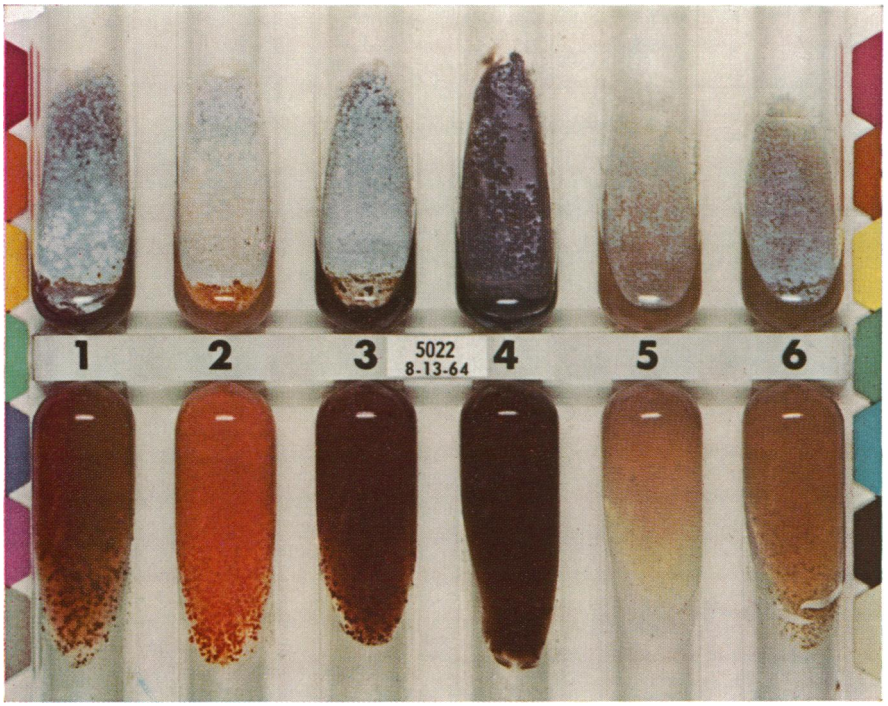

FIG. 1. *Ektacolor photograph of S. lomondensis.*

TABLE 1. *Reference color characteristics of S. lomondensis*

Medium	*Color Harmony Manual*, 3rd ed., 1948 (5)	ISCC-NBS method of designating colors and a dictionary of color names, Circular 553, 1955 (6)
Bennett's agar		
Surface	3ca(g) pearl pink, shell to 15cb(g) cloud blue	73gm pale yellow orange 184m very pale blue; 189gm bluish white
Reverse	5ng(g) brick red, henna to 5pg(g) henna, light copper, brown, russet, rust brown	43n moderate reddish brown; 55g strong brown; 55 gm strong brown
Pigment	5pg(g) henna, light copper, brown, russett, rust brown	55gm strong brown
Czapek's sucrose agar		
Surface	15ba(g) blue tint	184m very pale blue; 189gm bluish white.
Reverse	2fb(g) bamboo, buff, straw, wheat	87g moderate yellow; 89m pale yellow
Pigment	trace 4ca(g) flesh pink, shell pink, tearose	28g light yellowish pink; 31gm pale yellowish pink
Maltose tryptone agar		
Surface	15ba(m) blue tint	184m very pale blue; 189gm bluish white
Reverse	3ni(g) clove brown	77m moderate yellowish brown; 95g moderate olive-brown
Pigment	pale 3ni(g) clove brown	77m moderate yellowish-brown; 95g moderate olive-brown

dulcitol and D-sorbitol. There was no growth on the control, salicin, phenol, cresol, sodium formate, sodium oxalate, sodium tartrate, or sodium salicylate.

Temperature. The culture grows at temperatures of 18 to 37 C. It does not grow at 55 C. The optimal temperature is 37 C.

Antibiotic-producing properties. The culture

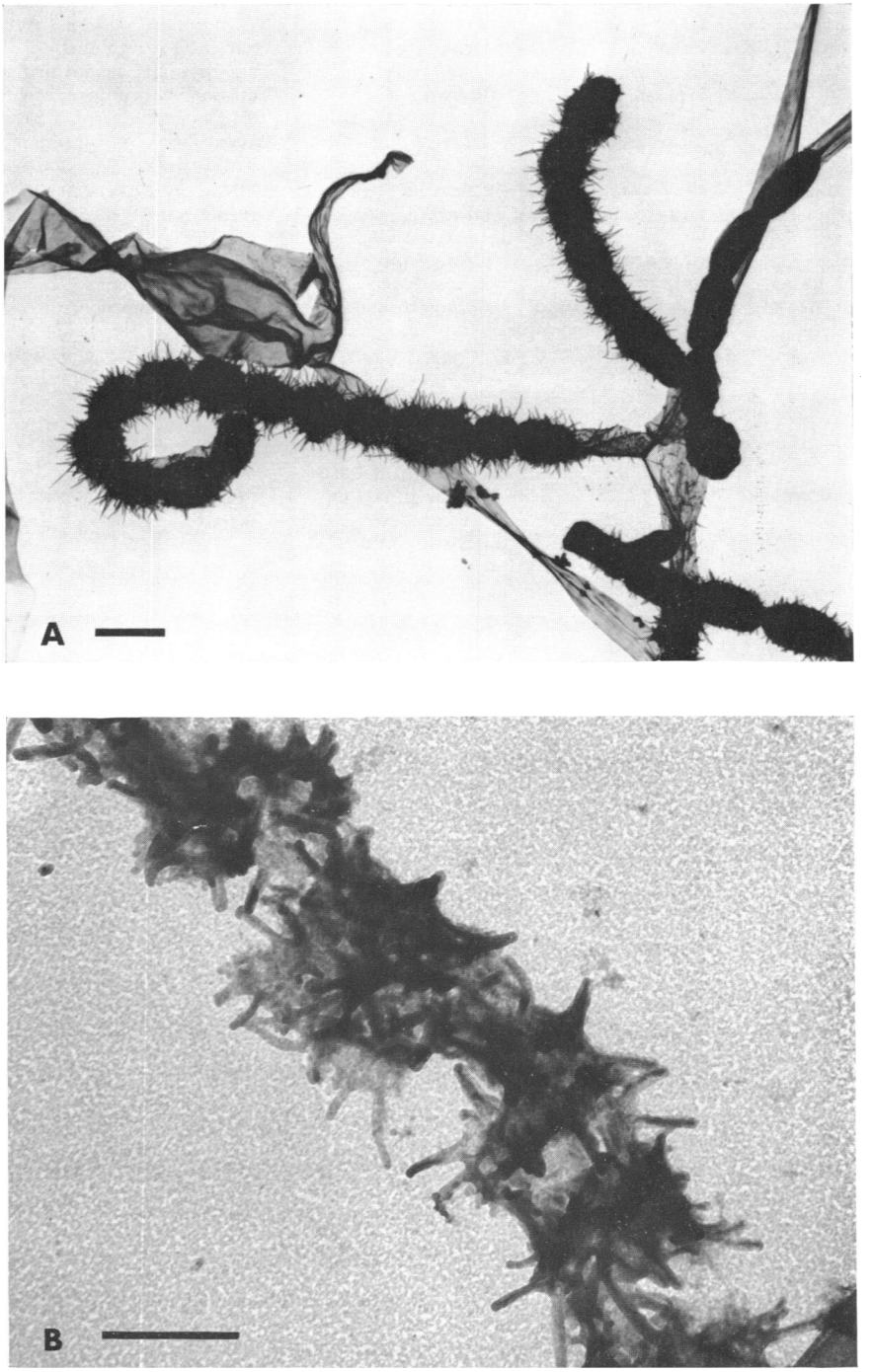

FIG. 2. *Electron micrographs of spores of S. lomondensis. Each index mark equals 1 μm. (A) S. lomondensis, whole spore mount; (B) S. lomondensis, carbon repligraph.*

TABLE 2. *Cultural and physiological characteristics of S. lomondensis*

Medium	Surface	Reverse	Other
Agar			
Peptone-iron	Trace gray	Brown	Brown pigment; melanin positive
Calcium malate	Gray-green	Yellow-green	No pigment; malate solubilized
Glucose asparagine	Peach	Orange	Orange-tan pigment
Skim milk	Gray-white	Tan-brown	Tan pigment; casein not solubilized
Tyrosine	Gray-blue	Brown	Tan-brown pigment; tyrosine solubilized.
Xanthine	Gray-green	Green	Pale yellow-tan pigment; xanthine solubilized.
Nutrient starch	Blue-gray	Pale olive-yellow	Yellow pigment; starch hydrolyzed.
Yeast extract-malt extract	Gray-orange	Orange	Orange-tan pigment
Bennett's	Blue-gray-peach	Orange-tan	Orange-tan pigment
Czapek's sucrose	Pale blue-gray	Peach-green	Pale peach pigment
Maltose-tryptone	Peach-blue	Orange-tan	Orange-tan pigment
Gelatin			
Plain			Brown pigment; 50% liquefied
Nutrient			Brown pigment; 50% liquefied
Broth			
Nutrient nitrate	Blue-gray on surface ring	Trace colorless vegetative growth at base	Yellow-tan pigment; nitrate reduced to nitrite
Synthetic nitrate		Trace pink-tan vegetative throughout	Orange pigment; nitrate reduced to nitrite
Litmus milk	White on brown surface ring		No peptonization; no coagulation; *p*H 6.7.

TABLE 3. *Fermentation titer and pH pattern of lomofungin production by S. lomondensis*

Age (hr)	*P. oxalicum* (biounits/ml)	*p*H
0	0	7.5
48	3.5	6.7
72	4.9	6.9
96	6.0	7.0
120	4.8	7.0

produces the antibiotic lomofungin (lomondomycin, U-24,792).

Source. Soil.

Type culture. UC-5022, NRRL 3252.

S. lomondensis may be placed in the Blue series of the RF, RA, S sections of Pridham et al. (8). However, none of the cultures in these sections shows the variability of this culture. Furthermore, the system of Pridham et al. does not indicate the vegetative and pigment color of the organisms.

The new organism could be placed in the *Caeruleus* series of Baldacci (1), the *Coerulescens* series of Gauze (3), the *prasinus* or *azureus-glaucus* grouping of Hütter (4), or the *viridochromogenes* (blue spore) series discussed by Trejo and Bennett (9).

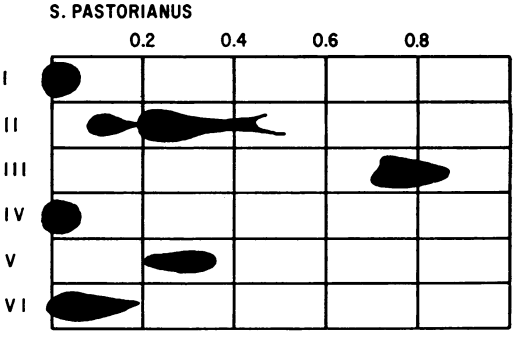

FIG. 3. *Paper chromatographic patterns of lomofungin. Solvent systems: (I) n-butyl alcohol–water (84:16), developed for 16 hr; (II) n-butyl alcohol–water (84: 16) plus 0.25% p-toluenesulfonic acid, developed for 16 hr; (III) n-butyl alcohol–acetic acid–water (2:1:1), developed for 16 hr; (IV) n-butyl–water (84:16) plus 2% piperidine, developed for 16 hr; (V) n-butyl–water (4:96), developed for 5 hr; (VI) n-butyl alcohol–water (4:96) plus 0.25% p-toluenesulfonic acid, developed for 5 hr.*

The placement of *S. lomondensis* in any of these categories and the subsequent equation of this organism with validly established species which differ from this culture in pigment production, spore type, and antibiotic production would in-

TABLE 4. *Antibacterial spectrum of lomofungin in Brain Heart Infusion broth*

Organism	Minimal inhibitory concn (μg/ml)
Staphylococcus aureus UC-76	62
S. aureus UC-552	62
Streptococcus hemolyticus UC-152	62
S. faecalis UC-3235	62
Escherichia coli UC-51	62
Proteus vulgaris UC-93	125
Klebsiella pneumoniae UC-57	62
Salmonella schottmuelleri UC-126	31
Pseudomonas aeruginosa UC-95	62
Bacillus subtilis UC-564	31
Diplococcus pneumoniae UC-41	16

TABLE 5. *Antifungal spectrum of lomofungin in Whiffen's agar medium*

Organism	Minimal inhibitory concn[a] (μg/ml)
Nocardia asteroides UC-2052	100
Blastomyces dermatitidis UC-1911	100
Coccidioides immitis UC-1119	100
Geotrichum sp. UC-1207	100[b]
Hormodendrum compactum UC-1222	100
Phialophora verrucosa UC-1807	100
Cryptococcus neoformans UC-1139	100
Histoplasma capsulatum UC-1220	100
Sporotrichum schenckii UC-1364	100
Monosporium apiospermum UC-1248	100
Trichophyton rubrum UC-1458	100
T. interdigitale UC-1399	1,000
Candida albicans Ab UC-1077	1,000
T. violaceum UC-1459	10[b]
T. mentagrophytes UC-4797	100
Microsporum canis UC-1395	100
T. asteroides UC-4775	100
T. mentagrophytes UC-4860	100

[a] Tested at concentrations of 1,000, 100, 10, and 1 μg/ml.
[b] Partial.

dicate acceptance of an oversimplification of the complex problem of differentiation of *Streptomyces* species.

None of the systems covers the microscopic and cultural characterization problems of the cultures considered. They do represent excellent efforts to solve the characterization problem.

S. lomondensis is differentiated from the cultures in the systems discussed by color pattern, sporophore type, spore pattern, and antibiotic production. It is readily distinguishable from named *Streptomyces* species in the Upjohn culture collection and, as far as can be determined, from those described in the literature. The distinctive characteristics of this culture warrant its designation as a new species, *Streptomyces lomondensis* sp. n.

It is proposed that *S. lomondensis*, described here and deposited as NRRL 3252, be designated the type strain.

Fermentation studies. A typical fermentation pattern at 28 C is shown in Table 3.

Paper chromatography. Lomofungin is distinguishable from all available similar antibiotics by paper chromatography. Its paper chromatogram pattern in six solvent systems is shown in Fig. 3.

In vitro spectra. Table 4 shows the in vitro antibacterial spectrum, with inhibition of gram-positive and gram-negative microorganisms at concentrations from 16 to 125 μg/ml. Lomofungin inhibits a wide spectrum of human pathogenic fungi at concentrations of 100 to 1,000 μg/ml (Table 5).

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