Streptomyces longwoodensis sp. nov.

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A new species of *Streptomyces* is described for which the name *S. longwoodensis* is proposed. The organism produces a polyether ionophorous antibiotic, lysocellin. It is characterized by its gray spore mass color, spiral spore chain with smooth spores, chromogenic reaction on ISP media 1 and 6, and carbon utilization characteristics. It differs from all streptomycetes described previously on the basis of its carbon utilization, protein hydrolysis, and pigment production. The type strain of *S. longwoodensis* is Roche X-14537 (= ATCC 29251).

Strain X-14537 was found in an antibiotic screen at Hoffmann-La Roche Inc., Nutley, N.J. The following report contains a description of this organism and a discussion of its taxonomic position.

MATERIALS AND METHODS

Bacterial strains. Strain X-14537 (= ATCC 29251) was isolated in our laboratory from soil from Longwood Gardens in Kennett Square, Pa. The following type strains were compared against our new isolate: *Streptomyces misionensis* ATCC 14991, *S. griseofuscus* ATCC 23916, *S. galbus* NRRL 2283, and *S. murinus* NRRL B2286.

Methods. The organisms were cultivated on standard ISP media (Difco) as described by Shirling and Gottlieb (9) as well as on the media used for the following tests: sodium chloride tolerance (5); decomposition of adenine, xanthine, tyrosine, and hypoxanthine (3); hydrolysis of hippurate (4) and casein (5); peptonization of skim milk (Gordon and Smith [5], modified without agar); hydrolysis of starch (actinomycetes broth [Difco] plus 0.25% soluble starch and 2.0% agar); and gelatin hydrolysis (Skerman [10], modified with actinomycetes broth [Difco] plus 2.0% agar in place of meat infusion agar); and reduction of nitrate (5). All tests were run at 28°C. Color determinations were made after 2 weeks of incubation.

Carbon utilization was determined by the method of Shirling and Gottlieb (9). A 24-h-old ISP-1 broth culture of each organism was homogenized and centrifuged to obtain a washed suspension for inoculation. The ability of the organisms to grow at 10, 28, 37, 45, and 50°C was investigated by inoculating broth of ISP-1 (Difco) medium. Cell wall analysis was performed by the method of Becker et al. (1).

Production of lysocellin, a monocarboxylic polyether ionophorous antibiotic, was determined on a chloroform extract of whole broth. The evaporated residue was resuspended to yield a 40-fold concentration. Thin-layer chromatograms were run in three solvent systems, bioautographed, and sprayed with vanillin reagent. Lysocellin was detectable as a water-insoluble bioactive compound that stained green with vanillin reagent.

RESULTS

Microscopic examination. Strain X-14537 produces a substrate mycelium, which does not fragment into spores, and an aerial mycelium, which later forms spore chains. After 14 days of incubation at 28°C, the spore chains appear spiral in form with greater than 10 spores per chain (range: 5 to >50). Spores are smooth and range in size from 1.20 by 0.32 μm to 1.80 by 0.74 μm (Fig. 1 and 2). The cell wall of this organism contains the L isomer of diaminopimelic acid, which, together with the above-mentioned characteristics, places this organism in the genus *Streptomyces* (6).

Macroscopic examination. In Table 1 are summarized the amount of growth, degree of sporulation, spore mass color, color of reverse-substrate mycelium, and presence of a soluble pigment produced by strain X-14537 on various solid media.

Physiological characteristics. Strain X-14537 did not hydrolyze hippurate; it decomposed adenine, hypoxanthine, and tyrosine but not xanthine; and it slowly peptonized skim milk.

Table 2 reports the results of carbon utilization by strain X-14537 and compares the results with those of the type strains of *Streptomyces misionensis*, *S. griseofuscus*, *S. galbus*, and *S. murinus*.

Table 3 is a list of diagnostically important properties, mostly metabolic, that are useful for differentiating strain X-14537 from closely related organisms.

DISCUSSION

A comparison of the description of strain X-14537 with those of the *Streptomyces* species described in *Bergey's Manual* (2), H. Nonomura's key for classification (7), and Pridham and Lyons' classification (8) showed that no

FIG. 1. *S. longwoodensis* X-14537. Spiral spore chains on ISP-3 agar; 14 days of incubation. $\times 3,000$. Scanning electron microscope.

FIG. 2. *S. longwoodensis* X-14537. Smooth spores on ISP-3 agar; 14 days of incubation. $\times 10,800$. Scanning electron microscope.

TABLE 1. *Cultural characteristics of strain X-14537*

Agar medium	Amt of growth; degree of sporulation	Spore mass color ^a	Color of reverse-substrate mycelium ^a
Yeast malt extract (ISP-2) ^b	Moderate to abundant growth; well sporulated	Mostly 2fe (covert gray); tufts of <i>b</i> (oyster white)	3ni (clove brown) at center; 3ie (camel) at edge
Oatmeal (ISP-3) ^b	Moderate growth; well sporulated	Between 2fe (covert gray) and 3fe (silver gray)	2dc (natural string)
Inorganic salts starch (ISP-4) ^b	Moderate growth; moderate sporulation	<i>c</i> (light gray) toward center; 2fe (covert gray) at edge	2dc (natural string) at center; 2ba (pearl) at edge
Glycerol asparagine (ISP-5) ^b	Poor growth; moderate to sparse sporulation	3fe (silver gray) where dense growth; 2fe (covert gray) where moderate growth; 3be (pearl) where isolated colonies	3ig (beige brown) where dense growth; 3gc (light tan) where isolated colonies
Czapek-Dox ^c	Moderate growth; sparse sporulation	<i>e</i> (gray) where densely sporulated; 2dc (natural) where sparsely sporulated	3fe (silver gray)
Bennett ^d	Moderate growth; well sporulated	2ba (pearl) where densely sporulated; <i>d</i> (light gray) where sparsely sporulated	3ni (clove brown) where dense; 3ie (camel) at edge
Sabouraud dextrose (Difco)	Poor growth; sparse sporulation	2ba (pearl) where sporulated	2gc (covert tan)
<i>Thermoactinomyces</i> fermentation medium ^e	Moderate to abundant growth; well sporulated, slightly hygroscopic	Mostly 2fe (covert gray); with some <i>b</i> (oyster white)	3lg (mustard tan) toward center; 3ie (camel) toward edge
Sporulation (ATCC medium 5) ^f	Moderate growth; well sporulated; some isolated non-sporulated colonies	Mostly 2fe (covert gray); some isolated colonies of 3ba (pearl)	3pn (dark brown) toward center; 2ih (dark covert gray) and 2ba (pearl) at edge
Amidex ^g	Moderate growth; moderate to abundant sporulation	Mostly 2fe (covert gray) with some areas of <i>a</i> (white)	3pl (mustard brown) toward center; 3lg (adobe brown) at edge
Starch casein ^h	Moderate growth; moderate to abundant sporulation	<i>d</i> (light gray) with tufts of <i>b</i> (oyster white)	2gc (covert tan)

^a The color scheme used was that taken from the *Color Harmony Manual*, 4th ed., 1958 (Container Corporation of America, Chicago).

^b Media recommended by the International *Streptomyces* Project (9).

^c Czapek-Dox broth (BBL) to which 1.5% agar was added.

^d *Thermoactinomyces* fermentation medium (Difco) to which 1.5% agar was added. This medium is no longer commercially available. The composition according to Difco is as follows: tryptone, 0.5%; yeast extract, 0.2%; Soytone, 0.2%; white potato dextrin, 1.0%; D-mannitol, 0.5%; MgSO₄, 0.02%; Fe(NH₄)₂SO₄, 0.001%; ZnCl₂, 2.1 µg/ml; MnCl₂, 1.8 µg/ml; CuSO₄, 0.2 µg/ml; Co(NO₃)₂, 0.5 µg/ml; H₃BO₃, 0.6 µg/ml; pH 7.2.

^e Yeast extract, 0.1%; beef extract, 0.1%; *N*-Z-amine A (enzymatic hydrolysate of casein from Humko-Sheffield Chemical Co., Lyndhurst, N.Y.), 0.2%; glucose, 1.0%; pH 7.3.

^f American Type Culture Collection Catalogue of Strains, 12th ed., 1976. (American Type Culture Collection, Rockville, Md.)

^g Amidex (Corn Products Co., Decatur, Ill.), 1%; *N*-Z-amine A, 0.2%; beef extract, 0.1%; yeast extract, 0.1%; CaCl₂ · 2H₂O, 0.0014%; agar, 2%; pH 7.3.

^h Soluble starch, 1%; casein, 0.1%; K₂HPO₄, 0.05%; MgSO₄, 0.05%; agar, 1.5%; pH 7.4.

TABLE 2. *Comparison of carbon utilization by strain X-14537 and related strains*

Carbon source	Growth response ^a of:				
	X-14537	<i>S. misionensis</i> ATCC 14991	<i>S. griseofuscus</i> ATCC 23916	<i>S. galbus</i> NRRL 2283	<i>S. murinus</i> NRRL B2286
D-Glucose	++	++	++	++ YP ^b	++ YP
D-Xylose	++	++	+	++ YP	+ YP
L-Arabinose	++	++	++	++ YP	—
L-Rhamnose	—	—	—	—	—
D-Fructose	++	+	+	++ YP	++ YP
D-Galactose	++	++	++	++ YP	++ YP
Raffinose	± to —	— to ± ^c	—	—	—
D-Mannitol	++	++	++	++ YP	++ YP
<i>i</i> -Inositol	++	± ^c	—	++ YP	—
Salicin	+	—	±	± to —	±
Sucrose	—	—	—	—	—
Cellulose	—	—	—	—	—

^a —, Negative response; ±, doubtful response; +, more growth than on carbon control but less than on glucose; ++, positive response equal to the amount of growth on glucose.

^b YP, Yellow diffusible pigment.

^c Reported as + in *Bergey's Manual*, 8th ed. (2).

TABLE 3. Comparison of metabolic characteristics of strain X-14537 and related strains

Test	X-14537	<i>S. misionensis</i> ATCC 14991	<i>S. griseofuscus</i> ATCC 23916	<i>S. galbus</i> NRRL 2283	<i>S. murinus</i> NRRL B2286
ISP-6 darkening	+	—	—	+	—
Melanin, ISP-7	—	—	—	—	—
Casein hydrolysis	+	+	+	—	+
Gelatin hydrolysis	—	+	+	—	+
Starch hydrolysis	+	+	+	+	+
NaCl (%) tolerance	5	7	7	7	5
Growth range temp (°C)	10–45	10–45	10–37	10–45	10–37
ISP-1 darkening	+	—	—	+	Yellow-orange
Reverse-side pigment	—	—	—	Yellow to orange	Yellow-brown
Soluble pigment	—	—	—	Yellow	Yellow
Streptomycin sensitivity (10- μ g disk)	+	+	+	+	+
Lysocellin production	+	—	—	—	—
Nitrate reduction	—	—	—	—	—
Hygroscopic property	Slight on <i>Thermoactinomyces</i> fermentation agar	+ on <i>Thermoactinomyces</i> fermentation agar	—	—	+ on ISP-3 and <i>Thermoactinomyces</i> fermentation agar

known species are identical to X-14537 based on the following combination of criteria: gray spore mass color, spiral spore chain form, smooth spore surface, chromogenic reaction on ISP media 1 and 6, and carbon utilization characteristics. Organisms that resemble this strain are *Streptomyces misionensis* ATCC 14991, *S. griseofuscus* ATCC 23916, *S. galbus* NRRL 2283, and *S. murinus* NRRL B2286. However, the strains of *S. misionensis*, *S. griseofuscus*, and *S. murinus* can be differentiated from X-14537 on the basis of carbon utilization, gelatin hydrolysis, and darkening of ISP-1 and ISP-6 media. Of the four nomen species cited above, strain X-14537 most closely resembles *S. galbus*, although there are minor differences in carbon utilization and pronounced differences in the reverse-side and soluble pigments. Of the strains examined, only X-14537 produced lysocellin in quantities of 20 μ g/ml. The others did not produce lysocellin above the minimal detectable quantity of 1 μ g/ml of whole broth. This fact, together with other biochemical and nutritional characteristics, indicates that strain X-14537 (= ATCC 29251) belongs to a new species, for which the name *Streptomyces longwoodensis* is proposed (long. wood. en'sis. M. L. gen. n. *longwoodensis* of Longwood; named for Longwood Gardens in Kennett Square, Pa., the origin of the soil from which this organism was first isolated).

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REPRINT REQUESTS

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