Streptomyces stramineus sp. nov., a New Species of the Verticillate Streptomycetes

D. P. LABEDA,^{1*} M. P. LECHEVALIER,² AND R. T. TESTA³

Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service,
U.S. Department of Agriculture, Peoria, Illinois 61604¹; R.R. 2, Box 2235, Morrisville, Vermont 05661²;
and Infectious Disease Section, Wyeth-Ayerst Research, Pearl River, New York 10965³

Strain NRRL 12292^T, which produces the bleomycin-like antibiotics LL-BO1208 α and LL-BO1208 β , forms umbels consisting of chains of smooth-surface ovoid spores that are borne on verticils on the aerial mycelia, indicating that it is a member of the verticillate group of the genus *Streptomyces* formerly classified in the genus *Streptovorticillium*. This strain was compared morphologically and physiologically to 54 other verticillate *Streptomyces* strains. The levels of DNA relatedness between strain NRRL 12292^T and 34 other verticillate *Streptomyces* strains, including strains representing at least 19 genetic species clusters, were also determined. Strain NRRL 12292^T is morphologically and physiologically distinct from the other verticillate strains studied, particularly because of the straw yellow color of its aerial mycelia and spore mass. DNA hybridization data support the uniqueness of this strain, since the levels of DNA relatedness between NRRL 12292^T and the other verticillate strains used in this study were low. Our data support designation of a new species, *Streptomyces stramineus*, whose type strain is NRRL 12292.

Streptomyces sp. strain NRRL 12292^T was isolated from a soil sample from South Dakota during the course of a natural-products screening program at Lederle Laboratories. This strain produces the antibiotics LL-BO1208 α and LL-BO1208 β , which are related to phleomycin. Morphological observations of the strain revealed that it produced spores from verticils on the aerial mycelium, which is typical of microorganisms previously described as members of the genus *Streptovorticillium*. The gross colonial morphology of strain NRRL 12292^T was distinctive when it was compared to the gross colonial morphology of other verticillate streptomycetes because of the straw yellow color of the aerial mass. The genus *Streptovorticillium* has been reunited with the genus *Streptomyces* based on 16S rRNA sequence similarity data (17). Strain NRRL 12292^T was therefore compared phenotypically with other verticillate streptomycetes formerly classified in the genus *Streptovorticillium* and was found to be a member of a new species. Subsequently, we determined levels of DNA relatedness spectrophotometrically between NRRL 12292^T and 34 representative strains belonging to 19 species of verticillate streptomycetes previously delineated (5) based on levels of DNA relatedness greater than 70%.

MATERIALS AND METHODS

Strains and cultivation conditions. The strains used in this study are listed in Table 1. Primary storage of strains was in refrigerated ampoules containing freeze-dried spore and vegetative mycelium suspensions in sterile beef serum. Working stock cultures were maintained on slants of synthetic starch agar or yeast extract-malt extract agar (15) and were stored at 4°C until they were needed. The inoculum used for physiological evaluation was grown in Bennett's broth (1 g of yeast extract per liter, 1 g of beef extract per liter, 2 g of NZamine type A per liter, 10 g of glucose per liter; pH 7.4). The biomass used for extraction of DNA was grown in tryptone-glucose-liver extract-yeast extract broth (12) with shaking at 200 rpm at 28°C.

Morphological observations. Gross morphological observations were made by using cultures grown for 14 days at 28°C on the standard media suggested by the International *Streptomyces* Project (ISP) (15). Micromorphology and sporulation were determined by light microscopy and scanning electron microscopy. The samples used for scanning electron microscopy were samples from 14-day cul-

tures on agar media that had been fixed overnight with osmium tetroxide vapors, postfixed for 1 h in 1% osmium tetroxide in Millonig's phosphate-buffered fixative (10), dehydrated through a graded acetone series, critical point dried from liquid CO₂, and sputter coated with gold-palladium. The samples were observed with a JEOL model JSM 6400V scanning electron microscope.

Chemotaxonomic methods. Diaminopimelic acid analysis was carried out by using the procedures of Becker et al. (1), as modified by Stanek and Roberts (16). The fatty acid content was determined by using biomass collected from Trypticase soy broth (BBL, Cockeysville, Md.) agar plates that had been incubated for 4 days at 28°C. Growth scraped from the plates was processed immediately or frozen at –20°C until it was processed. Fatty acid methyl esters were obtained from the biomass by saponification, methylation, and extraction by using the method of Miller and Berger (9). The fatty acid methyl esters were separated and quantified by using a model 9898A gas chromatograph (Hewlett-Packard, Inc., Avondale, Pa.) fitted with a methyl phenyl silicone fused silica capillary column (25 m by 0.2 mm; Hewlett-Packard) and Microbial Identification System software (MIDI, Inc., Newark, Del.). Peaks were automatically integrated and fatty acid identities and percentages were determined by the Microbial Identification System software.

Physiological tests. Physiological tests, including tests to determine production of acid from carbohydrates, utilization of organic acids, growth in the presence of glycerol, lysozyme, salicylate, and 5% NaCl, and hydrolysis and decomposition of adenine, guanine, hypoxanthine, tyrosine, xanthine, casein, esculin, urea, and nitrate, were performed by using the media of Gordon et al. (2). Phosphatase activity was evaluated by using the method of Kurup and Schmitt (4). The tests to determine utilization of carbohydrates as the sole carbon source for growth were performed with the media of Pridham and Gottlieb (11).

DNA isolation, purification, and hybridization. DNA was extracted from cells by the method of Marmur (7) after the cells were broken by passing them through a French pressure cell (SLM Instruments, Inc., Urbana, Ill.) at 10,000 lb/in². The DNA was further purified by chromatography on hydroxyapatite or by cesium chloride density gradient centrifugation (6). The guanine-plus-cytosine (G+C) content of the DNA was determined spectrophotometrically from the midpoint of thermal denaturation in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) (8). The levels of DNA relatedness between strains were calculated from C₀t_{0.5} values in 5× SSC supplemented with 20% dimethyl sulfoxide at 65°C (*T_m* – 23°C) by the method of Seidler et al. (13) and Seidler and Mandel (14) as described by Kurtzman et al. (3).

Statistical calculations. Statistical calculations on the DNA relatedness data were performed by using SAS for Windows, release 6.11 (SAS Institute, Inc., Cary, N.C.), and a FoxTech Guardian 150-MHz Pentium microcomputer (Fox Technologies, Inc., Yorkville, Ill.). The data were clustered by the average linkage algorithm with the SAS/STAT CLUSTER procedure, and a dendrogram was generated with SAS/GRAPH by using the macro GRAFTREE written and kindly provided by Dan Jacobs, University of Maryland.

RESULTS

Morphological and chemotaxonomic properties. The verticillate sporophores on the aerial mycelia of *Streptomyces* sp.

* Corresponding author. Mailing address: National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604.

TABLE 1. *Streptomyces* strains used in this study

Taxon	Strain	Characteristic(s) examined ^a
<i>S. abikoensis</i>	NRRL B-2113 ^T (= ATCC 12766 ^T)	DNA, PHYS
<i>S. ardens</i>	NRRL 2817 ^T (= ATCC 27417 ^T)	DNA, PHYS
<i>S. albireticuli</i>	NRRL B-5493 ^T (= ATCC 19721 ^T)	PHYS
<i>S. aspergilloides</i>	NRRL B-3355 ^T (= ATCC 14808 ^T)	PHYS
<i>S. aureoverisilis</i>	NRRL B-3325 ^T (= ATCC 15853 ^T)	DNA, PHYS
<i>S. baldacii</i>	NRRL B-3500 ^T (= ATCC 23654 ^T)	DNA, PHYS
<i>S. biverticillatus</i>	NRRL ISP-5272 ^T (= ATCC 23615 ^T)	DNA, PHYS
<i>S. blastmyceticus</i>	NRRL B-5480 ^T (= ATCC 19731 ^T)	DNA, PHYS
<i>S. caespitosus</i>	NRRL 2564 ^T (= ATCC 27422 ^T)	PHYS
<i>S. cinnamoneus</i> subsp. <i>albosporeus</i>	NRRL B-5624 ^T (= ATCC 25186 ^T)	DNA, PHYS
<i>S. cinnamoneus</i> subsp. <i>azacoluta</i>	NRRL B-1699 ^T (= ATCC 12686 ^T)	DNA, PHYS
<i>S. cinnamoneus</i> subsp. <i>cinnamoneus</i>	NRRL B-1285 ^T (= ATCC 11874 ^T)	DNA, PHYS
<i>S. cinnamoneus</i> subsp. <i>lanosus</i>	ATCC 25187 ^T	PHYS
<i>S. cinnamoneus</i> subsp. <i>sparsus</i>	ATCC 25185 ^T	PHYS
<i>S. distallicus</i>	NRRL 2886 ^T (= DSM 40846 ^T)	DNA, PHYS
<i>S. ehimensis</i>	NRRL B-1967 ^T (= ATCC 23903 ^T)	PHYS
<i>S. eurocidicus</i>	NRRL B-1676 ^T (= ATCC 27428 ^T)	DNA, PHYS
<i>S. fervens</i>	NRRL 2755 ^T (= ATCC 27429 ^T)	DNA, PHYS
<i>S. flavopersicus</i>	NRRL 2820 ^T (= ATCC 19756 ^T)	DNA, PHYS
<i>S. griseocarneus</i>	NRRL B-1068 ^T (= ATCC 12628 ^T)	DNA, PHYS
<i>S. griseoverticillatus</i>	NRRL B-12432 ^T (= ATCC 27436 ^T)	DNA, PHYS
<i>S. hachijoensis</i>	NRRL B-3106 ^T (= ATCC 19769 ^T)	DNA, PHYS
<i>S. hirosimensis</i>	NRRL B-5484 ^T (= ATCC 19722 ^T)	DNA, PHYS
<i>S. kashmirensis</i>	NRRL B-3103 ^T (= ATCC 27439 ^T)	PHYS
<i>S. kentuckensis</i>	NRRL B-1831 ^T (= ATCC 12691 ^T)	DNA, PHYS
<i>S. kishiwadensis</i>	NRRL B-12326 ^T (= ATCC 25464 ^T)	PHYS
<i>S. ladakanum</i>	NRRL 3191 ^T (= ATCC 27441 ^T)	DNA, PHYS
<i>S. lavenduligriseus</i>	NRRL B-3173 ^T (= ATCC 13306 ^T)	DNA, PHYS
<i>S. lilacinus</i>	NRRL B-1968 ^T (= ATCC 23930 ^T)	PHYS
" <i>S. luteoreticuli</i> "	NRRL B-12435 (= ATCC 27446)	DNA, PHYS
<i>S. luteosporeus</i>	NRRL 2401 ^T (= ATCC 33049 ^T)	PHYS
<i>S. luteoverticillatus</i>	NRRL B-1995 ^T (= ATCC 23933 ^T)	PHYS
<i>S. mashuensis</i>	NRRL B-8164 ^T (= ATCC 23934 ^T)	DNA, PHYS
<i>S. mobaraensis</i>	NRRL B-3729 ^T (= ATCC 29032 ^T)	DNA, PHYS
<i>S. morookaense</i>	NRRL B-12429 ^T (= ATCC 19166 ^T)	PHYS
<i>S. netropsis</i>	NRRL 2268 ^T (= ATCC 23940 ^T)	DNA, PHYS
<i>S. olivoreticuli</i>	NRRL B-2091 ^T (= ATCC 23943 ^T)	PHYS
<i>S. olivoverticillatus</i>	NRRL B-1994 ^T (= ATCC 25480 ^T)	DNA, PHYS
<i>S. orinoci</i>	NRRL B-3379 ^T (= ATCC 23202 ^T)	PHYS
<i>S. parvisporogenes</i>	NRRL B-5464 ^T (= ATCC 12568 ^T)	PHYS
" <i>S. pentaticus</i> subsp. <i>jenensis</i> "	NRRL B-12088 (= DSM 40848)	DNA, PHYS
" <i>S. reticulusruber</i> "	NRRL B-1484 (= ATCC 25607)	DNA, PHYS
<i>S. roseoverticillatus</i>	NRRL B-1993 ^T (= ATCC 19807 ^T)	DNA, PHYS
<i>S. roseoverticillatus</i> subsp. <i>albosporeus</i>	ATCC 25189 ^T	PHYS
" <i>S. rubroverticillatus</i> "	NRRL B-16433 (= DSM 41489)	DNA
<i>S. salmonis</i>	NRRL B-1472 ^T (= DSM 40895 ^T)	DNA
<i>S. septatus</i>	NRRL 2974 ^T (= ATCC 27464 ^T)	PHYS
<i>S. sporiferus</i>	ATCC 25188 ^T	PHYS
" <i>S. taitoense</i> "	NRRL B-16435 (= ATCC 29758)	DNA, PHYS
" <i>S. takataensis</i> "	NRRL B-12516 (= ATCC 27469)	DNA, PHYS
" <i>S. tropicalensis</i> "	NRRL B-12481 (= ATCC 17963)	DNA, PHYS
<i>S. thioluteus</i>	ATCC 12310 ^T	PHYS
<i>S. waksmanii</i>	NRRL B-1707 ^T (= ATCC 12629 ^T)	DNA, PHYS

^a DNA, level of DNA relatedness; PHYS, physiological profile.

strain NRRL 12292^T were observed on many media by light microscopy, as shown in Fig. 1, and were also clearly visible on a scanning electron micrograph (Fig. 2). Each of the verticils produced umbels consisting of straight chains of ovoid smooth-

surface spores (0.5 by 1.0 µm). This morphology is typical of strains previously assigned to the genus *Streptoverticillium*.

The gross colonial morphology of a 14-day growth of strain NRRL 12292^T on ISP media is shown in Table 2. The straw

TABLE 2. Morphological characteristics of *S. stramineus* NRRL 12292^T

Medium	Growth	Aerial mycelium-spore mass color	Vegetative mycelium color	Soluble pigments
Yeast extract-malt extract agar (ISP medium 2)	Good	Yellow	Tan	Light brown
Inorganic salts-soluble starch agar (ISP medium 4)	Good	Yellow	Yellow-white	None
Glycerol-asparagine agar (ISP medium 5)	Good	Yellow	Yellow	None
Oatmeal agar (ISP medium 3)	Good	Yellow	Yellow	None

FIG. 1. Light micrograph of a 24-day culture of *S. stramineus* NRRL 12292^T on Czapek agar. Note the umbels of spores borne on verticils on the aerial hyphae.

FIG. 2. Scanning electron micrograph of a 14-day culture of *S. stramineus* NRRL 12292^T on yeast extract-malt extract agar. Note the umbels of smooth-surface ovoid spores borne on verticils on the aerial mycelia. Bar = 1 μ m.

TABLE 3. Differential physiological properties of verticillate streptomycetes

DNA relatedness cluster	Taxon	Strain	Hydrolysis of:								Growth in the presence of:		Assimilation of:			
			Casein	Xanthine	Adenine	Starch	Phosphate	Urea	Nitrate	Esculin	5% NaCl	Lysozyme	Acetate	Citrate	Lactate	Propionate
<i>S. stramineus</i>	<i>S. stramineus</i>	NRRL 12292 ^T	+ ^a	+	—	+	+	—	—	+	+	+	+	+	—	+
<i>S. abikoensis</i>	<i>S. abikoensis</i>	NRRL B-2113 ^T	+	+	—	+	+	—	—	+	+	—	+	+	—	+
	" <i>S. takataensis</i> "	NRRL B-12516	+	+	—	+	+	—	—	+	—	+	+	+	—	+
	<i>S. waksmanii</i>	NRRL B-1707 ^T	+	+	—	+	—	—	—	+	—	—	+	+	—	+
<i>S. ardens</i>	<i>S. ardens</i>	NRRL 2817 ^T	+	—	—	—	+	—	—	+	+	v	+	+	+	+
<i>S. aureoverisilis</i>	<i>S. aureoverisilis</i>	NRRL B-3325 ^T	+	+	—	+	+	+	+	+	+	+	+	+	+	+
<i>S. baldaccii</i>	<i>S. baldaccii</i>	NRRL B-3500 ^T	+	+	—	+	+	—	—	+	+	+	+	+	—	+
	<i>S. biverticillatus</i>	NRRL B-2727 ^T	+	+	—	+	+	—	+	+	+	+	+	+	—	+
	<i>S. fervens</i>	NRRL 2755 ^T	+	+	—	+	+	—	—	—	+	—	+	+	—	+
	" <i>S. pentaticus</i> subsp. <i>jenensis</i> "	NRRL B-12088	+	+	—	—	+	—	—	+	+	—	+	+	—	+
	<i>S. roseovericillatus</i>	NRRL B-1993 ^T	+	+	—	+	+	—	—	+	+	—	+	+	—	+
<i>S. blastomyceticus</i>	<i>S. blastomyceticus</i>	NRRL B-5480 ^T	+	+	—	+	+	—	—	+	+	+	+	+	+	+
<i>S. cinnamoneus</i>	<i>S. cinnamoneus</i>	NRRL B-5624 ^T	+	+	—	+	+	—	—	—	+	+	+	+	—	+
	subsp. <i>albosporus</i>															
	<i>S. cinnamoneus</i> subsp. <i>cinnamoneus</i>	NRRL B-1285 ^T	+	+	—	—	+	—	—	+	+	+	+	+	—	+
<i>S. eurocidicus</i>	<i>S. eurocidicus</i>	NRRL B-1676 ^T	+	+	—	+	+	—	+	+	+	—	+	+	—	+
<i>S. griseocarneus</i>	<i>S. griseocarneus</i>	NRRL B-1068 ^T	+	+	—	+	+	—	—	+	—	+	+	+	—	+
<i>S. griseovericillatus</i>	<i>S. griseovericillatus</i>	NRRL B-12432 ^T	+	+	—	+	+	—	—	—	—	—	+	+	+	+
<i>S. hachijoensis</i>	<i>S. cinnamoneus</i>	NRRL B-1699 ^T	+	+	—	+	+	+	—	+	+	+	+	+	—	+
	subsp. <i>azacoluta</i>															
	<i>S. hachijoensis</i>	NRRL B-3106 ^T	+	+	—	+	+	—	—	—	+	—	+	+	—	+
	" <i>S. taiensis</i> "	NRRL B-16435	+	+	—	+	+	—	—	—	—	+	+	+	—	+
<i>S. hirosimensis</i>	<i>S. hirosimensis</i>	NRRL B-5484 ^T	+	+	—	+	+	—	+	+	+	+	+	+	—	+
<i>S. lavenduligriseus</i>	<i>S. lavenduligriseus</i>	NRRL B-3173 ^T	—	—	—	+	+	—	+	+	+	+	+	+	—	+
" <i>S. luteoreticuli</i> "	" <i>S. luteoreticuli</i> "	NRRL B-12435	—	+	—	+	+	—	+	+	v	+	+	+	+	+
<i>S. mashuensis</i>	<i>S. mashuensis</i>	NRRL B-3352 ^T	+	—	—	+	+	—	—	+	—	—	+	+	+	+
<i>S. mobaraensis</i>	<i>S. ladakanum</i>	NRRL 3191 ^T	+	+	—	—	+	—	+	+	+	+	+	—	—	+
	<i>S. mobaraensis</i>	NRRL B-3729 ^T	+	+	—	—	+	—	+	+	—	—	+	+	+	+
<i>S. netropsis</i>	<i>S. distallicus</i>	NRRL 2886 ^T	+	+	+	+	+	—	—	+	+	—	+	+	—	+
	<i>S. flavopersicus</i>	NRRL 2820 ^T	+	+	+	+	+	—	—	+	+	—	+	+	—	+
	<i>S. kentuckensis</i>	NRRL B-1831 ^T	+	+	+	+	+	—	—	+	—	—	+	+	—	+
	<i>S. netropsis</i>	NRRL 2268 ^T	+	+	—	+	+	—	—	+	+	—	+	+	—	+
<i>S. olivovericillatus</i>	<i>S. olivovericillatus</i>	NRRL B-1994 ^T	+	+	—	+	+	—	—	—	—	—	+	+	—	+
<i>S. salmonis</i>	" <i>S. reticulatus</i> "	NRRL B-1484	+	+	—	—	—	+	+	+	—	—	+	+	—	+
" <i>S. tropicalensis</i> "	" <i>S. tropicalensis</i> "	NRRL B-12481	+	+	—	+	+	—	—	+	—	+	+	+	—	+
Not determined	<i>S. albireticuli</i>	NRRL B-5493 ^T	+	+	—	+	+	+	+	+	+	+	+	+	—	+
Not determined	<i>S. albus</i>	NRRL 2401 ^T	+	—	—	+	+	—	—	—	+	+	+	+	—	+
Not determined	<i>S. aspergilloides</i>	NRRL B-3355 ^T	+	—	—	+	—	—	+	+	+	+	+	+	—	+
Not determined	<i>S. caespitosus</i>	NRRL 2564 ^T	+	—	—	+	+	—	—	+	+	+	+	+	—	+
Not determined	<i>S. cinnamoneus</i>	ATCC 25187 ^T	+	+	—	+	+	+	—	—	+	+	+	+	—	+
	subsp. <i>lanosus</i>															
Not determined	<i>S. cinnamoneus</i> subsp. <i>sparsus</i>	ATCC 25185 ^T	+	+	—	+	+	—	—	+	+	+	+	+	+	+
Not determined	<i>S. ehimensis</i>	NRRL B-1967 ^T	+	+	—	+	+	—	—	+	+	—	+	+	—	+
Not determined	<i>S. kashimirensis</i>	NRRL B-3103 ^T	—	+	—	+	+	—	—	+	+	+	+	+	+	+
Not determined	<i>S. kishiwadensis</i>	NRRL B-12326 ^T	+	+	—	+	+	—	—	+	—	—	+	+	+	+
Not determined	<i>S. lilacinus</i>	NRRL B-1968 ^T	+	—	—	+	+	—	—	+	—	—	+	+	—	+
Not determined	<i>S. luteovericillatus</i>	NRRL B-1995 ^T	+	—	—	+	+	—	—	+	—	—	+	+	—	+
Not determined	<i>S. morookaense</i>	NRRL B-12429 ^T	—	—	—	+	—	—	+	+	+	+	+	+	+	+
Not determined	<i>S. olivoreticuli</i>	ATCC 31159 ^T	+	—	—	+	+	—	—	+	+	—	—	+	+	+
Not determined	<i>S. olivoreticuli</i>	NRRL B-2091 ^T	+	—	—	+	+	—	—	—	+	—	+	+	—	+
Not determined	<i>S. orinoci</i>	NRRL B-3379 ^T	+	—	—	+	+	—	—	+	+	—	+	+	—	—
Not determined	<i>S. parvisporogenes</i>	NRRL B-5464 ^T	+	+	—	+	+	—	—	+	—	+	+	—	—	+
Not determined	<i>S. rectiverticillatus</i>	NRRL B-12369 ^T	+	+	—	+	+	—	+	+	+	+	+	+	—	+
Not determined	<i>S. roseovericillatus</i> subsp. <i>albosporus</i>	ATCC 25189 ^T	+	+	—	+	—	—	—	+	+	+	+	+	—	+
Not determined	<i>S. septatus</i>	NRRL 2974 ^T	+	+	—	+	+	—	—	—	—	+	+	+	—	+
Not determined	<i>S. sporiferus</i>	ATCC 25188 ^T	+	+	—	+	+	—	—	+	+	+	+	+	+	+
Not determined	<i>S. thioluteus</i>	ATCC 12310 ^T	+	+	—	+	+	—	—	+	+	—	+	+	+	+

^a +, positive; —, negative; v, variable.

yellow color and cottony nature of the aerial mycelia and spore mass of this strain on most media are particularly distinctive.

Strain NRRL 12292^T was found to contain the L isomer of diaminopimelic acid, which is typical of members of the family

Streptomycetaceae. The predominant fatty acids found in whole-cell methanolysates were 15:0 anteiso (47%), 16:0 iso (15%), and 14:0 iso (11%), which is also typical of streptomycetes.

Physiology. The differential physiological properties of *Streptomyces* sp. strain NRRL 12292^T and the other strains used in

TABLE 3—Continued

Acid produced from:																		Growth on the following compounds as sole carbon sources:					Aerial mycelium color			
Adonitol	Cellobiose	Fructose	Galactose	Inositol	Lactose	Maltose	Mannitol	Mannose	Melibiose	Rafinose	Rhamnose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose	β-Methyl-xyloside	Growth at 10°C	Growth at 42°C	Melanin produced from tyrosine	Arabinose	Fructose		Mannitol	Salicin	Xylose
+	-	+	-	+	-	+	+	+	-	-	-	-	+	-	+	+	-	+	+	-	-	+	+	-	-	Yellow
-	-	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	Cinnamon
-	-	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	Off-white
-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	White
-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	Light gray
-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	Pink-beige
-	-	+	-	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	Cinnamon pink
-	-	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	Bright fluorescent pink
-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-	+	+	+	+	-	+	+	Off-white
-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	Light cinnamon
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	Pinkish brown
-	-	+	+	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	Bald
-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	Cinnamon
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	Cinnamon
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	Yellow
-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	Bald
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	Cinnamon
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	Light khaki tan
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	Medium beige
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	Beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	Pinkish brown
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	-	-	Gray
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	+	-	Gray
-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	+	-	Sparse white
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Bald
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Mouse gray
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Gold
-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Dusty pink
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Light beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Greenish gray-cinnamon
-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Sparse white
-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Green-gray
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Off-white
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Light yellowish white
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Pinkish brown
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Gray
-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Off-white
-	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	Cinnamon
-	+	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	Grayish pink
-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	Gray-cinnamon
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Pink-beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Cinnamon
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Sparse white
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Gray
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Gray-white
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Light beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Light beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Pink-beige
-	-	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-	Pink-red
-	+	-	+	+	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	+	+	+	Gray-beige
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	-	Bald
-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-	-	Beige

this study are shown in Table 3. Strain NRRL 12292^T is physiologically distinct from the other verticillate strains studied.

DNA relatedness. The levels of DNA relatedness of *Streptomyces* sp. strain NRRL 12292^T to the other verticillate strains included in this study are shown in Table 4. Figure 3 is a graphic representation of the relatedness of strain NRRL 12292^T to the other strains after average linkage clustering was

performed. Figure 3 shows that *Streptomyces* sp. species NRRL 12292^T clusters separately from all of the other taxa studied.

DISCUSSION

The morphological properties of strain NRRL 12292^T (i.e., production of spore-bearing umbels on verticils formed on the

TABLE 4. Levels of DNA relatedness between NRRL 12292^T and other verticillate *Streptomyces* strains

DNA relatedness cluster ^a	Taxon	Strain	% DNA relatedness to NRRL 12292 ^T
<i>S. abikoensis</i>	<i>S. abikoensis</i>	NRRL B-2113 ^T	8
	" <i>S. rubroverticillatus</i> "	NRRL B-16433	16
	" <i>S. takataensis</i> "	NRRL B-12516	0
	<i>S. waksmanii</i>	NRRL B-1707 ^T	11
<i>S. arduus</i>	<i>S. arduus</i>	NRRL 2817 ^T	13
<i>S. aureoverisilis</i>	<i>S. aureoverisilis</i>	NRRL B-3325 ^T	18
<i>S. baldaccii</i>	<i>S. baldaccii</i>	NRRL B-3500 ^T	0
	<i>S. biverticillatus</i>	NRRL ISP-5272 ^T	29
	<i>S. fervens</i>	NRRL 2755 ^T	0
	" <i>S. pentaticus</i> subsp. <i>jenensis</i> "	NRRL B-12088	0
	<i>S. roseoverticillatus</i>	NRRL B-1993 ^T	0
<i>S. blastmyceticus</i>	<i>S. blastmyceticus</i>	NRRL B-5480 ^T	31
<i>S. cinnamoneus</i>	<i>S. cinnamoneus</i> subsp. <i>albosporeus</i>	NRRL B-5624 ^T	2
	<i>S. cinnamoneus</i> subsp. <i>cinnamoneus</i>	NRRL B-1285 ^T	12
<i>S. eurocidicus</i>	<i>S. eurocidicus</i>	NRRL B-1676 ^T	0
<i>S. griseocarnaeus</i>	<i>S. griseocarnaeus</i>	NRRL B-1068 ^T	3
<i>S. griseoverticillatus</i>	<i>S. griseoverticillatus</i>	NRRL B-12432 ^T	0
<i>S. hachijoensis</i>	<i>S. cinnamoneus</i> subsp. <i>azacoluta</i>	NRRL B-1699 ^T	0
	<i>S. hachijoensis</i>	NRRL B-3106 ^T	2
	" <i>S. taiensis</i> "	NRRL B-16435	1
	<i>S. hiroshimensis</i>	NRRL B-5484 ^T	18
<i>S. lavendulogriseus</i>	<i>S. lavendulogriseus</i>	NRRL B-3173 ^T	12
<i>S. luteoreticuli</i>	" <i>S. luteoreticuli</i> "	NRRL B-12435	0
<i>S. mashuensis</i>	<i>S. mashuensis</i>	NRRL B-8164 ^T	0
<i>S. mobaraensis</i>	<i>S. ladakanum</i>	NRRL 3191 ^T	8
	<i>S. mobaraensis</i>	NRRL B-3729 ^T	3
<i>S. netropsis</i>	<i>S. distallicus</i>	NRRL 2886 ^T	26
	<i>S. flavopersicus</i>	NRRL 2820 ^T	9
	<i>S. kentuckensis</i>	NRRL B-1831 ^T	14
	<i>S. netropsis</i>	NRRL 2268 ^T	11
<i>S. olivoverticillatus</i>	<i>S. olivoverticillatus</i>	NRRL B-1994 ^T	0
<i>S. salmonis</i>	" <i>S. reticulusruber</i> "	NRRL B-1484	20
	<i>S. salmonis</i>	NRRL B-1472 ^T	16
<i>S. tropicalensis</i>	" <i>S. tropicalensis</i> "	NRRL B-12481	0

^a DNA relatedness clusters described by Labeda (5).

aerial mycelia), as shown in Fig. 1 and 2, clearly place this strain with the verticillate streptomycetes, which were formerly classified in the genus *Streptoverticillium*. The unique straw yellow color of the aerial mycelia and spores of this strain (Table 2) distinguishes it from all other verticillate species; *Streptomyces albireticuli* and *Streptomyces eurocidicus* are the only other species with aerial mycelia and/or spores that are yellow. As Table 3 shows, strain NRRL 12292^T differs physiologically from the previously described verticillate taxa, including *S. albireticuli* and *S. eurocidicus*, which supports designation of this organism as a member of a new species. The lack of DNA relatedness between strain NRRL 12292^T and 34 representative verticillate streptomycetes, as shown by the data in Table 4 and as shown graphically after average linkage clustering in the dendrogram in Fig. 3, confirms that this strain represents a new species, for which the name *Streptomyces stramineus* is proposed because of the color of the aerial mycelium and spores. A formal description of the species is given below.

Description of *Streptomyces stramineus* sp. nov. *Streptomyces stramineus* (stra.min'e.us. L. adj. *stramineus*, straw colored, re-

ferring to the color of the aerial mycelium and spore mass). The substrate mycelium is yellow to yellowish brown. Aerial mycelium forms verticils on which umbels consisting of straight chains of ovoid, smooth-surface, straw yellow spores (0.5 by 1.0 μ m) are produced. Soluble pigments that range from yellowish to shades of brown are produced on several media, such as yeast extract-malt extract agar. Casein, esculin, gelatin, hypoxanthine, starch, tyrosine, and xanthine are hydrolyzed; adenine and urea are not hydrolyzed. Phosphatase is produced; nitrate is not reduced. Melanin pigments are not produced from tyrosine. Growth occurs in the presence of glycerol, 5% NaCl, and lysozyme; no growth occurs in the presence of salicylate. Acetate, citrate, malate, propionate, pyruvate, and succinate are assimilated; benzoate, lactate, mucate, oxalate, and tartrate are not assimilated. Acid is produced from adonitol, dextrin, fructose, glucose, glycerol, inositol, maltose, mannitol, mannose, sorbitol, and trehalose; no acid is produced from arabinose, cellobiose, dulcitol, erythritol, galactose, melibiose, α -methyl-D-glucoside, raffinose, rhamnose, salicin, sucrose, xylose, or β -methyl-D-xyloside. Growth occurs on fruc-

FIG. 3. Dendrogram based on average linkage clustering of levels of DNA relatedness among verticillate *Streptomyces* species, including *S. stramineus*. *S. stramineus* is distinct from the other taxa studied.

tose, glucose, inositol, and mannitol as the sole carbon source but not on galactose, raffinose, rhamnose, salicin, or xylose. Growth occurs at 10 and 42°C, but not 52°C. The G+C content of the DNA is 75 mol% (as determined by the thermal denaturation method). Isolated from a grassland soil sample from South Dakota. Produces the antibiotics LL-BO1208 α and LL-BO1208 β . The type strain of *S. stramineus* is NRRL 12292.

ACKNOWLEDGMENTS

The able technical assistance of F. L. Baker in preparing the scanning electron micrographs and the assistance of E. N. Hoekstra in isolating and purifying DNA and in performing many of the DNA hybridizations are gratefully acknowledged.

REFERENCES

1. Becker, B., M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier. 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl. Microbiol.* **12**:421–423.
2. Gordon, R. E., D. A. Barnett, J. E. Handershan, and C. H.-N. Pang. 1974. *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int. J. Syst. Bacteriol.* **24**:54–63.
3. Kurtzman, C. P., M. J. Smiley, C. J. Johnson, L. J. Wickerham, and G. B. Fuson. 1980. Two new and closely related heterothallic species, *Pichia amylophila* and *Pichia mississippiensis*: characterization by hybridization and deoxyribonucleic acid reassociation. *Int. J. Syst. Bacteriol.* **30**:208–216.
4. Kurup, P. V., and J. A. Schmitt. 1973. Numerical taxonomy of *Nocardia*. *Can. J. Microbiol.* **19**:1035–1048.
5. Labeda, D. P. 1996. DNA relatedness among verticil-forming *Streptomyces* species (formerly *Streptovorticillium* species). *Int. J. Syst. Bacteriol.* **46**:699–703.
6. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* **3**:208–218.
8. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109–118.
9. Miller, L., and T. Berger. 1985. Bacterial identification by gas chromatography of whole cell fatty acids. Hewlett-Packard application note 228-41. Hewlett-Packard Co., Avondale, Pa.
10. Millonig, G. 1961. Advantages of a phosphate buffer for osmium solutions in fixation. *J. Appl. Physiol.* **32**:1637–1640.
11. Pridham, T. G., and D. Gottlieb. 1948. The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. *J. Bacteriol.* **56**:107–114.
12. Pridham, T. G., and A. J. Lyons. 1980. Methodologies for *Actinomycetales* with special reference to streptomycetes and streptovorticillia, p. 153–224. In A. Dietz and D. W. Thayer (ed.), *Actinomycete taxonomy*. Special Publication no. 6. Society for Industrial Microbiology, Arlington, Va.
13. Seidler, R. J., M. D. Knittel, and C. Brown. 1975. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. *Appl. Microbiol.* **29**:819–825.
14. Seidler, R. J., and M. Mandel. 1971. Quantitative aspects of deoxyribonucleic acid renaturation: base composition, state of chromosome replication, and polynucleotide homologies. *J. Bacteriol.* **106**:608–614.
15. Shirling, E. B., and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **16**:313–340.
16. Stanek, J. L., and G. D. Roberts. 1974. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl. Microbiol.* **28**:226–231.
17. Witt, D., and E. Stackebrandt. 1990. Unification of the genera *Streptovorticillium* and *Streptomyces*, and emendment of *Streptomyces* Waksman and Henrici 1943, 339^{AL}. *Syst. Appl. Microbiol.* **13**:361–371.