Streptomyces cellulolyticus sp. nov., a New Cellulolytic Member of the Genus *Streptomyces*

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Actinomycete strain LX^T (T = type strain), which decomposes cellulose, was identified as a member of the genus *Streptomyces* on the basis of morphological characteristics and the chemotype of the cell wall. The key characteristics of this organism are rectiflexibiles spore chains, a nonfragmenting vegetative mycelium, a warty spore surface, a type I cell wall, white to pink spore masses, and a lack of formation of soluble pigments (including melanin). These results indicate that strain LX^T represents a distinct *Streptomyces* species, for which the name *Streptomyces cellulolyticus* is proposed. The type strain is strain LX, which has been deposited in the China General Microbiological Culture Collection Center as strain AS. 41332.

Cellulose, the major component of plant biomass, is a linear polymer of anhydroglucose units linked by β -1,4-glucoside bonds. Hydrogen binding with and between multiple layers of closely packed cellulose results in the formation of cellulose microfibrils. A variety of fungi and bacteria convert this insoluble substrate into soluble cellooligomers and ultimately to cellobiose and glucose.

Actinomycetes are widely distributed in soil, where they play an important role in the degradation of the lignocellulose of plant cell walls (3, 11). However, lignocellulose degradation in natural substrates is largely attributed to fungi, and the importance of actinomycetes in this process may be underestimated. Thus far, well-known cellulolytic actinomycetes include members of the genera *Cellulomonas* and *Thermomonospora* (2, 4, 7, 9). In the genus *Streptomyces*, cellulose-degrading activity has been found in some strains (6, 10, 17).

In a search of soil samples for cellulolytic bacteria, a streptomycete strain designated LX^T (T = type strain) was obtained, and this strain completely degraded filter paper, α -cellulose, and Avicel in 2 or 3 days. In addition, almost no reducing sugar was detected during incubation of strain LX^T in medium containing cellulose as the sole carbon source. In this paper, I present a morphological and physiological description of this strain. The data show that this isolate should be classified as a member of a new species of the genus *Streptomyces*, and the name *Streptomyces cellulolyticus* sp. nov. is proposed.

MATERIALS AND METHODS

Isolation. Soil samples to isolate organisms were obtained from the campus of Shandong University in Jinan, People's Republic of China. A 1-g (fresh weight) portion of a soil sample was suspended in 2 ml of sterile distilled water, and aliquots of the resulting suspension were inoculated onto cellulose agar plates by directly plating 10-fold serially diluted samples. After incubation for 3 days at 30°C, several different types of colonies were observed on the plates. One colony of a strain that produced a large clearing zone, designated LX^T, was purified and subcultured on slants of cellobiose agar which contained nutrient salts medium supplemented with 0.2% (wt/vol) cellobiose and 0.1% (wt/vol) cellulose (Whatman CF11). Nutrient salts medium contained (per liter) 0.2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.75 g of KNO_3 , 0.5 g of K_2HPO_4 , 0.02 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.04 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 2 g of peptone. Cellulose agar plates were prepared by placing medium containing 4% Whatman CF11 and 1.5% (wt/vol) agar over nutrient salts medium containing 1.5% (wt/vol) agar in plates.

Cultural and morphological characterization. A medium that contained 10 g of yeast extract per liter and 10 g of glucose per liter and was adjusted to pH 7.2

(yeast extract-glucose medium) was used for general cultivation of actinomycete strains. The media used for morphological characterization of strain LX^T were those described by Shirling and Gottlieb (15).

Cultural characteristics of cells in various media were recorded after incubation for 14 days at 30°C by the methods recommended by Shirling and Gottlieb (15). Morphological observations were made with a light microscope (model CH; Olympus Optical Co., Ltd.) by using the method of Shirling and Gottlieb (15). The surfaces of spores and spore chains were examined by scanning electron microscopy with a JEOL model JEM 1200 electron microscope. The samples used for scanning electron microscopy were prepared by the method of Eguchi et al. (5).

Physiological characteristics. Melanoid pigment production was examined on peptone-yeast extract-iron agar (ISP medium 6), on tyrosine agar (ISP medium 7), and in tryptone-yeast extract broth (ISP medium 1) (15).

Carbon utilization was determined on plates containing ISP basal medium 9 (15) to which filter-sterilized carbon sources were added to a final concentration of 1.0%. The plates were incubated at 30°C and read after 14 days.

The ability of isolate LX^T to utilize amino acids, including L-asparagine, L-cysteine, L-threonine, L-glutamic acid, L-valine, L-arginine, and L-histidine (each at a concentration of 0.1% [wt/vol]), was determined in a basal medium containing 0.3% (wt/vol) cellobiose, 0.05% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001% (wt/vol) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% (wt/vol) K_2HPO_4 , and 1.2% (wt/vol) agar. Results were determined after 14 days.

The temperature range for growth was determined in nutrient salts medium containing 0.3% (wt/vol) cellobiose (nutrient salts-cellobiose medium) at temperatures between 5 and 50°C. The minimum pH that allowed growth was determined in nutrient salts-cellobiose medium by the method of Williams et al. (18).

Sodium chloride tolerance was determined at concentrations of 1 to 12% in nutrient salts-cellobiose agar. Antibiotic susceptibility was determined with susceptibility disks (diameter, 8 mm) consisting of filter paper saturated with an antibiotic solution; the antibiotics tested included dimethylchlorotetracycline (500 $\mu\text{g/ml}$), vancomycin (50 $\mu\text{g/ml}$), kanamycin (50 $\mu\text{g/ml}$), rifampin (50 $\mu\text{g/ml}$), aminobenzylpenicillin (50 $\mu\text{g/ml}$), and penicillin G (10 IU/ml). The disks were placed on the surfaces of nutrient salts-cellobiose medium plates seeded with a 2% vegetative inoculum.

Catalase activity was determined by bubble formation in a 3% hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Nitrate reduction, indole production, hydrogen sulfide production, and hydrolysis of gelatin were studied as described by Lanyi (12). Other phenotypic characteristics were determined by the method described by Smibert and Krieg (16). All tests were performed at 30°C.

Cell wall analysis. The chemotypes of cell walls were determined as described previously (1). Whole-cell sugars were determined by the method of Nakagaito et al. (14).

RESULTS

Morphological characteristics. Strain LX^T was an aerobic gram-positive bacterium which decomposed crystalline cellulose. This new isolate grew well on various organic media, but grew only moderately well on yeast extract-malt extract agar and did not grow on sucrose-nitrate agar or peptone-yeast extract-iron agar (Table 1). The reverse sides of colonies were

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TABLE 1. Cultural characteristics of strain LX^T

Medium	Amt of growth	Color of:		Production of soluble pigment
		Aerial mycelium	Vegetative mycelium	
Tryptone-yeast extract broth (ISP medium 1)	Abundant	White	Yellow	None
Yeast extract-malt extract agar (ISP medium 2)	Moderate	White	Yellow	None
Oatmeal agar (ISP medium 3)	Abundant	White, pink	Colorless	None
Inorganic salts-starch agar (ISP medium 4)	Abundant	Pink	Light yellow	None
Glyccrol-asparagine agar (ISP medium 5)	Abundant	Pink	Brown	None
Peptone-yeast extract-iron agar (ISP medium 6)	None			
Tyrosine agar (ISP medium 7)	Abundant	White	Brown	None
Glucose-asparagine agar	Abundant	Pink	Light yellow	None
Yeast extract-glucose agar	Abundant	Light pink	Yellow	None
Czapek's solution agar	Abundant	Pink	Brown	None
Nutrient agar	Abundant	White	Light yellow	None
Sucrose-nitrate agar	None			

yellow on several organic media but were brown on tyrosine agar, glycerol-asparagine agar, and Czapek's solution agar and were colorless on oatmeal agar. The aerial spore mass was white to pink. On all standard agar media, the aerial mycelium of strain LX^T formed straight to flexuous (rectiflexibiles) spore chains which consisted of 20 or more oval spores (2.1 to 2.3 by 2.5 to 2.7 μm) and did not form retinaculiaperti or spirales spore chains. The surfaces of the spores were warty (Fig. 1). The substrate mycelium was composed of long branching filaments that were 0.8 to 1.0 μm in diameter. No fragmentation of the substrate mycelium was observed. No soluble pigments (including melanoid pigments) were formed.

Physiological characteristics. The physiological characteristics of strain LX^T were as follows: oxidase activity and indole production were negative; catalase activity, acid reaction in milk, reduction of nitrate, and production of H₂S were positive; casein, cellulose, starch, and esculin were decomposed; gelatin was not liquefied; and no soluble pigments (including melanin) were formed.

Growth occurred in the presence of up to 10% sodium chloride. The optimum pH for growth was 7.2, but growth occurred at pH 4 to 10. The temperature range at which growth occurred was 10 to 40°C, and optimum growth occurred at 30°C. Strain LX^T used D-glucose, D-fructose, L-arabinose, sucrose, D-xylose, raffinose, and i-inositol, but not L-rhamnose,

as the sole carbon source. Tests for utilization of amino acids showed that asparagine, L-cysteine, and L-threonine served as nitrogen sources. Strain LX^T was resistant to dimethylchlorotetracycline, vancomycin, kanamycin, rifampin, and aminobenzylpenicillin and was susceptible to penicillin G.

Analysis of cell wall hydrolysates of strain LX^T revealed that the cell walls contained LL-diaminopimelic acid and glycine, but no characteristic sugars were found. This information indicates that the cell wall type is type I and that the sugar pattern type is type C (8).

DISCUSSION

The morphological and cultural characteristics, as well as the cell wall and sugar pattern types, provide evidence that strain LX^T should be assigned to the genus *Streptomyces*.

A comparison of isolate LX^T with the previously described *Streptomyces* species (13) showed that only one of the previously described species has rectiflexibiles spore chain morphology and warty spore surfaces; this species, *Streptomyces lomondensis*, produces a melanoid pigment. In addition, *S. lomondensis*, but not isolate LX^T, also forms retinaculiaperti or spirales spore chains. The spore mass of isolate LX^T is white to pink, while the *S. lomondensis* spore mass is blue. Casein is degraded by isolate LX^T, but not by *S. lomondensis*. Isolate LX^T was similar to *Streptomyces hygroscopicus* and *Streptomyces cellulolyticus* in some physiological and cultural test reactions; however, isolate LX^T could be separated from these species on the basis of spore chain form, spore surface type, and spore mass color. I believe that strain LX^T represents a new species of the genus *Streptomyces*. Thus, I propose the name *Streptomyces cellulolyticus* for strain LX^T. A description of this new species is given below.

Description of *Streptomyces cellulolyticus* sp. nov. *Streptomyces cellulolyticus* (cel.lu.lo.ly'ti.cus. M. L. n. *cellulosum*, cellulose; Gr. adj. *lyticus*, dissolving; M. L. adj. *cellulolyticus*, decomposing cellulose).

Spore chains are rectiflexibiles, with 20 or more spores per chain. The spores are oval and are 2.1 to 2.3 by 2.5 to 2.7 μm . The spore surface is warty. Mycelia do not fragment into coccoid or bacillary structures. The branching substrate mycelium is yellow on yeast extract-malt extract agar, inorganic salts-starch agar, glucose-asparagine agar, and nutrient agar; brown on glycerol-asparagine agar, tyrosine agar, and Czapek's solution agar; and colorless on oatmeal agar. The aerial spore mass is white to pink.

Soluble pigments, including melanin, are not produced. The organism is positive for catalase activity and production of H₂S

FIG. 1. Scanning electron micrograph showing the warty surfaces of spores of strain LX^T (= AS. 41332^T). The organism was grown on yeast extract-glucose agar at 30°C for 14 days.

and negative for oxidase activity and indole production. Nitrate is reduced. Starch, casein, and esculin are hydrolyzed. Gelatin is not liquefied. Cellulose is decomposed. D-Glucose, D-fructose, L-arabinose, sucrose, D-xylose, raffinose, and D-inositol are utilized for growth, but L-rhamnose is not utilized. L-Asparagine, L-cystine, and L-threonine can be used as nitrogen sources. Good growth occurs at pH 7.2, and the optimum temperature is 30°C. Growth occurs in the presence of up to 10% NaCl. Isolate LX^T is susceptible to penicillin G, but not to dimethylchlorotetracycline, vancomycin, kanamycin, rifampin, and aminobenzylpenicillin. The cell wall chemotype is chemotype I, and the cell wall contains LL-diaminopimelic acid and glycine; no characteristic sugars are detected as whole-cell sugars.

The type strain is strain LX, which has been deposited in the China General Microbiological Culture Collection Center as strain AS. 41332.

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