Streptomyces lacticiproducens sp. nov., a lactic acid-producing streptomycete isolated from the rhizosphere of tomato plants

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A novel actinomycete, designated strain GIMN4.001^T, was isolated from the rhizosphere of tomato plants grown in Guangzhou, China. The strain produced greyish white aerial mycelia, lactic acid and a large quantity of double diamond-shaped crystals on potato dextrose agar and yeast extract-malt extract agar. The colour of the substrate mycelium was not sensitive to pH. Microscopic observations revealed that strain GIMN4.001^T produced verticillate chains of cylindrical spores. Chemotaxonomic data confirmed that strain GIMN4.001^T belonged to the genus *Streptomyces*. Melanin pigments were not produced. No antibacterial activity was observed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* or *Candida albicans*, but inhibitory activity was observed against *Penicillium citrinum*. 16S rRNA gene sequence analysis revealed that strain GIMN4.001^T was related most closely to *Streptomyces morookaense* ATCC 19166^T (98.9 % similarity) and *Streptomyces lavenduligriseus* ATCC 13306^T (98.7 %). Levels of DNA–DNA relatedness between strain GIMN4.001^T and the type strains of these species were low (14–20 %). Furthermore, strain GIMN4.001^T could be differentiated from *S. morookaense*, *S. lavenduligriseus* and other closely related species of the genus *Streptomyces* based on morphological, physiological and biochemical characteristics. On the basis of its physiological and molecular properties, strain GIMN4.001^T is considered to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces lacticiproducens* sp. nov. is proposed. The type strain is GIMN4.001^T (=CCTCC M208214^T=NRRL B-24800^T).

Members of the genus *Streptomyces* are ubiquitous, can be isolated easily on simple laboratory media, have broad metabolic abilities and can produce antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents. They have thus been utilized in various fields, especially in the food and pharmaceutical industries (Bérdy, 2005). A novel strain of the genus *Streptomyces*, designated GIMN4.001^T, was isolated from the rhizosphere of tomato plants grown in Guangzhou, China, in July 2008, by inoculating samples onto potato dextrose agar (Atlas, 2004) and incubating for 5–7 days at 28 °C. The strain was observed to produce large quantities of double diamond-shaped crystals on potato dextrose agar and yeast extract-malt extract agar (ISP 2).

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GIMN4.001^T is GQ184344.

A supplementary figure is available with the online version of this paper.

International *Streptomyces* Project (ISP) media were prepared according to the methods of Shirling & Gottlieb (1966). Morphological and physiological characteristics were determined as recommended by Williams *et al.* (1989), and morphological observations of spores and mycelia were made by light microscopy (DM RAR; Leica) as well as scanning electron microscopy (Philips FEI-XL30 and Hitachi S-3000N). Physiological tests were performed at 28 °C unless indicated otherwise. Melibiose, glucose, sucrose, D-fructose, xylose, D-galactose, arabinose, inositol and D-mannitol were tested as sole carbon source at concentrations of 0.1 % (w/v) and were filter-sterilized. Colour determination was referenced against Kornerup & Wanscher (1978).

Analysis of the isomer of diaminopimelic acid (DAP) and the whole-cell sugar composition followed the procedure described by Hasegawa *et al.* (1983) with the exception that dried cells were used instead of colonies from agar plates. Fatty acid methyl esters were prepared by the trimethyl sulphonium hydroxide method (Butte, 1983) and were

analysed by GC (6890; Hewlett Packard) by using the Microbial Identification software package (Sasser, 1990). The base composition of the genomic DNA of strain GIMN4.001^T was determined in 0.1 × SSC according to the method of Mandel & Marmur (1968). Genomic DNA was extracted (Cui *et al.*, 2001) and the 16S rRNA gene sequence was amplified by PCR by using universal bacterial 16S rRNA gene primers. The forward primer F27 (5'-AGAGTTTGTATCCTGGCTCAG-3') and reverse primer 1522R (5'-AAGGAGGTGATCCAGCCGCA-3') were adapted from primers pA and pH of Edwards *et al.* (1989). The 16S rRNA gene was sequenced with an automated capillary DNA sequencing system (ABI 3730) and a BigDye Terminator cycle sequencing kit. DNA relatedness studies were performed according to the fluorometric microdilution plate method (Ezaki *et al.*, 1988; Sawabe *et al.*, 1998).

Strain GIMN4.001^T grew well on ISP 2, inorganic salts-starch agar (ISP 4) and potato dextrose agar. It exhibited moderate growth on glycerol-asparagine agar (ISP 5) and glucose-asparagine agar, poor growth on Gause's synthetic agar media (Atlas, 1993), and no growth on oatmeal agar (ISP 3) or starch agar media. Diffusible pigments were not observed on any of the various different media (Table 1). Double diamond-shaped crystals were produced on various agar media (see Supplementary Fig. S1, available in IJSEM Online), but were not observed in liquid media.

Morphological features of strain GIMN4.001^T were observed on ISP 2, ISP 4 and ISP 5 media after incubation for 2 weeks at 28 °C. The novel strain exhibited morphological characteristics typical of the genus *Streptomyces*. Microscopy revealed a branched mycelium with verticils. The aerial mycelium produced verticillate chains of spores that were cylindrical and smooth (see Supplementary Fig. S2).

Chemotaxonomic analysis showed that the cell wall contained LL-DAP, typical of cell wall type I (Lechevalier & Lechevalier, 1970), and whole-cell hydrolysates contained mannose, ribose and galactose. Fatty acid analysis showed that strain GIMN4.001^T contained straight-chain, and iso- and anteiso-branched components: anteiso-C_{15:0} (38.70 %), iso-C_{16:0} (12.55 %), anteiso-C_{17:0} (11.63 %),

iso-C_{15:0} (9.75 %), C_{16:0} (8.54 %), iso-C_{14:0} (4.64 %), iso-C_{17:0} (2.85 %) and cyclo C_{17:0} (2.25 %). The G + C content of the genomic DNA of strain GIMN4.001^T was 70.91 mol%.

The almost-complete 16S rRNA gene sequence was determined for strain GIMN4.001^T. Searches of the GenBank database with this sequence via the BLAST program (Altschul *et al.*, 1997) showed its similarity to many species of the genus *Streptomyces*. Strain GIMN4.001^T showed 98.88 % similarity (over 1408 bases) to the 16S rRNA gene sequence of *Streptomyces morookaense* NBRC 13416^T, 98.67 % similarity (over 1406 bases) to that of *Streptomyces lavenduligriseus* ATCC 13306^T and less than 98 % similarity to those of the type strains of other species of the genus *Streptomyces*.

A phylogenetic tree of 16S rRNA gene sequences of members of the genus *Streptomyces* was constructed by using the neighbour-joining method of Saitou & Nei (1987) with CLUSTAL W (version 1.81; Thompson *et al.*, 1994) and MEGA (version 3.1; Kumar *et al.*, 2001) (Fig. 1). For the neighbour-joining analysis, a distance matrix was calculated according to Kimura's two-parameter correction model. This tree shows the close phylogenetic association between strain GIMN4.001^T and recognized members of the genus *Streptomyces*.

The morphological and physiological characteristics of strain GIMN4.001^T, for example cell-wall type, whole-cell sugar pattern and fatty acid profile, were consistent with those of members of the genus *Streptomyces*. Strain GIMN4.001^T produced lactic acid (determined by GC-MS), a large quantity of double diamond-shaped crystals, greyish brown substrate mycelium and a greyish white spore mass of cylindrical spores in verticillate chains. Melanin was not produced on tyrosine agar (ISP 7). Comparison of the cultural characteristics of strain GIMN4.001^T and its closest phylogenetic neighbours (Table 2), however, revealed significant differences.

S. morookaense was unable to grow on ISP 2, ISP 3, ISP 4 or ISP 5 media and did not produce double diamond-shaped crystals; *S. lavenduligriseus* differed from the novel strain in that melanin pigments were produced on ISP 7. Despite

Table 1. Cultural characteristics of strain GIMN4.001^T on various media

ISP media (Shirling & Gottlieb, 1966) are described in the text. No growth occurred on ISP 3 medium. The colour of mycelium was compared according to Kornerup & Wanscher (1978).

Media	Growth	Sporulation	Colour of:	
			Aerial mycelium	Substrate mycelium
ISP 2	Good	Moderate	Greyish brown	Yellowish white
ISP 4	Good	Moderate	Greyish yellow	Greenish yellow
ISP 5	Moderate	Moderate	Greyish white	Greenish brown
Glucose-asparagine agar	Moderate	Moderate	White	Yellow
Gause's synthetic agar	Poor	Moderate	Yellowish white	Yellowish white
Potato dextrose agar	Good	Grey-white	Greyish white	Greyish yellow

Fig. 1. Unrooted neighbour-joining phylogenetic tree constructed from *Streptomyces* 16S rRNA gene sequences. The 16S rRNA gene sequence of strain GIMN4.001^T was aligned with all sequences for members of the genus *Streptomyces* available in GenBank and a relevant subset of this alignment is shown. *Actinomadura hibisca* JCM 9627^T was used as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) of >50 % are given at nodes. The minimum-evolution and maximum-parsimony methods were also used for tree construction. Branches marked with an asterisk were conserved in all three methods. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cultural characteristics of strain GIMN4.001^T and its closest phylogenetic relatives

Taxa: 1, GIMN4.001^T; 2, *S. morookaense*; 3, *S. lavenduligriseus*. Data for reference species were taken from Hatano *et al.* (2003) and Shirling & Gottlieb (1972). All taxa were negative production of diffusible pigment and for growth on sucrose as the sole carbon source. All taxa had smooth spore surfaces, gave a positive result for milk coagulation and were positive for growth on D-fructose, glucose and inositol as the sole carbon source. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3
Colony colour on ISP 2	Greyish white	Pale yellow–green	Greyish yellow
Spore shape	Cylindrical	Oval or long round	Oval
Spore chain morphology	Verticillate	Verticillate	Rectiflexibles to retinaculiaperti
Milk peptonization	–	–	+
Starch hydrolysis	–	ND	–
H ₂ S production	–	–	+
Melanin	–	–	+
Nitrate reduction	+	–	w
Growth on sole carbon source			
Arabinose	+	–	+
D-Galactose	–	+	ND
D-Mannitol	–	+	+
Melibiose	+	–	+
Xylose	+	–	+

the high level of 16S rRNA gene sequence similarity between strain GIMN4.001^T and the type strains of *S. morookaense* and *S. lavenduligriseus*, they showed different morphological characteristics, cultural characteristics and carbon-utilization patterns (Table 2). Levels of DNA–DNA relatedness between strain GIMN4.001^T and *S. morookaense* ATCC 19166^T and *S. lavenduligriseus* ATCC 13306^T were 14.43 and 20.0 %, respectively, values well below the 70 % threshold proposed by Wayne *et al.* (1987) for species delineation. Thus, DNA–DNA hybridization studies confirmed that strain GIMN4.001^T represented a novel species.

Other species of the genus *Streptomyces* showing >98 % 16S rRNA gene sequence similarity to GIMN4.001^T had significant differences when grown on ISP media. For example, *Streptomyces luteireticuli* differed based on the production of yellowish and greenish yellow pigment on ISP media 2, 3, 4 and 5 (Hatano *et al.*, 2003), and *Streptomyces thioluteus* differed in that aerial mycelium was not produced on these media (Shirling & Gottlieb, 1972).

These results support the classification of strain GIMN4.001^T as representing a novel species of the genus *Streptomyces*, for which the name *Streptomyces lacticiproducens* sp. nov. is proposed.

Description of *Streptomyces lacticiproducens* sp. nov.

Streptomyces lacticiproducens (lac.ti.ci.pro'du.cens. N.L. n. *acidum lacticum* lactic acid; L. part. adj. *producens* producing; N.L. part. adj. *lacticiproducens* producing lactic acid).

Aerobic, Gram-positive, catalase-positive actinomycete that forms a yellowish white aerial mycelium and greyish brown substrate mycelium. Verticillate chains of cylindrical smooth-surfaced spores are produced. Diffusile pigments are not produced. Melanin pigments are not produced on ISP 7. Very good growth occurs on ISP 2, ISP 4 and potato dextrose agar; moderate growth on ISP 5 and glucose-asparagine agar; poor growth on Gause's synthetic agar media; no growth on ISP 3 or starch agar media. The substrate mycelium is greyish brown on ISP 2, greenish yellow on ISP 4 and ISP 5, yellow on glucose-asparagine agar, yellowish white on Gause's synthetic agar and greyish yellow on potato dextrose agar. The cell wall contains LL-DAP (cell wall type I). No inhibitory activity is observed against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 6538, *Bacillus subtilis* ATCC 6633 or *Candida albicans* ATCC 10231, but inhibitory activity is observed against *Penicillium citrinum* AS3.2788. Melibiose, glucose, D-fructose, xylose, inositol and arabinose are utilized as sole carbon source. Negative for production of H₂S.

The type strain, GIMN4.001^T (=CCTCC M208214^T=NRRL B-24800^T), was isolated from the rhizosphere of tomato plants grown in Guangzhou, China. The DNA G + C content of the type strain is 70.91 mol%

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