Leifsonia ginsengi sp. nov., isolated from ginseng root

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A Gram-positive, rod-shaped, non-motile bacterium, designated strain wged11^T, was isolated from the root of ginseng, and its taxonomic position was established using a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences showed that this organism formed a robust clade with recognized species of the genus *Leifsonia*. Strain wged11^T was characterized by a high content of ω -cyclohexylundecanoic and anteiso- and iso-branched saturated fatty acids, MK-11 as the major menaquinone and DL-2,4-diaminobutyric acid in its cell-wall peptidoglycan. The DNA G + C content of strain wged11^T was 66.4 mol%. Levels of similarity between the 16S rRNA gene sequence of strain wged11^T and those of the type strains of other members of the genus *Leifsonia* ranged from 94.7 to 97.6 %. The mean level of DNA–DNA relatedness between strain wged11^T and *Leifsonia poae* DSM 15202^T, its nearest phylogenetic neighbour, was 35.3 %. Based on these findings, strain wged11^T (= CGMCC 4.3491^T = JCM 13908^T) is proposed as the type strain of a novel species of the genus *Leifsonia*, *Leifsonia ginsengi* sp. nov.

The genus *Leifsonia* was proposed by Evtushenko *et al.* (2000) and at the time of writing comprises eight recognized species: *Leifsonia poae* (Evtushenko *et al.*, 2000), *Leifsonia aquatica* (Leifson, 1962), *Leifsonia xyli* (Davis *et al.*, 1984), *Leifsonia naganoensis*, *Leifsonia shinshuensis* and *Leifsonia cynodontis* (Suzuki *et al.*, 1999), and *Leifsonia rubra* and *Leifsonia aurea* (Reddy *et al.*, 2003). Members of the genus *Leifsonia* are characterized by coryneform cell morphology, peptidoglycans based upon 2,4-diaminobutyric acid, MK-11 as the major menaquinone, phosphatidylglycerol and diphosphatidylglycerol as principal phospholipids, a high content of anteiso- and iso-branched saturated fatty acids, and a DNA G + C content in the range 66–73 mol% (Evtushenko *et al.*, 2000). During an investigation on the diversity of endophytic bacteria of ginseng, a novel strain, designated wged11^T, was isolated. Genotypic and phenotypic data indicated that this strain represents a novel species of the genus *Leifsonia*.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain wged11^T is DQ473536.

A table comparing carbon source utilization between strain wged11^T and recognized species of the genus *Leifsonia* is available as supplementary material in IJSEM Online.

Strain wged11^T was isolated on plates of nitrogen-free medium (Von Bulow & Döbereiner, 1975) that had been seeded with a tissue suspension of ginseng roots and incubated at 28 °C for 7 days. Healthy 3-year-old ginseng roots were collected from Fusong county, Jilin Province, China. The root tissue suspension was prepared according to the following procedure. Roots were separated from the soil and washed with tap water, surface-sterilized with 75 % ethanol for 3 min and 2.6 % sodium hypochlorite solution for 3–5 min, and then rinsed with sterile double distilled water (ddH₂O). The surface-sterilized root mass was pulverized in a ceramic mortar and diluted with sterile ddH₂O using the standard dilution plating technique. The new isolate and reference strain *L. poae* DSM 15202^T were maintained on LB medium or corynebacterial (CB) agar (Zgurskaya *et al.*, 1993) slants at 4 °C and as a glycerol suspension (20 %, v/v) at –20 °C. Biomass for chemotaxonomic and molecular systematic studies was prepared by growing the strains in shake flasks of CB medium (l^{–1}: 10 g casein peptone, 5 g yeast extract, 5 g glucose and 5 g NaCl; pH 7.0) at 28 °C for 3–5 days. Cells were harvested by centrifugation, washed with ddH₂O and freeze-dried before use in chemical studies.

Colony properties of the isolates were observed on CB agar. Cell morphology was examined by light microscopy and

transmission electron microscopy (TEM). Presence of flagella was observed by TEM. Cell motility was examined by phase-contrast microscopy of cells from exponentially growing cultures. Acid production from carbohydrates was examined by using the modified method of Hugh & Leifson (1953), as described by Gledhill & Casida (1969). Utilization of a variety of substrates as sole carbon source was tested using a GP2 Microplate (Biolog). The ability of strain wged11^T to grow in the presence of nine antibiotics was examined by placing 6-mm antibiotic discs centrally in plates of CB agar seeded with 200 µl of 3-day-old CB medium culture, and inhibition zones were observed after 3–5 days incubation at 28 °C.

Cell walls were prepared as described by Streshinskaya *et al.* (1979). Analysis of peptidoglycan amino acids was carried out according to the methods given by Schleifer & Kandler (1972) and Bousfield *et al.* (1985). Cell-wall sugars were identified according to the methods described by Maltsev *et al.* (1992). Cellular menaquinones were extracted and purified as described by Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). Non-hydroxylated fatty acids were extracted, purified, methylated, identified and quantified by GC with the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The results were validated by GC-MS given that monoenoic fatty acid was found as a major component in the profile.

A loop of biomass was scraped off the agar plate, suspended in 20 µl ddH₂O and lysed by boiling for 10 min and freezing for 5 min. Following centrifugation, the supernatant was used as the template for PCR with the universal primers 27F and 1492R (Lane, 1991). Automated sequencing was performed by using an ABI Big Dye Primer cycle sequencing ready reaction kit and Applied Biosystems 3730 DNA sequencer.

The program CLUSTAL_X v1.8 (Thompson *et al.*, 1997) was used for multiple alignment of 16S rRNA gene sequences of

type strains of members of the genus *Leifsonia* together with sequences of related species retrieved from GenBank. Three tree-making algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Eck & Dayhoff, 1966; Fitch, 1971) and maximum-likelihood (Felsenstein & Churchill, 1996), from the PHYLIP package v3.6 were used to infer the phylogenetic evolutionary trees. The resulting unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings.

Preparation of genomic DNA was carried out according to the method of Marmur (1961). The G+C content of the DNA of strain wged11^T was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* K-12 as a control. Levels of DNA–DNA relatedness between the new isolate and the reference strains were determined by using the reassociation rate method (Dong *et al.*, 2000).

An almost complete 16S rRNA gene sequence (1485 nt) for strain wged11^T was determined. Although a comparative 16S rRNA gene sequence analysis (BLAST search; <http://www.ncbi.nlm.nih.gov/blast>) revealed strain wged11^T to be related most closely to *Agreia pratensis* DSM 14226^T (98 %, 1267/1293 nt), strain wged11^T fell within the radiation of the cluster of the genus *Leifsonia* in the phylogenetic tree generated with the PHYLIP package (Fig. 1). It formed a separate phyletic line and showed highest 16S rRNA gene sequence similarity to *L. poae* DSM 15202^T (97.6 %). The 16S rRNA gene sequence similarity between strain wged11^T and *A. pratensis* was 96.8 %, and that between strain wged11^T and the type strains of other *Leifsonia* species, except *L. aurea* (94.7 %), was between 96 and 96.5 %. According to the criterion of Ludwig *et al.* (1998) used for genus definition (95 % 16S rRNA gene sequence similarity), strain wged11^T can be classified as representing a member of the genus *Leifsonia*. Its phylogenetic distinctiveness suggested that it represents a taxon separate from recognized *Leifsonia* species.

Fig. 1. Phylogenetic tree showing the relationship of strain wged11^T with other species within the genus *Leifsonia* and adjacent species. The tree is based on an alignment of almost complete 16S rRNA gene sequences and was constructed using the neighbour-joining method (Saitou & Nei, 1987). Asterisks indicate branches that were also recovered using the maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein & Churchill, 1996) algorithms. The consensus phylogenetic tree was produced following bootstrap sampling of 1000 datasets. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 0.02 substitutions per nucleotide position.

Morphological, cultural, physiological and biochemical characteristics of strain wged11^T are given in the species description, and in Table 1 and Supplementary Table S1 (available in IJSEM Online). Amino acid composition (alanine, 2,4-diaminobutyric acid, glutamic acid and glycine) of the cell-wall peptidoglycan, cell-wall sugars (mannose, galactose, glucose, rhamnose) and menaquinone profile (MK-11 as the major component, with a minor amount of MK-10 and a trace amount of MK-12) are in agreement with the description of the genus *Leifsonia*. The major cellular fatty acid profile of strain wged11^T [ω -cyclohexylundecanoic acid (52.0 %), anteiso-C_{15:0} (19.6 %), anteiso-C_{17:0} (10.5 %) and iso-C_{16:0} (8.3 %); data from GC-MS], except the unusual ω -cyclohexylundecanoic acid (identified as C_{18:1} ω 7cis in the MIDI Sherlock system), is consistent with the type strains of recognized members of the genus *Leifsonia*. ω -Cyclohexylundecanoic acid was also found as a major component of the cellular fatty acids in *Curtobacterium pusillum* (Kawaguchi *et al.*, 1986); however, strain wged11^T formed a robust clade with recognized members of the genus *Leifsonia* but not with recognized members of the genus *Curtobacterium* (Fig. 1). At the same time, the mean level of DNA–DNA relatedness (35.3 %) is low enough to distinguish strain wged11^T from the type strain of *L. poae*, its nearest phylogenetic neighbour. In addition, strain wged11^T showed differences in several phenotypic characteristics from recognized species of the genus *Leifsonia* (see Table 1 and Supplementary Table S1 in IJSEM Online). Therefore, on the basis of the data presented, strain wged11^T is considered to represent a novel species of the genus *Leifsonia*, for which the name *Leifsonia ginsengi* sp. nov. is proposed.

Description of *Leifsonia ginsengi* sp. nov.

Leifsonia ginsengi (gin.sen'gi. N.L. gen. n. *ginsengi* of ginseng, the source of the type strain of this species).

Colonies on CB agar are white to yellow, circular, convex, glistening, opaque, butyrous and 1–2 mm in diameter. Cells are Gram-positive, non-spore-forming, aerobic, straight or curved rods (length 1.2–2.5 μ m, width 0.6–0.8 μ m). Flagella are not found by TEM. Cell motility is not detected by phase-contrast microscopy. Cell growth occurs between 4 and 37 °C; optimum growth is at 26–30 °C. Cell growth is observed at 1 % but not at 3 % NaCl. Positive for catalase, hydrolysis of starch and indole production. Production of H₂S is weak. Negative for oxidase, urease, gelatinase and the Voges–Proskauer reaction. Cells are susceptible to tetracycline (10 μ g ml^{−1}), erythromycin (15 μ g ml^{−1}), rifampicin (30 μ g ml^{−1}) and chloramphenicol (10 μ g ml^{−1}), but resistant to ampicillin (10 μ g ml^{−1}), lincomycin (15 μ g ml^{−1}), penicillin (10 U ml^{−1}), streptomycin (10 μ g ml^{−1}) and kanamycin (15 μ g ml^{−1}). Acid is produced from fructose, mannose, glucose, arabinose, sucrose and maltose, but not from ribose. Dextrin (weak), Tween 40 (weak), D-cellobiose, α -D-glucose, maltose, D-mannitol, D-ribose, D-sorbitol, sucrose, turanose, D-xylose, acetic acid (weak), γ -hydroxybutyric acid, 2'-deoxyadenosine, pyruvic acid methyl ester, 2,3-butanediol (weak), D-mannose, pyruvic acid, glycerol, D-fructose, adenosine and thymidine are used as sole carbon sources for energy and growth. Cell-wall peptidoglycan contains alanine, 2,4-diaminobutyric acid, glutamic acid and glycine. Cell-wall sugars are galactose, glucose, mannose and rhamnose. The major menaquinone is MK-11, with a minor amount of MK-10 and a trace amount of MK-12. The fatty

Table 1. Characteristics that differentiate strain wged11^T and *L. poae* from each other and from other recognized species of the genus *Leifsonia*

Taxa: 1, strain wged11^T; 2, *L. poae* DSM 15202^T; 3, *L. rubra* (data from Reddy *et al.*, 2003); 4, *L. aurea* (Reddy *et al.*, 2003); 5, *L. aquatica* (Reddy *et al.*, 2003); 6, *L. xyli* subsp. *cynodontis* (Suzuki *et al.*, 1999); 7, *L. naganoensis* (Suzuki *et al.*, 1999); 8, *L. shinshuensis* (Suzuki *et al.*, 1999). +, Positive or present; w, weakly positive; −, negative or absent; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Yellow	Yellow	Red	Yellow	Yellow	Yellow	White	White
Motility	−	+	−	−	+	−	−	−
Growth temperature (°C)	4–37	4–37	0–22	0–30	7–37	7–35	7–37	7–42
Oxidase, H ₂ S production	−, w	−	−	−	+	−	NR	NR
Starch hydrolysis	+	−	−	−	+	+	+	+
Methyl red test	−	+	−	−	−	+	NR	NR
Voges–Proskauer test	−	−	−	+	−	+	NR	NR
Major menaquinone(s)	MK-11	MK-11	MK-11	MK-11	MK-11, MK-10	MK-11, MK-12	MK-11, MK-10	MK-11, MK-12
Cell-wall sugars*	Gal, Glc, Man, Rha	Gal, Glc, Rib, Rha	Gal, Glc, Rib, Rha	Gal, Glc, Rib	Gal, Glc, Rib, Rha, Fuc	Gal, Rha, Fuc	NR	NR
DNA G + C content (mol%)	66.4	69.5	66	64	70.3	69.4	70.5	70.7

*Fuc, Fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose.

acid profile comprises mainly ω -cyclohexylundecanoic acid (52.0 %), anteiso- $C_{15:0}$ (19.6 %), anteiso- $C_{17:0}$ (10.5 %) and iso- $C_{16:0}$ (8.3 %).

The DNA G + C content of the type strain is 66.4 mol%. The type strain, wged11^T (=CGMCC 4.3491^T=JCM 13908^T), was isolated from a tissue suspension of a surface-sterilized ginseng root.

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