Williamsia phyllosphaerae sp. nov., isolated from the surface of Trifolium repens leaves

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A Gram-positive-staining, non-endospore-forming actinobacterium, designated C7T, was isolated from the leaf surface of Trifolium repens. On the basis of 16S rRNA gene sequence analysis, strain C7T was shown to belong to the genus Williamsia and was most closely related to Williamsia maris SJS0289/JJS1T (98.0 % 16S rRNA gene sequence similarity), Williamsia deligens IMMIB RIV-956T (96.4 %) and Williamsia serinedens IMMIB SR-4T (95.7 %). The quinone system consisted predominantly of the menaquinones MK-9(H2), MK-8(H2) and MK-7(H2). The major components in the polar lipid profile were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol. Mycolic acids were present. These chemotaxonomic traits and the major fatty acids, which were C16:1ω7c, C16:0, C18:0, C18:1ω9c and tuberculostearic acid, supported the affiliation of strain C7T with the genus Williamsia. Physiological and biochemical analysis revealed clear differences between strain C7T and its closest phylogenetic neighbours. Therefore, strain C7T represents a novel species, for which the name Williamsia phyllosphaerae sp. nov. is proposed. The type strain is C7T (=CCUG 60465T=CCM 7855T).

The strain was preserved in nutrient broth (Oxoid) with 20 % (v/v) glycerol at −80 °C or by lyophilization.

Cell morphology was determined by phase-contrast microscopy as described by Kämpfer & Kroppenstedt (2004). Gram-staining was performed as described by Gerhardt et al. (1994).

The 16S rRNA gene sequence of strain C7T (1372 bp) was determined and analysed as described previously (Kämpfer & Kroppenstedt, 2004), using MEGA version 4 (Tamura et al., 2007) as well as ARB (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse et al., 2007). Genetic distances were calculated on the basis of pairwise comparisons and cluster analyses were performed with neighbour joining as well as maximum likelihood with fastDNAm (Olsen et al., 1994) using ARB. Tree reconstruction using the maximum-likelihood method was performed with a 30 % conservation filter (only alignment columns in which the frequency of the most abundant nucleotide is ≥30 % were included) as well as without filters. A maximum-parsimony tree was calculated with MEGA4. No significant differences could be observed between these trees. A neighbour-joining tree (without filters) is shown in Fig. 1. All of the nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Sequence similarity calculations on the basis of pairwise comparison indicated that the closest relatives of strain C7T.
were *W. maris* SJS0289/JSI1<sup>T</sup> (98.0 % 16S rRNA gene sequence similarity), *W. deligens* IMMB RIV-956<sup>T</sup> (96.4 %) and *W. serinedens* IMMB SR-4<sup>T</sup> (95.7 %). All other members of the genus *Williamsia* revealed <95.5 % 16S rRNA gene sequence similarity with strain C7<sup>T</sup>.

For analysis of mycolic acids, quinones and polar lipids, cells were grown in nutrient broth for 48 h at 28 °C in shake flasks at 180 r.p.m. The occurrence of mycolic acids was determined by TLC as described by Minnikin et al. (1975). Menaquinones were extracted and analysed as described by Collins et al. (1979) and Groth et al. (1996). Polar lipids were extracted by the method of Minnikin et al. (1979) and identified by two-dimensional TLC as described by Collins & Jones (1980). Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996).

Mycolic acids were detected in the extracts of strain C7<sup>T</sup>. The quinone system consisted predominantly of MK-9(H₂); minor amounts of MK-8(H₂) and MK-7(H₂) were also detected. The ratio of MK-9(H₂)/MK-8(H₂)/MK-7(H₂) was 81 : 10 : 6. The polar lipid profile was composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and three unknown polar lipids (Fig. 2), which is consistent with the polar lipid profile reported for the genus *Williamsia* (Kämpfer et al., 1999; Stach et al., 2004; Pathom-aree et al., 2006; Yassin & Hupfer, 2006). The fatty acid profile of strain C7<sup>T</sup> was similar to those reported for the genus *Williamsia* (Table 1) and was composed mainly of the fatty acids C<sub>16:1</sub>ω7c, C<sub>16:0</sub>, C<sub>18:0</sub>, C<sub>18:1</sub>ω9c and tuberculostearic acid. The results from the analysis of mycolic acids, quinones, polar lipids and fatty acid were in excellent agreement with the traits listed in the description for the genus *Williamsia* (Kämpfer et al., 1999).

Physiological characters were determined with methods as described previously (Kämpfer et al., 1991) and are given in Table 2 and the species description. Strain C7<sup>T</sup> was clearly different from its closest phylogenetic neighbours: strain C7<sup>T</sup> utilized pyruvate as a sole carbon source but did not utilize D-mannose, maltose, trehalose, D-xylose, azelate, citrate or suberate.

DNA–DNA hybridization was performed between strain C7<sup>T</sup> and *W. maris* DSM 44693<sup>T</sup> and *W. deligens* DSM 44902<sup>T</sup> using the method described by Ziemke et al. (1998), with a minor variation in the nick translation step, where 2 μg DNA was labelled at 15 °C for 3 h. We assumed a DNA G+C content of 65 mol% for strain C7<sup>T</sup>. Strain C7<sup>T</sup> showed relatively low DNA–DNA relatedness with *W. maris* DSM 44693<sup>T</sup> (29.4 %, reciprocal 35.5 %) and *W. deligens* DSM 44902<sup>T</sup> (25.9 %, reciprocal 20.0 %).

On the basis of phylogenetic, chemotaxonomic and phenotypic analysis, we propose that strain C7<sup>T</sup> represents a novel species in the genus *Williamsia*, with the name *Williamsia phyllosphaerae* sp. nov.

**Description of *Williamsia phyllosphaerae* sp. nov.**

*Williamsia phyllosphaerae* (phyl.lo.spha’erae. Gr. neut. n. phyllon leaf; L. fem. n. sphaera ball, sphere; N.L. gen. fem. n. phyllosphaerae of the phyllosphere).
Gram-positive-staining, coccoid–rod-like cells, about 1.0–1.5 μm in diameter. Oxidase- and catalase-positive and showing an aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on TSA, R2A agar and nutrient agar at 25–30 °C. The quinone system consists of MK-9(H2), MK-8(H2) and MK-7(H2). The polar lipid profile is composed of diphosphatidylglycerol, phosphatidyethanolamine, phosphatidylglycerol, phosphatidylinositol and three unknown polar lipids. Mycolic acids are present. The major fatty acids are C16:1ω7c, C16:0, C18:0, C18:1ω9c and tuberculostearic acid. Utilizes D-glucose, D-fructose, sucrose, D-mannitol, D-sorbitol, acetate, propionate, fumarate, pyruvate, 4-hydroxybutyrate and L-malate, but does not utilize N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, p-arbutin, cellubiose, D-galactose, D-glucurionate, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, salicin, trehalose, D-xylene, adonitol, myo-inositol, maltitol, putrescine, cis-acminocitrate, trans-acminocitrate, adipate, 4-amino butyrate, glutarate, itaconate, DL-lactate, mesaconate, oxoglutarate, L-α-amylase, L-asparagine, L-aspartate, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. Hydrolyses aesculin, p-nitrophenyl (NP)-α-D-glucopyranoside, pNP-β-D-glucopyranoside, bis-pNP-phosphate, pNP-phenylphosphonate, pNP-phosphorylcholine and L-alanine-pNA and utilization of D-glucose, D-fructose, sucrose, D-mannitol, D-sorbitol, acetate, propionate, fumarate, 4-hydroxybutyrate and L-malate. All strains are negative for hydrolysis of ascinulin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, bis-pNP-phosphate, pNP-phenylphosphonate, pNP-phosphorylcholine and L-alanine-pNA and utilization of D-glucose, D-fructose, sucrose, D-mannitol, D-sorbitol, acetate, propionate, fumarate, 4-hydroxybutyrate and L-malate. All strains are negative for hydrolysis of oNP-β-D-galactopyranoside, pNP-β-D-glucuronic acid, pNP-β-D-glucopyranoside and L-glutamate-γ-3-carboxy-pNA and utilization of N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, L-arabinose, p-arbutin, cellubiose, D-galactose, D-glucurionate, melibiose, L-rhamnose, D-ribose, salicin, adonitol, myo-inositol, putrescine, trans-acminocitrate, adipate, 4-amino butyrate, glutarate, itaconate, DL-lactate, mesaconate, oxoglutarate, L-α-amylase, L-asparagine, L-aspartate, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

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Table 1. Cellular fatty acid compositions of strain C7T and its closest phylogenetic neighbours

Strains: 1, C7T; 2, W. maris DSM 44693T; 3, W. deligens DSM 44902T.

All data were taken from this study. TBSA, Tuberculostearic acid.

Table 2. Physiological properties of strain C7T and its closest phylogenetic neighbours

Strains: 1, C7T; 2, W. maris DSM 44693T; 3, W. deligens DSM 44902T.

All data were taken from this study. All strains are positive for oxidase- and catalase-positive and showing an aerobic respiratory metabolism. Good growth occurs after 3 days of incubation on TSA, R2A agar and Thuringen and Brandenburg (according to 72 BbgNatSchG).

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**References**


