Williamsia limnetica sp. nov., isolated from a limnetic lake sediment

Anil Sazak and Nevzat Sahin

Correspondence

Nevzat Sahin

nsahin@omu.edu.tr

Department of Biology, Faculty of Art and Science, Ondokuz Mayıs University, 55139 Kurupelit-Samsun, Turkey

An actinomycete, strain L1505^T, was isolated from a limnetic lake sediment and found to have morphological, biochemical, physiological and chemotaxonomic properties consistent with its classification in the genus *Williamsia*. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain L1505^T formed a distinct lineage within the genus *Williamsia*. The isolate belonged to a cluster containing *W. muralis* MA140/96^T, *W. marianensis* MT8^T and *W. faeni* N1350^T, with which the isolate shared 99.0–98.2% 16S rRNA gene sequence similarity.

Genotypic and phenotypic data also indicated that the isolate was different from known members of the genus *Williamsia*. On the basis of these data, strain 1505^T is considered to represent a novel species of the genus *Williamsia*, for which the name *Williamsia limnetica* sp. nov. is proposed (type strain L1505^T=DSM 45521^T=NRRL B-24829^T=KCTC 19981^T).

The genus *Williamsia* was proposed by Kämpfer *et al.* (1999) to accommodate an unusual actinomycete. At the time of writing, the genus *Williamsia* contained six recognized species, isolated from environmental samples [*Williamsia maris* (Stach *et al.*, 2004), *Williamsia marianensis* (Pathomaree *et al.*, 2006), *Williamsia serinedens* (Yassin *et al.*, 2007) and *Williamsia faeni* (Jones *et al.*, 2010)] and clinical materials [*Williamsia muralis* (Kämpfer *et al.*, 1999) and *Williamsia deligens* (Yassin & Hupfer, 2006)]. Members of the genus *Williamsia* form a clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009) and are characterized by a number of chemical markers, including the presence of *meso*-diaminopimelic acid and the sugars arabinose, galactose, mannose and ribose (chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The predominant menaquinone types are MK-9(H₂), MK-8(H₂) (Kämpfer *et al.*, 1999; Stach *et al.*, 2004; Yassin & Hupfer, 2006; Yassin *et al.*, 2007) and MK-7(H₂) and the mycolic acids have carbon chain lengths ranging from C₅₀ to C₅₈. The predominant fatty acids are octadecenoic acid, hexadecanoic acid, hexadecenoic acid and tuberculostearic acid. The polar lipid profile has major amounts of phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol (phospholipid type II; Lechevalier *et al.*, 1977).

An actinomycete, designated L1505^T, was isolated from sediment of Iznik Lake, in the Marmara region in Turkey.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain L1505^T is HQ157192.

Three supplementary figures are available with the online version of this paper.

Sediment samples were collected from the lake using a dredge at a depth of 3 m. Samples were aseptically subsampled and stored at –20 °C until use. Strain L1505^T was recovered from a suspension of the sample inoculated onto starch-casein agar (Küster, 1959) supplemented with (ml^{–1}) filter-sterilized cycloheximide (50 µg), nystatin (50 µg) and rifampicin (0.5 µg). The organism was maintained on glucose-yeast extract malt-extract agar slopes (l^{–1} distilled water: 4 g glucose, 4 g yeast extract, 10 g malt extract; pH 7.2) at room temperature and in 20% (v/v) glycerol at –20 °C.

The colony, micro-morphological and staining characteristics of the isolate were determined using standard procedures, as described by Isik *et al.* (1999). The morphological and cultural characteristics were determined using ISP media 2–7 (Shirling & Gottlieb, 1966), modified Bennett's agar (Jones, 1949), Czapek's agar (Weyland, 1969), glucose-yeast extract-malt extract agar (Shirling & Gottlieb, 1966), nutrient agar (Difco) and trypticase soy agar (Difco). Colour name charts from the National Bureau of Standards (Kelly, 1964) were used to designate mycelial colours. The isolate and the most closely related members of the genus *Williamsia* (*W. muralis* DSM 44343^T, *W. marianensis* DSM 44944^T and *W. faeni* DSM 45372^T) were examined for a range of phenotypic properties using well-established procedures (Gordon *et al.*, 1974; Williams *et al.*, 1983; Isik *et al.*, 1999). Enzyme activities (β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H₂S, urease, tryptophan deaminase, amygdalin and catalase) were examined qualitatively using the API 20 E system (bioMérieux).

Strain L1505^T showed morphological properties consistent with its assignment to the genus *Williamsia*. The isolate formed smooth reddish-orange colonies on glucose-yeast extract malt-extract agar. Cells were rod- and coccoid-like

(Fig. S1, available in IJSEM Online), Gram-staining-positive and non-acid-alcohol-fast. The isolate grew well on most of the media tested, but not on ISP 3 or ISP 4. Physiologically, strain L1505^T clearly differed from the reference strains. The isolate could be differentiated from *W. muralis* DSM 44343^T by assimilation of (+)-D-cellobiose, dextrin, maltose, (+)-D-melezitose, (+)-D-melibiose and xylose, acid production from inositol and sorbitol, degradation of adenine and RNA, and catalase, β -galactosidase (ONPG) and tryptophan deaminase activities. The

physiological properties that differentiated strain L1505^T from the reference strains are shown in Table 1.

Biomass for chemotaxonomic study of strain L1505^T was prepared in glucose-yeast extract-malt extract broth with shaking at 160 r.p.m. for 10 days at 28 °C. Cells were harvested by centrifugation, washed in distilled water, reharvested and freeze dried. Whole-cell hydrolysates were analysed for diaminopimelic acid isomers using TLC (Staneck & Roberts, 1974) and for sugars using the method of Mikami & Ishida (1983). Polar lipids were extracted and analysed by the method of Minnikin *et al.* (1984) as modified by Kroppenstedt & Goodfellow (1991). The predominant isoprenoid quinones were extracted and purified by the method of Tindall (1990a, b) and determined by reversed-phase HPLC (Wu *et al.*, 1989). Mycolic acid composition was determined at the DSMZ (Braunschweig, Germany) by using the standard Microbial Identification System (Microbial ID) for automated GC analysis, as described previously (Klatte *et al.*, 1994). To quantify the whole-cell fatty acids, cells were first grown in 20 ml trypticase soy broth (TSB) with shaking at 150 r.p.m. at 28 °C, then, after 5 days, 5 ml starter culture was inoculated into 50 ml TSB, which was incubated as before for 5 days. After harvesting by cellulose membrane filtration (0.45 μ m), 200 mg wet cells were placed in an extraction tube. Cellular fatty acids were extracted, derivatized to their fatty acid methyl esters and analysed using the Microbial Identification System and a 6890N GC with a G2614A autosampler and a 6783 injector (Agilent Technologies) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Peaks were analysed using TSBA version 5.0. The DNA G+C content was determined following the procedure developed by Gonzalez & Saiz-Jimenez (2005).

The chemotaxonomic properties of the isolate were consistent with its assignment to the genus *Williamsia* (Kämpfer *et al.*, 1999). Whole-cell hydrolysates contained *meso*-diaminopimelic acid as the only diamino acid of the peptidoglycan and arabinose and galactose as the major cell-wall sugars (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). The polar lipid profile was composed of phosphatidylethanolamine, phosphatidylinositol, diphosphatidylglycerol and three unknown polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977) (Fig. S2). The quinone system consisted of MK-8(H₂) (48.7%), MK-9(H₂) (34.6%) and MK-7(H₂) (16.5%) (Fig. S3). The major fatty acids were C_{18:1}9c (30.68%), C_{16:0} (28.86%), C_{16:1}9t (16.98%) and 10-methyl C_{18:0} (14.03%). Small amounts of C_{18:0} (4.68%), C_{14:0} (2.67%) and C_{16:1} (1.01%) were also found. The mycolic acid major-chain lengths ranged from C₅₂ to C₅₈ (12% C₅₂, 2% C₅₃, 31% C₅₄, 4% C₅₅, 38% C₅₆, 2% C₅₇ and 11% C₅₈). The G+C content of the DNA was 65.1 mol%.

Preparation of genomic DNA and amplification of the 16S rRNA gene were carried out following Chun & Goodfellow (1995) and sequencing was performed using an automatic sequencer (3730XL; ABI PRISM). The identification of phylogenetic neighbours and the calculation of pairwise

Table 1. Phenotypic characteristics that differentiate strain L1505^T from its closest phylogenetic neighbours in the genus *Williamsia*

Strains: 1, L1505^T; 2, *W. muralis* DSM 44343^T; 3, *W. marianensis* DSM 44944^T; 4, *W. faeni* DSM 45372^T. +, Positive; $\pm$, weakly positive; –, negative.

Characteristic	1	2	3	4
Degradation of:				
Adenine	+	–	–	–
Casein	–	–	+	–
Gelatin	–	–	–	+
Hypoxanthine	–	–	+	–
RNA	+	–	+	+
Starch	–	–	+	+
Tween 20	–	+	–	–
Uric acid	+	–	+	+
Sole carbon sources (1 %)				
Adonitol	–	+	–	+
(–)-D-Arabinose	–	–	+	+
(–)-D-Cellobiose	+	–	+	+
(–)-D-Sorbitol	–	+	+	+
(+)-D-Galactose	+	–	–	+
(+)-D-Melezitose	+	–	–	–
(+)-D-Melibiose	+	–	+	+
Dextrin	+	–	–	–
Inulin	–	–	–	+
Lactose	–	–	–	+
Maltose	+	–	–	+
<i>myo</i> -Inositol	–	+	–	+
Raffinose	–	–	–	+
Xylitol	–	–	+	+
Xylose	+	–	+	+
Sole nitrogen sources (0.1 %)				
α -Isoleucine	+	–	+	–
D-Phenylalanine	–	–	–	+
L-Methionine	–	–	–	+
L-Phenylalanine	–	–	–	+
L-Serine	+	–	+	+
L-Threonine	–	$\pm$	+	–
Biochemical tests				
Aesculin	+	–	–	+
Allantoin	–	–	$\pm$	+

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the position of strain L1505^T in the genus *Williamsia*. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Asterisks indicate that the corresponding nodes were recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per site.

16S rRNA gene sequence similarities were achieved using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequence of strain L1505^T with those of related taxa retrieved from public databases. Phylogenetic trees were inferred using neighbour-joining (Saitou & Nei, 1987) and maximum parsimony (Fitch, 1971) in MEGA version 3 (Kumar *et al.*, 2004) and maximum likelihood (Felsenstein, 1981) in the PHYLIP suite of programs (Felsenstein, 1993). The evolutionary distance model of Jukes & Cantor (1969) was used to generate evolutionary distance matrices for the neighbour-joining algorithm. The topologies of the resultant trees were evaluated using bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset with the CONSENSE and SEQBOOT options in the PHYLIP package.

An almost-complete (1460 nt) 16S rRNA gene sequence for strain L1505^T was obtained and compared with corresponding sequences from representatives of genera in the suborder *Corynebacterineae*. Strain L1505^T contained signature nucleotides that are characteristic for members of the family *Nocardiaceae* (Stackebrandt *et al.*, 1997), except at positions 139–224 (A:U), 843 (U) and 1189 (U) (*Escherichia coli* numbering system). Furthermore, strain L1505^T contained signature nucleotides that are shared with other members of the genus *Williamsia* [at positions 293–304 (G:C), 307 (C) and 1007–1022 (C:G)] (Yassin & Hupfer, 2006; Yassin *et al.*, 2007). The phylogenetic tree based on the neighbour-joining algorithm showed that strain L1505^T formed a cluster with *W. muralis* MA140/96^T, *W. marianensis* MT8^T and *W. faeni* N1350^T (Fig. 1). Strain L1505^T exhibited 16S rRNA gene sequence similarity of 99.0 % (14 nt differences over 1455 nt), 98.8 % (16 nt differences over 1433 nt) and 98.29 % (24 nt differences over 1406 nt) with *W. muralis* MA140/96^T, *W. marianensis* MT8^T and *W. faeni* N1350^T, respectively.

DNA–DNA relatedness between strain L1505^T and the reference strains was determined by the identification service at the DSMZ. DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) incorporating the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted

6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). DNA–DNA relatedness was calculated as the mean of duplicate determinations. The taxonomic integrity of the isolate was supported by the hybridization study. Strain L1505^T exhibited 18.8 % DNA–DNA relatedness to *W. muralis* DSM 44343^T and *W. marianensis* DSM 44944^T and 22.4 % DNA–DNA relatedness to *W. faeni* DSM 45372^T. These values are clearly well below the 70 % threshold proposed by Wayne *et al.* (1987) for the delineation of separate species.

It is apparent from the genotypic, chemotaxonomic and phenotypic data that strain L1505^T represents a novel species of the genus *Williamsia*. The name *Williamsia limnetica* sp. nov. is proposed.

Description of *Williamsia limnetica* sp. nov.

Williamsia limnetica (lim.ne'ti.ca. N.L. fem. adj. *limnetica* limnetic, isolated from lake sediment, the source of the organism).

Forms smooth, reddish-orange colonies on glucose-yeast extract malt-extract agar at 28 °C after 7 days. Cells are Gram-positive-staining rod- and coccoid-like elements (0.6–1.0 µm wide, 1.4–1.8 µm long) and are non-acid-alcohol-fast. Grows well on ISP 2, ISP 5, ISP 6, ISP 7, modified Bennett's agar, Czapek's agar, glucose-yeast extract-malt extract agar, nutrient agar and trypticase soy agar. Grows at 10–37 °C (optimum 28 °C), but not at 4 or 45 °C, and grows with 0–4 % (w/v) NaCl. Positive for β-galactosidase (ONPG), catalase and urease, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and citrate and H₂S production. Tests for nitrate reduction and aesculin and urea hydrolysis are positive, but those for allantoin and arbutin hydrolysis are negative. Degrades adenine, DNA, RNA and uric acid, but does not degrade casein, chitin, elastin, gelatin, guanine, hypoxanthine, starch, Tweens 20 and 80, xanthine or xylan. As sole sources of carbon for energy and growth, assimilates (–)-D-cellobiose, dextrin, (–)-D-fructose, (+)-D-galactose, maltose, D-mannitol, (+)-D-mannose, (+)-D-melezitose, (+)-D-melibiose, (+)-L-rhamnose, (–)-D-glucose, sucrose and xylose, but does not assimilate adonitol, (–)-D-arabinose, inulin, lactose, (–)-D-salicin, (–)-D-sorbitol, (–)-L-sorbose, L-glutamate, *myo*-inositol, raffinose or xylitol. As sole sources of nitrogen, utilizes

isoleucine, glycine, L-alanine, L-arginine, leucine, L-histidine, L-proline, L-serine and L-valine, but does not utilize D-phenylalanine, L-cysteine, L-hydroxyproline, L-methionine, L-phenylalanine or L-threonine. The quinone system consists of MK-8(H₂), MK-9(H₂) and MK-7(H₂). The major fatty acids are octadecenoic acid (C_{18:1}9c), hexadecanoic acid (C_{16:0}) and tuberculostearic acid (10-methyl C_{18:0}). Mycolic acids with 52–58 carbons are present.

The type strain, L1505^T (=DSM 45521^T=NRRL B-24829^T=KCTC 19981^T), was isolated from limnetic lake sediment of Iznik Lake, Bursa, Turkey. The genomic DNA G+C content of the type strain is 65.1 mol%.

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