Williamsia maris sp. nov., a novel actinomycete isolated from the Sea of Japan

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The taxonomic position of a marine actinomycete, strain SJS0289/JS1^T, was determined using a polyphasic taxonomic approach. The organism, which had phenotypic properties consistent with its classification in the genus *Williamsia*, formed a distinct clade in the 16S rRNA gene tree together with the type strain of *Williamsia muralis*, but was readily distinguished from this species using DNA–DNA relatedness and phenotypic data. The genotypic and phenotypic data show that the organism merits recognition as a new species of *Williamsia*. The name proposed for the new species is *Williamsia maris*; the type strain is SJS0289/JS1^T (=DSM 44693^T=JCM 12070^T=KCTC 9945^T=NCIMB 13945^T).

The genus *Williamsia* was proposed by Kämpfer *et al.* (1999) and belongs to the mycolic-acid-containing group of actinomycetes, that is to the suborder *Corynebacterineae* Stackebrandt *et al.* 1997. This taxon also contains the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania* and *Tsukamurella* together with the genus *Turicella* which lacks mycolic acids (Goodfellow *et al.*, 1998, 1999). Members of these taxa form a distinct phyletic line in the 16S rRNA gene tree and can be distinguished from one another using a combination of phenotypic markers (Goodfellow *et al.*, 1999). The genus *Williamsia* is currently based on a single species, *Williamsia muralis*, which contains an organism that was isolated from indoor building material of a children's day care centre in Finland (Kämpfer *et al.*, 1999).

Members of the suborder *Corynebacterineae* are usually associated with terrestrial habitats, notably soil (Orchard *et al.*, 1977; Wang *et al.*, 1999), though corynebacteria, dietziae, gordoniae, mycobacteria and rhodococci have been recovered from various depths in the Pacific Ocean (Colquhoun *et al.*, 1998a). Analysis of 16S rRNA gene sequences of representative isolates from marine habitats indicates the occurrence of novel taxa. It is important to establish the species richness of such strains in the marine ecosystem in order to establish the functional roles of specific taxa and to choose high-quality biological material for biotechnological purposes (Colquhoun *et al.*, 1998b; Brandão *et al.*, 2001).

The present study was designed to establish the taxonomic status of an actinomycete, strain SJS0289/JS1^T, which was isolated from the Sea of Japan and considered to be closely related to the genus *Gordonia* (Colquhoun *et al.*, 1998a). The organism was the subject of a polyphasic study and found to form a distinct phyletic line that is most closely related to *W. muralis*. It is proposed that the organism be recognized as a new species designated *Williamsia maris*.

Sediment was collected by the unmanned submersible Dolphin 3K from the Sea of Japan at a depth of 289 m in August 1991 (JAMSTEC dive 3K# 121). The sediment sample was stored at -180°C until transfer to the UK for subsequent analysis. Strain SJS0289/JS1^T was isolated from a suspension of the sediment sample on M3 agar (Rowbotham & Cross, 1977) supplemented with cycloheximide and nystatin (each at $100\text{ }\mu\text{g ml}^{-1}$). Typical orange–pink colonies resembling gordoniae were seen after incubation for 10 days at 30°C under atmospheric pressure. Strain SJS0289/JS1^T was tested for purity then maintained on glucose/yeast extract agar (GYEA) plates (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20%, v/v) at -20°C .

Biomass for the chemotaxonomic and molecular systematic studies was prepared by growing the strain in GYE broth in shake flasks for 10 days at 28°C . The biomass for the chemotaxonomic studies was washed in distilled water and freeze-dried; that for the molecular systematics investigations was washed in NaCl/EDTA buffer (0.1 M EDTA pH 8.0, 0.1 M NaCl) and stored at -20°C until needed. Standard HPLC and TLC procedures were used to determine the diagnostic isomers of diaminopimelic acid (A₂pm; Stanek & Roberts, 1974), major whole-organism sugars (Schaal, 1985), predominant isoprenologues (Collins,

Published online ahead of print on 8 August 2003 as DOI 10.1099/ijs.0.02767-0.

The GenBank accession number for the 16S rRNA gene sequence of *Williamsia maris* SJS0289/JS1^T is AB010909.

1994) and muramic acid type (Uchida *et al.*, 1999). The test strain and the type strain of *W. muralis* were examined for their ability to grow on a range of carbohydrates as sole sources of carbon for energy and growth using Stevenson's basal medium (Stevenson, 1967). The colonial, micro-morphological and staining properties of the test strain were determined using standard procedures, as described by Isik *et al.* (1999).

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of strain SJS0289/JS1^T were carried out as described previously (Brandão *et al.*, 2001). The resultant 16S rRNA gene sequence (i.e. 1478 nt) was aligned manually with corresponding sequences of representatives of the genera classified in the suborder *Corynebacterineae* retrieved from the DDBJ/EMBL/GenBank databases using the PHYDIT program (Chun, 1995). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were generated after Jukes & Cantor (1969). The topologies of the resultant trees were evaluated by bootstrap analyses

(Felsenstein, 1985) of the neighbour-joining datasets on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP package. DNA–DNA relatedness studies were performed between strain SJS0289/JS1^T and *W. muralis* DSM 44343^T by the identification service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), as described by Kim *et al.* (1999).

Strain SJS0289/JS1^T showed a range of properties that was consistent with its classification in the genus *Williamsia* (Kämpfer *et al.*, 1999). The organism is an aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete which forms short rods and coccoid-like elements, and small, round, convex, orange colonies on GYEA after 5 days at 28 °C. It contains *meso*-A₂pm and arabinose, galactose, mannose and ribose as major sugars [wall chemotype IV *sensu* Lechevalier & Lechevalier (1970)], *N*-glycolated muramic acid residues, dihydrogenated menaquinone with nine isoprene units as the predominant isoprenologue, major amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol as major polar lipids (phospholipid type II; Lechevalier *et al.*, 1977), and mycolic acids which co-migrate with those from *W. muralis* DSM 44343^T. Comparison of the almost-complete 16S rRNA gene sequence of the test strain

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing relationships between strain SJS0289/JS1^T and representatives of the suborder *Corynebacterineae*. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods. The numbers at the nodes indicate the level of bootstrap support (%) based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 55% are shown. F and P indicate branches that were also recovered using the least-squares and maximum-parsimony methods, respectively. Bar, 0.1 substitutions per nucleotide position.

with those of corresponding sequences of representatives of the suborder *Corynebacterineae* confirmed that it belongs to the genus *Williamsia* (data not shown).

Analysis of the 16S rRNA gene sequence of strain SJS0289/JS1^T for the presence of signature nucleotides revealed that it contained all those expected for the suborder *Corynebacterineae* Stackebrandt *et al.* 1997. Furthermore, in our alignment, both *W. muralis* and strain SJS0289/JS1^T contained five of the 11 signature nucleotides defined for the family *Gordoniaceae* [*W. muralis* was previously reported to contain only one (Kämpfer *et al.*, 1999)]. The isolation of a second *Williamsia* species allows signature nucleotides to be highlighted: the pattern of 16S rRNA gene signatures for the genus *Williamsia* consists of nucleotides at positions 293–304 (G-C), 307 (C), 602–636 (U-G), 603–635 (C-G), 612–628 (U-A), 1124–1149 (G-C) and 1445–1457 (C-G). As the genus contains only two species, each based on a single strain, the pattern of signature nucleotides may need to be updated as new species are added.

It is evident from Fig. 1 that strain SJS0289/JS1^T is most closely related to the type strain of *W. muralis*. This association is supported by the results obtained using all four treeing algorithms and by the very high bootstrap value. The two organisms shared a 16S rRNA gene sequence similarity of 96.6%, a value that corresponds to 45 nt differences out of 1458 locations available for alignment. The two organisms shared a DNA–DNA homology of 23.7%, a value well below the 70% cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). It is clear from Table 1 that the two strains can be separated on the basis of their ability to grow on a range of sole carbon compounds.

It is apparent from the genotypic and phenotypic data that strain SJS0289/JS1^T represents a new species of the genus *Williamsia*, for which the name *Williamsia maris* is proposed.

Description of *Williamsia maris* sp. nov.

Williamsia maris (mar'is. L. gen. n. *maris* of the sea).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete which forms short rods and coccoid-like elements and produces round, convex, orange colonies on glucose/yeast extract agar after 5 days incubation at 28 °C; the colonies assume a pinkish hue after 5–10 days incubation. Grows well on modified Bennett's and tryptic soy agars. Growth occurs between 10 and 37 °C, with an optimum around 28 °C. Uses glycogen, glycerol, *meso*-inositol, (–)-L-rhamnose, (–)-L-sorbose and (+)-D-xylose as sole sources of carbon for energy and growth but not (+)-D-arabitol, (–)-L-arabitol, dextran, dextrin, dulcitol, (+)-D-galactose, inulin, α -lactose, (+)-D-melezitose, α -D-methyl glucopyranoside, (+)-D-raffinose, (–)-D-ribose, (–)-D-salicin, xylan or xylitol. Additional sole carbon source properties are shown in Table 1.

Table 1. Phenotypic properties that distinguish strain SJS0289/JS1^T from *W. muralis* DSM 44343^T

Both strains used (–)-L-fructose, (+)-D-glucose, (–)-D-sorbitol and (+)-D-sucrose but neither used (+)-L-arabinose, (+)-D-cellobiose, (+)-D-maltose, (+)-D-melibiose or salicin. –, Negative; +, positive.

Character	SJS0289/JS1 ^T	DSM 44343 ^T
Utilization of sole carbon sources:		
Adonitol	–	+
(–)-D-Mannitol	+	–
(+)-D-Mannose	–	+
<i>meso</i> -Inositol	–	+
(+)-D-Trehalose	+	–
(+)-D-Xylose	+	–

The type and only strain of the species is SJS0289/JS1^T (=DSM 44693^T=JCM 12070^T=KCTC 9945^T=NCIMB 13945^T). Isolated from sediment collected from the Sea of Japan at a depth of 289 m.

Acknowledgements

This work was supported by the UK Natural Environmental Research Council (grants NER/T/S/2000/00614 and NER/T/S/2000/00616). The authors are indebted to Professor Koki Horikoshi and the Japan Marine Science and Technology Centre, Yokosuka, for providing the deep-sea sediments.

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