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Genome-based reclassification of *Williamsia marianensis* as a later heterotypic synonym of *Williamsia muralis*

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Abstract

The present study used whole-genome data to clarify the taxonomic assignment of two closely related *Williamsia* species. Genomic information for 10 type strains was available at the time of conducting this analysis. One group of type strains was found to be conspecific, namely *Williamsia muralis* Kämpfer *et al*. 1999 and *Williamsia marianensis* Pathom-aree *et al*. 2006. The 16S rRNA gene sequences showed 99% similarity between these type strains. Whole-genome-based comparisons showed that *Williamsia muralis* DSM 44343^T and *Williamsia marianensis* DSM 44944^T shared 98.07% average nucleotide identity, 98.29% average amino acid identity and 84.80% digital DNA–DNA hybridization values. These values exceeded the threshold values for bacterial species delineation. Further, phylogenomic analysis based on the core genomes of the strains under study confirmed that *Williamsia muralis* DSM 44343^T and *Williamsia marianensis* DSM 44944^T formed a monophyletic clade. Based on this evidence, we propose the reclassification of *Williamsia marianensis* Pathom-aree *et al*. 2006 as a later heterotypic synonym of *Williamsia muralis* Kämpfer *et al*. 1999.

Williamsia is a genus within the family *Gordoniaceae* of the class *Actinomycetes* [1]. This group includes actinomycetes with atypical cell morphology and mycolic acids with 50–56 carbon chain lengths. Members of the genus *Williamsia* are Gram-positive, aerobic and can be rod- or coccoid-shaped. They form smooth colonies, which are orange-red pigmented in some species, and can grow at temperatures between 10 and 37°C. Currently, the genus comprises 13 recognized species that have been isolated from human clinical specimens and environmental resources [2]. The most commonly reported sources of *Williamsia* infection include pulmonary infection [3], bacteraemia [4], endophthalmitis [2] and perinatal sepsis [5].

As of September 2023, 13 species of the genus *Williamsia* have been taxonomically characterized and their names are validly published [6]. With the availability of whole-genome sequencing, comparison of taxon-specific, conserved gene contents is a valuable tool for examining taxonomic assignments. In this study, we performed phylogenomic analyses of the 10 available genomes of *Williamsia* species, excluding *Williamsia aurantica*, *Williamsia spongiae* and *Williamsia faeni* for which genome sequences were unavailable.

Analyses based on phylogenomics and overall genome relatedness indices indicated that *Williamsia marianensis* Pathom-aree *et al*. 2006 is a later heterotypic synonym of *Williamsia muralis* Kämpfer *et al*. 1999.

The phylogenetic comparison of strains (*n*=13) was performed based on 16S rRNA gene sequencing using *Corynebacterium diphtheriae* as an outgroup to root the tree. The evolutionary distances were computed using the Jukes–Cantor model [7]. Evolutionary analyses were conducted in MEGA11 (Fig. S1, available in the online Supplementary Material) [8]. The results were comparable when the tree was inferred using the maximum-likelihood approach (Fig. 1) [9]. In the 16S rRNA gene-based phylogenetic tree, *W. marianensis* DSM 44944^T and *W. muralis* DSM 44343^T formed a monophyletic clade with a gene sequence similarity value of 99%. This observation was also supported by phylogenetic trees inferred using Kimura's two-parameter model, the unweighted pair group method with arithmetic mean, the maximum-parsimony method and the

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Abbreviations: AAI, Average Amino Acid Identity; AF, Alignment Fraction; ANIb, BLAST based Average Nucleotide Identity; dDDH, digital DNA-DNA Hybridization; iTOL, Interactive Tree of Life; MAFFT, Multiple Alignment using Fast Fourier Transform; NCBI, National Centre for Biotechnology Information.
Six supplementary figures and three supplementary tables are available with the online version of this article.