**Williamsia herbipolensis** sp. nov., isolated from the phyllosphere of *Arabidopsis thaliana*

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A Gram-stain-positive, non-endospore-forming actinobacterium (ARP¹) was isolated from the phyllosphere of *Arabidopsis thaliana*. On the basis of 16S rRNA gene sequence phylogeny strain ARP¹ was placed into the genus *Williamsia* and the closest related species were *Williamsia phyllosphaerae* (98.5 % 16S rRNA gene sequence similarity), *Williamsia deligens* (98.5 %), *Williamsia maris* (98.3 %) and *Williamsia serinedens* (98.2 %). Genome-based comparison indicated a clear distinction to the type strains of those species with pairwise average nucleotide identities (ANI) between 76.4–78.4 %. The quinone system of strain ARP¹ consisted predominantly of menaquinones MK-9(H₂), MK-7(H₂) and MK-8(H₂), and the polar lipid profile contained the major compound diphosphatidylglycerol, and moderate amounts of phosphatidylethanolamine, phosphatidylglycerol and numerous unidentified lipids. Mycolic acids were present. These chemotaxonomic traits and the major fatty acids, which were C₁₆:₁ω-7c, C₁₆:₀, C₁₈:₀, C₁₈:₁ω-9c and tuberculostearic acid supported the affiliation of strain ARP¹ to the genus *Williamsia*. Genotypic, physiological and biochemical testing revealed clear differences of strain ARP¹ to the most closely related species of the genus *Williamsia*. Therefore strain ARP¹ represents a novel species of this genus, for which the name *Williamsia herbipolensis* sp. nov. is proposed. The type strain is ARP¹ (=DSM 4687²=LMG 28679¹).

The genus *Williamsia* was proposed by Kämpfer et al. (1999) to accommodate an unusual mycolic-acid-containing actinomycete isolated from indoor wall material. At that time the genus was proposed as a new genus of the suborder *Corynebacterineae* distinct to the family *Gordoniaceae*. Based on the phylogenetic analysis performed by Zhi et al. (2009), the genus *Williamsia* was placed together with the genera *Millisia* and *Skermania* between the families *Nocardiaceae* and *Gordoniaceae* but was assigned to the emended family *Nocardiaceae*. At the time of writing, the taxon contains eight species with validly published names, *Williamsia deligens* (Yassin & Hupfer, 2006), *Williamsia faeni* (Jones et al., 2010), *Williamsia maris* (Stach et al., 2004), *Williamsia muralis* (Kämpfer et al., 1999), *Williamsia mariannensis* (Pathom-aree et al., 2006), *Williamsia serinedens* (Yassin et al., 2007), *Williamsia phyllosphaerae* (Kämpfer et al., 2011) and *Williamsia limnetica* (Sazak & Sahin, 2012). The type strains of these species were isolated from very different sources such as human blood, soil, hay meadow, deep sea sediment, indoor building materials and the phyllosphere of plants. All species of this genus belong to the mycolic-acid-containing group of the actinomycetes and are grouped into the order *Corynebacteriales*, which forms a distinct phylogenetic lineage in the 16S rRNA gene tree (Zhi 2009).

Abbreviation: ANI, average nucleotide identity.

The GenBank/EMBL/DDJB accession number of the 16S rRNA gene sequences of strain ARP¹ is KP676047. The Whole Genome Shotgun (WGS) project number of strain ARP¹ in Genbank is BioProject PRJNA272726 with accession number JXYP00000000. The locus tag of the 16S rRNA gene sequence is Ga0077730_144.
sequence similarities to \textit{Williamsia phyllosphaerae} (Williamsia maris) \textit{Arabidopsis thaliana} rRNA gene sequence (locus tag: Ga0077739\_144; 1520 bp) of the genome of strain ARP1$^T$ (Horn et al., 2016). The sequence was identical to the partial gene sequence obtained by PCR amplification and Sanger sequencing (GenBank accession no. KP676047; 1273 nt). Pairwise sequence similarities to next closest related type strains were obtained by EzTaxon analysis (Kim et al., 2012). Detailed phylogenetic analyses were performed in the \textit{ARB} software package release 5.2 (Ludwig et al., 2004). The 16S rRNA gene sequence of strain ARP1$^T$ was aligned in the \textit{SILVA} Incremental Aligner (\textit{SINA}; v1.2.11; Pruesse et al., 2012) and added to the phylogenetic tree of the ‘living tree project’ (LTPs; Yarza et al., 2008) database release LTPs119 (November 2014) using the parsimony quick add marked module in \textit{ARB}. Type strains of all species of the genus \textit{Williamsia} and all species of next closest related genera of the family \textit{Nocardiaceae} (Zhi et al., 2009) were included in phylogenetic analysis. Type strains of species of the genus \textit{Dietzia} (member of the closely related family \textit{Dietziaceae}) were selected as the outgroup. The alignment of selected sequences was corrected manually considering the secondary structure of the 16S rRNA. Phylogenetic trees were calculated based on 16S rRNA gene sequence positions 85 to 1459 [numbering according to the \textit{Escherichia coli} rrnB numbering; Brosius et al. (1978)], which were covered by all sequences included in the analysis. Phylogenetic trees were calculated with three tree-making algorithms: the maximum-likelihood method using \textit{RAxML} version 7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis and PhyML with the HKY85 nucleotide substitution model (Hasegawa et al., 1985); the maximum-parsimony method using \textit{DNAPARS} v 3.6 (Felsenstein, 2005); and the neighbour-joining method using \textit{ARB} neighbour-joining and the Jukes-Cantor correction model (Jukes & Cantor, 1969). All phylogenetic trees, except the PhyML tree, were based on 100 resamplings (bootstrap analysis; Felsenstein, 1985).

Strain ARP1$^T$ shared highest pairwise 16S rRNA gene sequence similarities to \textit{Williamsia phyllosphaerae} C7$^T$ (98.5\%), \textit{Williamsia deligens} IMMB RIV-956$^T$ (98.5\%), \textit{Williamsia maris} SJS0289/JS1$^T$ (98.3\%) and \textit{Williamsia serinedens} IMMB SR-4$^T$ (98.2\%), followed by \textit{Gordonia soli} CC-AB07$^T$ (97.5\%), \textit{Gordonia hankookensis} ON-33$^T$ (97.1\%) and \textit{Williamsia sterculiae} CPCC 203464$^T$ (97.1\%). Sequence similarities of strain ARP1$^T$ to all other type strains of species of the genera \textit{Williamsia} and \textit{Gordonia} were below 97\%. Phylogenetic trees calculated with different treeing methods clearly showed the placement of strain ARP1$^T$ into the monophyletic cluster of the genus \textit{Williamsia} (Fig. 1). In contrast to species of the genus \textit{Williamsia}, species of the genus \textit{Gordonia} formed a monophyletic cluster only in the maximum-likelihood tree generated with PhyML. In the other calculated phylogenetic trees the genus \textit{Gordonia} did not form a clear monophyletic cluster and the type strains of \textit{Gordonia soli} and \textit{Gordonia hankookensis} clustered closer to the \textit{Williamsia} cluster than to other species of the genus \textit{Gordonia}. Strain ARP1$^T$ was placed within the genus \textit{Williamsia} in a sub-cluster together with \textit{Williamsia deligens}, \textit{Williamsia serinedens}, \textit{Williamsia phyllosphaerae} and \textit{Williamsia maris}. The clustering of strain ARP1$^T$ was stable among the phylogenetic trees calculated by different algorithms (Fig. 1) and supported by high bootstrap values in the neighbour-joining tree (data not shown).

Whole genome comparisons were performed for strain ARP1$^T$ and the four closest related type strains of species of the genus \textit{Williamsia}. The genome sequences of \textit{Williamsia deligens} DSM 44902$^T$, \textit{Williamsia maris} DSM 44693$^T$ and

\begin{fig}
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Phylogenetic placement of strain ARP1$^T$ based on 16S rRNA gene sequence analysis. The maximum-parsimony tree was generated in \textit{ARB} including species of the next closest related genera of the family \textit{Nocardiaceae} beside species of the genus \textit{Williamsia}. Type strains of species of the genus \textit{Dietzia} (family \textit{Dietziaceae}) were used as the outgroup. Circles at nodes represent those nodes that were also present in the phylogenetic trees calculated with other treeing methods. Large circles represent those nodes which were also supported by a high bootstrap value (>70\%) in the maximum-likelihood tree generated with \textit{RAxML}. Numbers at nodes represent bootstrap values >70\% based on 100 replications. Numbers in cluster represent the numbers of type strain sequences included in a cluster. Numbers in parentheses represent other sequence accession numbers or locus tags (for strain ARP1$^T$), Bar, 0.10 nucleotide substitutions per nucleotide position.}
\end{fig}
**Williamsia serinedens** DSM 45037\(^{\text{T}}\) were sequenced as part of the Genomic Encyclopedia of type strains, phase I (Kyrpides et al., 2014). The genome sequence of *Williamsia phylosphaerae* C7\(^{\text{T}}\) was sequenced recently (unpublished data). The pairwise average nucleotide identities (ANIs) between the genome of strain ARP1\(^{\text{T}}\) and the genomes of the four reference strains were determined with the algorithm from Goris et al. (2007) using EzGenome (Kim et al., 2012). The ANI for strain ARP1\(^{\text{T}}\) to *Williamsia phylosphaerae* C7\(^{\text{T}}\) was 78.3714 % (reciprocal, 78.3457 %), to *Williamsia deligens* DSM 44902\(^{\text{T}}\) 76.4288 % (reciprocal, 76.4937 %), to *Williamsia maris* DSM 44693\(^{\text{T}}\) 78.2779 % (reciprocal, 78.1541 %) and to *Williamsia serinedens* DSM 45037\(^{\text{T}}\) 76.4417 % (reciprocal, 76.4075 %). All values were clearly below the proposed cut-off value of 95–96 % for the species boundary (Richter & Rosselló-Mora, 2009). The four species of the genus *Williamsia* also shared ANIs between 75.30–90.58 %.

For mycolic acid, quinone and polar lipid analyses of strain ARP1\(^{\text{T}}\), cells were grown in NB for 48 h at 28 °C in shake flasks at 180 r.p.m. Menaquinones and polar lipids were extracted and analysed applying the integrated method described by Tindall (1990a, b) and Altenburger et al. (1996). The occurrence of mycolic acids was determined by TLC as described by Frischmann et al. (2012). Mycolic acids were detected in the extracts of strain ARP1\(^{\text{T}}\) and were composed of significantly longer carbon chain-length than those of a species of the genus *Corynebacterium* which was used as a positive control for the presence of mycolic acids. This result is in line with the carbon chain-length reported for *Williamsia muralis* to contain 50–56 carbons (Kämpfer et al., 1999), whereas species of the genus *Corynebacterium* contain mycolic acids with 22–38 carbons (Collins et al., 1982).

The quinone system consisted of 61 % MK-9(H\(_2\)), 28 % MK-7(H\(_2\)) and 11 % MK-8(H\(_2\)).

![Fig. 2. Two-dimensional TLC of polar lipid extracts from strain ARP1\(^{\text{T}}\), stained with molybdatophosphoric acid. DPG, Diposphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidyglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; GL1–GL12, unidentified glycolipids; AL1, unidentified aminolipid; L1, unidentified polar lipid not containing a phosphate group, an amino group nor a sugar residue.]

The polar lipid profile was composed of the predominant lipid diposphatidylglycerol and moderate to minor amounts of phosphatidylethanolamine, phosphatidyglycerol, phosphatidylinositol, a phosphatidylinositol-mannoside, twelve unidentified glycolipids (GL1–GL12), an unidentified aminolipid (AL1) and one unidentified polar lipid (L1) (Fig. 2). It is worth mentioning here that so far the presence of glycolipids was not reported for any of the established species of the genus *Williamsia*.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain ARP1\(^{\text{T}}\) was similar to those previously reported for species of the genus *Williamsia* (Table 1). It was composed mainly of the fatty acids C\(_{16:1}\)ω7c, C\(_{14:0}\), C\(_{16:0}\), C\(_{18:1}\)ω9c and tuberculostearic acid. Results from analysis of mycolic acids, quinones, polar lipids and fatty acids are in excellent agreement with the traits listed in the genus description (Kämpfer et al., 1999) and hence, support the affiliation of strain ARP1\(^{\text{T}}\) to the genus *Williamsia*.

Results of the physiological characterization are given in Table 2 and the species description, with methods as described previously (Kämpfer et al., 1991). The data showed that strain ARP1\(^{\text{T}}\) was clearly different from the most closely related species of the genus *Williamsia*.

**Table 1.** Cellular fatty acid composition of strain ARP1\(^{\text{T}}\) and the type strains of the most closely related species of the genus *Williamsia*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{14:0})</td>
<td>10.7</td>
<td>0.9</td>
<td>5.7</td>
<td>9.5</td>
<td>6.7</td>
</tr>
<tr>
<td>C(_{15:0})</td>
<td>1.1</td>
<td>–</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C(_{16:1})ω7c</td>
<td>16.2</td>
<td>16.2</td>
<td>7.5</td>
<td>10.8</td>
<td>12.4</td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>–</td>
<td>1.3</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{18:1})ω9c</td>
<td>14.8</td>
<td>25.2</td>
<td>25.9</td>
<td>25.5</td>
<td>21.8</td>
</tr>
<tr>
<td>C(_{17:1})ω8c</td>
<td>–</td>
<td>–</td>
<td>0.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{18:1})ω9c</td>
<td>24.8</td>
<td>24.4</td>
<td>28.2</td>
<td>21.0</td>
<td>24.6</td>
</tr>
<tr>
<td>C(_{18:0})</td>
<td>10.5</td>
<td>2.9</td>
<td>8.1</td>
<td>9.5</td>
<td>11.2</td>
</tr>
<tr>
<td>TBSA*</td>
<td>21.3</td>
<td>28.4</td>
<td>18.3</td>
<td>23.2</td>
<td>22.0</td>
</tr>
</tbody>
</table>

*TBSA, tuberculostearic acid.*
Table 2. Physiological properties of strain ARP1<sup>T</sup> and the type strains of the most closely related species of the genus *Williamsia*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>5</th>
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<tr>
<td>Hydrolysis of:</td>
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<tr>
<td>2-Deoxythymidine-5'-pNP-phosphate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>pNP-phosphate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>pNP-phosphate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>l-Proline-pNA</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<td>Assimilation of:</td>
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<tr>
<td>d-Mannose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Maltose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>(+)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>L-Malate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>cis-Aconitate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Azelate</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Citrate</td>
<td>+</td>
<td>(+)</td>
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<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

On the basis of this polyphasic approach, we showed the assignment of strain ARP1<sup>T</sup> to the genus *Williamsia* and demonstrated the clear distinction of strain ARP1<sup>T</sup> from other species of the genus *Williamsia*. Thus we propose a novel species of the genus *Williamsia* named *Williamsia herbipolensis* sp. nov., with the type strain ARP1<sup>T</sup>.

**Description of Williamsia herbipolensis sp. nov.**

*Williamsia herbipolensis* (her.bi.pol.en’sis N.L. fem. adj. herbipolensis from Herbipolis, the Latin and medieval name of Würzburg, referring to the city where the sample was collected and the type strain isolated).

Coccoid to rod-like cells, about 1.0–1.5 µm in diameter. Gram-staining-positive, oxidase- and catalase-positive, 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 is composed of the major menaquinone MK-9(H<sub>4</sub>), followed by lower amounts of MK-7(H<sub>6</sub>) and MK-8(H<sub>6</sub>). The polar lipid profile contains the predominant lipid diphasphatidylglycerol and moderate to minor amounts of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol and phosphatidylglycerol. Ten unidentified glycolipids (GL1–GL12), one unidentified aminolipid (AL1), and one unidentified polar lipid (P1). Mycolic acids are present. Major fatty acids are C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:0</sub>, C<sub>18:1</sub>, C<sub>18:2</sub>, and C<sub>19:0</sub> and tuberculostearic acid. Utilizes d-glucose, d-fructose, d-mannose, sucrose, trehalose (weakly), d-xylol (weakly), d-mannitol, d-sorbitol, acetate, propionate, azelaic acid, fumarate and d-lactate, but does not utilize N-acetyl-d-glucosamine, N-acetyl-d-glucosamine, l-arabinose, p-arbutin, cellobiose, d-galactose, d-glucuronide, maltose, melibiose, d-rhamnose, d-ribose, salicin, adonitol, l-rhamnose, trans-aconitate, adipate, 4-aminoxytrate, glutarate, itaconate, mesaconate, oxoglutarate, l-α-alanine, β-alanine, l-aspartate, l-leucine, l-ornithine, l-phenylalanine, l-proline, l-serine, l-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

The type strain is ARP1<sup>T</sup> (=DSM 46872<sup>T</sup>=LMG 28679<sup>T</sup>) and was isolated from the phyllosphere of *Arabidopsis thaliana*. The genomic DNA G+C content of the type strain is 68.63 mol% (draft genome data).

**Acknowledgements**

We are grateful to Gundula Will, Maria Sowinski and Katja Grebing for excellent technical assistance and we thank Mitja N. P. Remus-Emsermann for insightful discussions. Many thanks to Professor Hans-Peter Klenk FRSB for providing us the genome sequences of *Williamsia maris* DSM 44693<sup>T</sup>, *Williamsia deligens* DSM 44902<sup>T</sup> and *Williamsia serinedes* DSM 45037<sup>T</sup>, which were sequenced as part of the Genomic Encyclopedia of type strains, phase I.

**References**


