Pseudomonas antimicrobica Attafuah and Bradbury 1990 is a junior synonym of Burkholderia gladioli (Severini 1913) Yabuuchi et al. 1993

Tom Coenye, Monique Gillis and Peter Vandamme

Laboratory of Microbiology, Universiteit Gent, K.-L. Ledeganckstraat 35, B-9000 Gent, Belgium

Comparison of the 16S rDNA sequence of Pseudomonas antimicrobica LMG 18920T with published 16S rDNA sequences from other pseudomonads indicated that Pseudomonas antimicrobica belongs to the genus Burkholderia, with Burkholderia gladioli, Burkholderia glumae and Burkholderia plantarii as its closest neighbours. DNA–DNA hybridizations confirmed that Pseudomonas antimicrobica and Burkholderia gladioli represent the same species. Strain LMG 18920T and other Burkholderia gladioli strains were also indistinguishable by SDS-PAGE of whole-cell proteins and had similar biochemical characteristics. The whole-cell fatty acid composition, however, was different from that of other Burkholderia gladioli strains. It is concluded that Pseudomonas antimicrobica is a later synonym of Burkholderia gladioli. As Burkholderia gladioli is known to cause infections in patients with cystic fibrosis and chronic granulomatous disease, the eventual use of strain LMG 18920T as a biological control agent should be approached with caution.

Keywords: Pseudomonas antimicrobica, Burkholderia gladioli, biocontrol, Burkholderia cepacia, taxonomy

The name Pseudomonas antimicrobica was proposed in 1989 for a micro-organism isolated from the mealy bug (Planococcoides njalensis) (Attafuah & Bradbury, 1989). The absence of arginine dihydrolase, failure to grow at 40 °C and the fact that the organism did not store poly-β-hydroxybutyrate in its cells suggested an affinity with rRNA group III of the genus Pseudomonas, but its phylogenetic position was not determined (Kersters et al., 1996). Pseudomonas antimicrobica LMG 18920T showed antagonism against a wide range of fungi and bacteria, including a number of well-known phytopathogens (Attafuah & Bradbury, 1989); additional testing by Walker et al. (1996) revealed that it was antagonistic to Botrytis cinerea, a plant pathogen that causes grey mould on various host plants, including numerous commercial crops. Tests are under way to determine whether Pseudomonas antimicrobica would be a suitable biological agent for the control of Botrytis cinerea (Walker et al., 1996). We compared the 16S rDNA sequence of Pseudomonas antimicrobica LMG 18920T with the published 16S rDNA sequences of reference strains of other pseudomonads. The high level of similarity to Burkholderia gladioli, Burkholderia glumae, Burkholderia plantarii and members of the Burkholderia cepacia complex prompted the polyphasic taxonomic study described herein.

Pseudomonas antimicrobica strain LMG 18920T (= NCIB 9898T) and Burkholderia reference strains have been described previously (Attafuah & Bradbury, 1989; Vandamme et al., 1997; Coenye et al., 1999a). All strains were grown aerobically on Trypticase soy agar (BBL) and incubated at 28 °C unless otherwise indicated. The 16S rDNA sequence of Pseudomonas antimicrobica LMG 18920T was retrieved from the GenBank database (accession no. AB021384) and compared with published 16S rDNA sequences of other pseudomonads. A phylogenetic tree was constructed with the GeneCompar 2.1 software package (Applied Maths), using the neighbour-joining method (Saitou & Nei, 1987). Approximately 1460 bases were used and all unknown bases were excluded from the calculations.

DNA was prepared as described by Pitcher et al. (1989) and DNA–DNA hybridizations were per-
formed with photobiotin-labelled probes in microplate wells, as described by Ezaki et al. (1989), using an HTS7000 Bio Assay Reader (Perkin-Elmer) for the fluorescence measurements. The hybridization temperature was 50 °C in 50% formamide. For SDS-PAGE of whole-cell proteins, strains were grown on nutrient agar (CM3; Oxoid) supplemented with 0.04% (w/v) KH$_2$PO$_4$ and 0.24% (w/v) Na$_2$HPO$_4$. 12H$_2$O (pH 6.8) and incubated aerobically at 28 °C. The preparation of whole-cell proteins and SDS-PAGE were performed as described previously (Pot et al., 1994). Densitometric analysis, normalization and
interpolation of the protein profiles, as well as numerical analysis using the Pearson product moment correlation coefficient, were performed using the GelCompar 4.2 software package (Applied Maths).

For fatty acid methyl ester analysis, a loopful of well-grown cells was harvested after an incubation period of 24 h at 28 °C; fatty acid methyl esters were then prepared, separated and identified using the Microbial Identification System (Microbial ID) as described previously (Vandamme et al., 1992). Phytopathogenicity was tested as described by Gonzalez & Vidaver (1979). *Burkholderia gladioli* strain LMG 2216ᵀ was used as a positive control. Sterile distilled water was used as a negative control.

The 16S rDNA sequence of *Pseudomonas antimicrobica* strain LMG 18920ᵀ was very similar to that of *Burkholderia gladioli* LMG 2216ᵀ (X67038) (97.8%), *Burkholderia glumae* LMG 2196ᵀ (U96931) (97.9%) and *Burkholderia plantarii* LMG 9035ᵀ (U96933) (98.2%) (Fig. 1). Levels of similarity to members of the *Burkholderia cepacia* complex (between 97.2 and 97.4%) and to other *Burkholderia* species (between 96.5% and 93.2%) were lower. Levels of similarity to representatives of other taxa belonging to the β-Proteobacteria were below 87.2%. These data unambiguously indicate that *Pseudomonas antimicrobica* belongs to the genus *Burkholderia*, with *Burkholderia gladioli*, *Burkholderia glumae* and *Burkholderia plantarii* as its closest neighbours. The DNA–DNA hybridization results revealed that the DNA–DNA binding value between *Pseudomonas antimicrobica* LMG 18920ᵀ and *Burkholderia gladioli* LMG 2216ᵀ was high (96%). The levels of DNA–DNA binding between *Pseudomonas antimicrobica* LMG 18920ᵀ and the type strains of *Burkholderia glumae* (14%) and *Burkholderia plantarii* (19%) were low. These data unambiguously showed that *Pseudomonas antimicrobica* LMG 18920ᵀ and *Burkholderia gladioli* represent the same taxon. Numerical analysis of the protein patterns revealed that the reference strains of *Burkholderia glumae* and *Burkholderia plantarii* each formed a separate cluster (Fig. 2). The protein patterns of the *Burkholderia gladioli* reference strains and strain LMG 18920ᵀ were very similar (Fig. 3), but, upon numerical analysis, two separate clusters of *Burkholderia gladioli* strains (one

### Table 1. Fatty acid composition (%) of the strains studied

<table>
<thead>
<tr>
<th>Strain</th>
<th>14:0</th>
<th>16:0</th>
<th>17:0</th>
<th>16:1 2-OH</th>
<th>16:0 3-OH</th>
<th>18:0</th>
<th>19:0 2-OH</th>
<th>18:1 2-OH</th>
<th>Summed feature: <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas antimicrobica</em> LMG 18920ᵀ</td>
<td>47</td>
<td>204</td>
<td>82</td>
<td>19</td>
<td>15</td>
<td>55</td>
<td>19</td>
<td>66</td>
<td>50</td>
</tr>
<tr>
<td><em>Burkholderia gladioli</em> (4)†</td>
<td>49±0.2</td>
<td>290±1</td>
<td>172±19</td>
<td>15±02</td>
<td>32±03</td>
<td>71±10</td>
<td>100±27</td>
<td>35±07</td>
<td>68±11</td>
</tr>
<tr>
<td><em>Burkholderia glumae</em> (3)‡</td>
<td>47±0.1</td>
<td>270±24</td>
<td>118±07</td>
<td>11±02</td>
<td>30±12</td>
<td>56±04</td>
<td>85±11</td>
<td>35±03</td>
<td>61±06</td>
</tr>
<tr>
<td><em>Burkholderia plantarii</em> (3)‡</td>
<td>45±0.1</td>
<td>336±27</td>
<td>252±01</td>
<td>14±01</td>
<td>63±05</td>
<td>12±01</td>
<td>103±23</td>
<td>44±20</td>
<td>52±08</td>
</tr>
</tbody>
</table>

* Summed feature 3 comprises 14:0 3-OH, 16:1 iso I, an unidentified fatty acid with an equivalent chain-length value of 10.928, or 12:0 ALDE, or any combination of these fatty acids. Summed feature 4 comprises 16:1 α7c or 15 iso 2-OH, or both. Summed feature 7 comprises 18:1 α7c, 18:1 α9t, or 18:1 α12t, or any combination of these fatty acids.

† Data are from Vandamme et al. (1997).
including strain LMG 18920T were formed (Fig. 2). The obvious reason for this subdivision is a high-density protein band in the region between 35 and 43 kDa, which is present in some strains but absent in others, as discussed previously (Coenye et al., 1999a). The type or reference strains of other Burkholderia species occupied separate positions. The following fatty acids were present in strain LMG 18920T: 14:0, 16:0, 17:0 cyclo, 16:1 2-OH, 16:0 3-OH, 18:0, 19:0 cyclo ω8c, 18:1 2-OH, summed feature 3 (comprising 14:0 3-OH, 16:1 iso 1, an unidentified fatty acid with an equivalent chain-length value of 10.928, or 12:0 ALDE, or any combination of these fatty acids), summed feature 4 (comprising 16:1 ω7c or 15 iso 2-OH or both) and summed feature 7 (comprising 18:1 ω7c, 18:1 ω9t, or 18:1 ω12t, or any combination of these fatty acids). Summed feature 3, summed feature 4 and summed feature 7 probably correspond to 14:0 3-OH, 16:1 ω7c and 18:1 ω7c, respectively, as these fatty acids have been reported in Burkholderia strains before (Stead, 1992; Vandamme et al., 1997). The fatty acid profile of LMG 18920T is most similar to those of Burkholderia gladioli, Burkholderia glumae and Burkholderia plantarii. When compared with other Burkholderia gladioli strains, strain LMG 18920T is characterized by lower amounts of 16:0 and 17:0 cyclo and a higher amount of summed feature 7 (Table 1). Strain LMG 18920T caused a soft-rot in onions that was comparable to the rot caused by Burkholderia gladioli LMG 2216T. Comparison of the previously published biochemical characteristics of strain LMG 18920T (Attafuah & Bradbury, 1989) and Burkholderia gladioli (Palleroni, 1984; Gillis et al., 1995; Coenye et al., 1999a) indicated that the phenotypic characteristics of strain LMG 18920T and Burkholderia gladioli were very similar, the only differences being the inability of strain LMG 18920T to utilize adipate, malate and m-tartrate and to accumulate poly-β-hydroxybutyrate, and the inability of Burkholderia gladioli to hydrolyse aesculin. However, care should be taken when comparing these data, as different methodologies have been used in these different studies. Our polyphasic taxonomic study unambiguously demonstrated that Pseudomonas antimonica and Burkholderia gladioli represent the same taxon; we conclude that Pseudomonas antimonica is a junior synonym of Burkholderia gladioli.

Strain LMG 18920T has attracted attention as a potentially useful organism in the biocontrol of Botrytis cinerea, the causative agent of grey mould (Walker et al., 1996). However, several Burkholderia gladioli strains have been involved in infections in compromised human hosts and patients with cystic fibrosis and chronic granulomatous disease are especially prone to infection with this micro-organism (Christenson et al., 1989; Ross et al., 1995; Hoare & Cant, 1996; Khan et al., 1996). In addition, Burkholderia gladioli is a well-known plant pathogen that is traditionally isolated from Gladiolus spp. and Iris sp. (Palleroni, 1984). It is currently impossible to distinguish between those Burkholderia gladioli isolates that are potentially useful for biocontrol purposes and those isolates capable of causing infections in compromised human hosts (Christenson et al., 1989; Coenye et al., 1999a, b); consequently, the eventual use of this organism as a biological control agent should be approached with caution.

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