Kribbella endophytica sp. nov., an endophytic actinobacterium isolated from the surface-sterilized leaf of a native apricot tree

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A new strain of the genus Kribbella, PIP 118T, was isolated from the leaf of an Australian native apricot tree (Pittosporum angustifolium), or Gumbi Gumbi in the indigenous language. This strain is an aerobic actinobacterium consisting of hyphae that fragment into short to elongated rod-like elements. Phylogenetic evaluation based on 16S rRNA gene sequence analysis placed this isolate as a member of the family Nocardioidaceae and most closely related to Kribbella antibiotica YIM 31530T (98.6 %) and Kribbella koreensis LM 161T (98.4 %). Chemotaxonomic data including cell wall components, major menaquinone and major fatty acids confirmed the affiliation of strain PIP 118T to the genus Kribbella. The results of the phylogenetic analysis, including physiological and biochemical studies in combination with DNA–DNA hybridization, allowed the genotypic and phenotypic differentiation of strain PIP 118T and members of the most closely related species with validly published names. The name proposed for the new species is Kribbella endophytica sp. nov. The type strain is PIP 118T (=DSM 23718T=NRRL B-24812T).

The genus Kribbella belongs to the family Nocardioidaceae and was first described by Park et al. (1999). At the time of writing, the genus Kribbella contained 17 species. Their geographical distribution is diverse. Most were isolated from soil: Kribbella flavida, K. sandramycini (Park et al., 1999), K. koreensis (Sohn et al., 2003), K. jejuniensis (Song et al., 2004) and K. ginsengisoli (Cui et al., 2010) from soils in Korea; K. antibiotica (Li et al., 2004), K. alba, K. yunnanensis (Li et al., 2006) and K. amoyensis (Xu et al., 2012) from Chinese soils; K. karoonensis, K. swartbergensis (Kirby et al., 2006) and K. hippodromi (Everest & Meyers, 2008) from soil in South Africa. K. catacumbae and K. sancticallisti (Urzi et al., 2008) were isolated from whitish-grey patina in the Roman catacombs. K. aluminosa was isolated from a medieval alum slate mine (Carlsohn et al., 2007). Two species have been reported as endophytes: K. solani was isolated from scab lesions of potato tuber (Song et al., 2004) and K. lupini was from the surface-sterilized root of Lupinus angustifolius (Trujillo et al., 2006).

During the course of our research on isolating endophytic actinobacteria from crop plants and native trees (Coombs et al., 2004), 2 Department of Medical Biotechnology, School of Medicine, Flinders University, Bedford Park SA 5042, Australia

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During the course of our research on isolating endophytic actinobacteria from crop plants and native trees (Coombs et al., 2004), a Kribbella-like strain PIP 118T was isolated from the leaf of a native apricot tree (Pittosporum angustifolium), a species endemic to Australia. In this paper, we describe the taxonomic position of this strain, including morphological, physiological, chemotaxonomic and phylogenetic characteristics. Strain PIP 118T was isolated from leaf samples of a native apricot tree collected from the grounds of Flinders University, Adelaide, South Australia, and processed within 4 h. The leaf was sterilized with 70 % ethanol and 6 % hypochlorite for 5 min each, rinsed several times with sterile water and then soaked with 10 % NaHCO₃ for 10 min followed by rinsing with sterile water. Leaf tissue was ground by using a pestle and mortar and spread onto manitol mung bean yeast extract mineral salt agar [mannitol 1.0 g, ground mung bean 15.0 g, yeast extract 0.5 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.2 g, CaCl₂·2H₂O 0.1 g, 1 ml trace salt solution (TS), agar 15.0 g, reverse osmosis water 1 l]. TS contained 7.7 M HCl 10 ml, FeCl₂·4H₂O 1.5 g, ZnCl₂ 70 mg, MnCl₂·4H₂O 100 mg, H₂BO₃ 6 mg, CoCl₂·6H₂O 190 mg, CuCl₂·2H₂O 2 mg, NiCl₂·6H₂O 24 mg, Na₂MoO₄·2H₂O 36 mg, per 990 ml water. The pH was adjusted to 7.2. The medium was supplemented with 20 μg nalidixic acid ml⁻¹ and 100 U nystatin to inhibit the growth of some bacteria and fungi, respectively. Plates were kept in plastic sealed boxes, which contained wet paper towels to maintain moisture, and
incubated at 27 °C. Strain PIP 118T emerged from the leaf tissue onto the isolation medium after incubation for 12 weeks.

Extraction of genomic DNA from strain PIP 118T and amplification and sequencing of the 16S rRNA gene were carried out as described previously (Coombs & Franco, 2003). The nearly complete resultant 16S rRNA gene sequence of strain PIP 118T (1421 bp) was analysed using BLAST (Altschul et al., 1997) and subsequently aligned with the 16S rRNA gene sequences of representatives of related genera available from GenBank/EMBL by using CLUSTAL_X (Thompson et al., 1997). The phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) tree-making methods by using the software package MEGA version 4 (Tamura et al., 2007). Pairwise distances for the neighbour-joining algorithm were calculated according to the Kimura two-parameter model (Kimura, 1980) and Min-mini heuristic (factor = 1) was applied in maximum-parsimony analysis. The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

The phylogenetic evaluation showed clearly that strain PIP 118T was a member of the genus Kribbella with the highest 16S rRNA gene sequence similarity value observed with K. antibiotica YIM 31530T (98.6%) followed by K. koreensis LM 161T (98.4%). The affiliation between strain PIP 118T and its closest neighbour, K. antibiotica YIM 31530T, was supported by both neighbour-joining and maximum-parsimony algorithms with bootstrap values of 55 and 38%, respectively (Figs 1 and S1 available in IJSEM Online). Also, strain PIP 118T was in the same cluster as Kribbella antibiotica YIM 31530T. The phylogenetic tree of strain PIP 118T and all species of the genus Kribbella with validly published names is shown in Fig. S2.

The level of DNA–DNA relatedness between strain PIP 118T and its two closest neighbours was determined according to the colorimetric microdilution plate method using biotinylated DNA (Kusunoki et al., 1991). The DNA hybridization rate was measured both by labelling the DNA of PIP 118T and then reciprocally of the type strains. The relatedness was calculated from quadruplicate hybridization experiments and expressed as a mean of the corresponding reciprocal values. The DNA–DNA relatedness between strain PIP 118T and K. antibiotica YIM 31530T was 22.4% and between PIP 118T and K. koreensis LM 161T was 19.1% (both of which are well below the 70% cut-off point for recognition of genomic species (Wayne et al., 1987). The SDSs with labelled PIP 118T DNA were 0.837 ± 0.04, 0.244 ± 0.02 and 0.212 ± 0.03 in quadruplicate wells of PIP118T, K. antibiotica and K. koreensis, respectively. The SDSs with labelled K. antibiotica DNA were 0.674 ± 0.06 and 0.231 ± 0.01 in quadruplicate wells of K. antibiotica and PIP118T, respectively. The SDSs with labelled K. koreensis DNA were 3.584 ± 0.04 and 0.260 ± 0.03 in quadruplicate wells of K. koreensis and PIP 118T, respectively.

The G+C (mol%) content of the DNA of strain PIP 118T was determined by HPLC (Mesbah et al., 1989) to be 67.4 mol%, which is close to those for other species of the genus with validly published names (68–71.3 mol%) (Li et al., 2006).

Polar lipids were extracted according to the protocol of Mininnik et al. (1984). Major lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylglycholine, which correspond to phospholipid type III (Fig. S3; Lechevalier et al., 1977). Diaminopimelic acid (DAP) was detected by TLC (Bousfield et al., 1985) and whole cell sugar was analysed by TLC (Hasegawa et al., 1983). ll-DAP was present and the whole cell sugar contained mannose, glucose, galactose and ribose while the whole cell sugar of the most closely related type strain, Kribbella antibiotica YIM 31530T, contained glucose, xylose and ribose. Extraction and purification of isoprenoid quinones was performed using the method of Collins et al. (1977) with analysis of the samples by reverse phase LC-MS employing UV detection and electrospray ionization mass spectrometry (ESI). The LC solvent system was 2-propanol: methanol (1:1) at a flow rate of 1.0 ml min−1. Strain PIP 118T contained MK-9(H4) (54%) as the predominant menaquinone and small amounts of MK-9(H2) (25.1%) and MK-9(H2) (20.8%). For the analysis of whole-cell fatty acids, strain PIP 118T and both the most

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**Fig. 1.** 16S rRNA gene-based neighbour-joining tree showing the phylogenetic relationships between Kribbella endophytica PIP 118T and selected micro-organisms belonging to the genus Kribbella, with Nocardiooides albus as the outgroup. Sequence length was 1386 bp. Asterisks indicate branches of the tree that were also recovered by using the maximum-parsimony algorithm (Fig. S1). The numbers on the branches indicate the percentage bootstrap values of 1000 replicates. Bar, 0.002 changes per nucleotide.
closely related Kribbella type strains were grown for 10 days at 25 °C in tryptic soya broth (Oxoid) in an Erlenmeyer flask at 150 r.p.m. and harvested by centrifugation. Washed cells (100 mg) were saponified, methylated and extracted and the fatty acid methyl esters (FAMEs) were determined by following the protocols described by Microbial Identification (MIDI) (Sasser, 2001). The Sherlock MIS SITE1 software version 6.0 was used for analysis. The whole-cell fatty acid pattern of strain PIP 118T is of the iso-anteiso-branched type (Table 1). The major cellular fatty acid of this strain is anteiso-C₁₅ : 0 (26.73 %). The pattern of cellular fatty acids for this strain was similar to those of the two closest type strains. However, PIP 118T had higher levels of iso-C₁₄ : 0 and iso-C₁₆ : 0 compared with cells of the type strains. Based on chemotaxonomic data, strain PIP 118T was different from other species of the genus Kribbella with validly published names but was sufficiently similar to allow the classification of this strain into the genus Kribbella.

Morphological characteristics of the isolate were observed on eight different media: ISP 2, ISP 3, ISP 4, ISP 5, ISP 7 (Shirling & Gottlieb, 1966; Atlas, 1993), Bennett’s agar, half-strength potato dextrose agar (HPDA) and nutrient agar (NA) (Atlas, 1993), and are described in Table S1. Mycelium was non-motile, extensively branched and fragmented into rod-shaped elements. Short to long rod spores (0.35 μm diameter and between 2.0 and 3.3 μm in length) were observed (Fig. S4). The strain did not produce diffusible pigments on any of the media used.

Acid production from carbohydrates and decomposition of adenine, hypoxanthine, L-tyrosine, xanithine, uric acid, urea, ascorulin and hippurate were evaluated according to the methods of Gordon et al. (1974). Hydrolysis of casein, starch and gelatin, catalase production, organic acid assimilation and use of phenolic compounds as sole carbon source were analysed as described by Kurup & Schmitt (1973). Growth at different temperatures (4, 15, 27, 37 and 45 °C), NaCl concentrations (1, 3, 5, 10, 15 and 20 %, w/v) and pH between 4 and 10 (in 1 pH unit intervals) was assessed after incubation at 27 °C for 7–14 days on ISP 2 medium (Kurup & Schmitt, 1973).

Physiological properties of PIP 118T and its closest neighbour, K. antibiotica YIM 31530T, were significantly different (Table 2) in terms of acid production from ducitol and sorbitol. K. antibiotica YIM 31530T could decompose hypoxanthine while PIP 118T could not. On the other hand, PIP 118T could hydrolyse hippurate, assimilate citrate and utilize toluene and cyclohexane as sole carbon source but the most closely related type strain could not. Moreover, PIP 118T could not grow at 3 % NaCl and pH 4 while K. antibiotica YIM 31530T could grow under these conditions.

Based on the results of this polyphasic taxonomy study, isolate PIP 118T is proposed as a novel species of the genus Kribbella, named Kribbella endophytica sp. nov.

### Table 1. Whole-cell fatty acid composition (%) of Kribbella endophytica PIP 118T and related Kribbella type strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₈ : 0</td>
<td>0.15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C₁₃ : 0</td>
<td>0.35</td>
<td>0.70</td>
<td>0.31</td>
</tr>
<tr>
<td>anteiso-C₁₃ : 0</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>iso-C₁₄ : 0</td>
<td>14.39</td>
<td>4.1</td>
<td>2.89</td>
</tr>
<tr>
<td>anteiso-C₁₄ : 0</td>
<td>0.26</td>
<td>0.23</td>
<td>0.1</td>
</tr>
<tr>
<td>C₁₄ : 0</td>
<td>0.1</td>
<td>–</td>
<td>0.14</td>
</tr>
<tr>
<td>iso-C₁₅ : 1 G</td>
<td>–</td>
<td>0.12</td>
<td>–</td>
</tr>
<tr>
<td>anteiso-C₁₅ : 1 A</td>
<td>–</td>
<td>0.2</td>
<td>–</td>
</tr>
<tr>
<td>anteiso-C₁₅ : 0 A</td>
<td>0.25</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C₁₅ : 0</td>
<td>10.44</td>
<td>16.32</td>
<td>14.59</td>
</tr>
<tr>
<td>anteiso-C₁₅ : 0</td>
<td>26.73</td>
<td>54.8</td>
<td>30.87</td>
</tr>
<tr>
<td>C₁₅ : 106c</td>
<td>1.12</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>C₁₆ : 1 iso H</td>
<td>1.97</td>
<td>0.64</td>
<td>0.41</td>
</tr>
<tr>
<td>iso-C₁₆ : 0</td>
<td>17.16</td>
<td>3.96</td>
<td>16.17</td>
</tr>
<tr>
<td>C₁₆ : 0</td>
<td>–</td>
<td>–</td>
<td>2.46</td>
</tr>
<tr>
<td>anteiso-C₁₆ : 0</td>
<td>0.2</td>
<td>–</td>
<td>0.42</td>
</tr>
<tr>
<td>anteiso-C₁₇ : 109c</td>
<td>0.18</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>iso-C₁₇ : 0</td>
<td>2.74</td>
<td>4.36</td>
<td>9.32</td>
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<tr>
<td>anteiso-C₁₇ : 0</td>
<td>1.96</td>
<td>1.95</td>
<td>5.3</td>
</tr>
<tr>
<td>C₁₇ : 108c</td>
<td>5.81</td>
<td>1.72</td>
<td>3.87</td>
</tr>
<tr>
<td>C₁₇ : 0 2-OH</td>
<td>2.26</td>
<td>3.89</td>
<td>1.64</td>
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<tr>
<td>C₁₆ : 1 2-OH</td>
<td>1.07</td>
<td>0.33</td>
<td>0.92</td>
</tr>
<tr>
<td>C₁₇ : 0 10-methyl</td>
<td>0.78</td>
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<td>–</td>
</tr>
<tr>
<td>iso-C₁₈ : 0</td>
<td>0.41</td>
<td>–</td>
<td>0.26</td>
</tr>
<tr>
<td>C₁₈ : 109c</td>
<td>0.63</td>
<td>0.11</td>
<td>0.59</td>
</tr>
<tr>
<td>C₁₈ : 107c</td>
<td>–</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>C₁₈ : 0</td>
<td>0.29</td>
<td>0.24</td>
<td>0.29</td>
</tr>
<tr>
<td>C₁₈ : 1 2-OH</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
</tr>
<tr>
<td>C₁₇ : 0 3-OH</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C₁₆ : 106c</td>
<td>1.75</td>
<td>0.98</td>
<td>2.2</td>
</tr>
</tbody>
</table>

### Description of Kribbella endophytica sp. nov.

*Kribbella endophytica* (en.do.phy’t.i.ca. Gr. pref. *endo* within; Gr. n. *phyton* pertaining to the original isolation from plant tissue).

Gram-stain-positive, aerobic, non-acid–alcohol-fast. Catalase-positive. The strain grows at temperatures between 15 and 27 °C. Good growth occurs between pH 5.0 and 10.0 and in the presence of 1 % (w/v) NaCl but not at 3 % (w/v) NaCl. Colony is wrinkled with a shiny surface. Substrate mycelium develops well on most media but aerial mycelium is formed on a few media. Mycelium was extensively branched and fragmented into rods. Chains of rod-shaped spores (0.35 diameter × 2.0–3.0 μm length) were observed. Additionally, the whole-cell fatty acid profile is shown in Table 1 and physiological properties are listed in Table 2. The DNA G+C content of this strain is 67.4 mol%.
**Table 2.** Differential characteristics between *Kribbella endophytica* PIP 118<sup>T</sup> and related species of *Kribbella*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tbody>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ducitol</td>
<td>–</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Adonitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>1,2 Propanediol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippurate</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid assimilation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td></td>
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<tr>
<td>Utilization of:</td>
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</tr>
<tr>
<td>Pyridine</td>
<td>+</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Toluene</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Benzene</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Growth in/at:</td>
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<td></td>
</tr>
<tr>
<td>3% NaCl</td>
<td>–</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>pH 4</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
</tbody>
</table>

The type strain, PIP 118<sup>T</sup> (= DSM 23718<sup>T</sup>=NRRL B-24812<sup>T</sup>), is an endophytic actinobacterium isolated from the leaf of a native apricot tree (*Pittosporum angustifolium*) which grows on the campus of Flinders University, Adelaide, South Australia.

**Acknowledgements**

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**References**


