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# *Pantoea rodasii* sp. nov., *Pantoea rwandensis* sp. nov. and *Pantoea wallisii* sp. nov., three novel *Pantoea* species isolated from *Eucalyptus*

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Footnote: The GenBank/EMBL/DDBJ accession numbers for sequences generated in this study are as follows: JF295053 - JF295058 (16S rRNA gene), JF295013 - JF295022 (*atpD*), JF295023 - JF295032 (*gyrB*), JF295033 - JF295042 (*infB*) and JF295043 - JF295052 (*rpoB*).

**Summary**

Several Gram-negative, facultatively anaerobic bacterial isolates were obtained from *Eucalyptus* seedlings showing symptoms of bacterial blight and dieback in Colombia, Rwanda and South Africa. Partial 16S rRNA gene sequencing, together with partial *gyrB* sequencing, placed the isolates in the genus *Pantoea* and indicated they constituted three novel species. Multilocus sequence analysis (MLSA) based on partial sequences of *gyrB*, *rpoB*, *infB* and *atpD* revealed *P. dispersa*, *P. eucrina* and *P. cypripedii* as their closest phylogenetic relatives. DNA-DNA hybridizations confirmed the classification of the isolates as three novel species and phenotypic tests allowed the differentiation of them from their closest phylogenetic neighbours. The names *Pantoea rodasii* sp. nov. (type strain LMG 26273$^T$ = BD 943$^T$), *Pantoea rwandensis* sp. nov. (type strain LMG 26275$^T$ = BD 944$^T$) and *Pantoea wallisii* sp. nov. (type strain LMG 26277$^T$ = BD 946$^T$) are proposed.

**Introduction**

*Pantoea ananatis* is reported as the causal agent of bacterial blight and dieback of *Eucalyptus* in South Africa. Young leaves present symptoms first, with leaf spots that become water-soaked and eventually form larger necrotic lesions. Trees either fail to survive or become multi-stemmed (Coutinho *et al*., 2002). In the last decade similar symptoms have been observed in nurseries and plantations in Uganda, Argentina and Uruguay. The bacteria isolated from these diseased trees were identified as belonging to three novel *Pantoea* species: *P. vagans*, *P. eucalypti* and *P. deleyi* (Brady *et al*., 2009). It has been suggested that a complex of *Pantoea* species may be responsible for bacterial blight and dieback in Africa and South America (Coutinho *et al*., 2011). The *Pantoea* species, *P. ananatis*, *P. vagans*, *P. eucalypti* and *P. deleyi*, have been isolated from a wide range of *Eucalyptus* species, hybrids and clones which is of concern for the forestry industry.

As part of an on-going isolation campaign in countries of Africa, South America and Asia, *Eucalyptus* seedlings are regularly examined for symptoms of bacterial blight and dieback. Bacterial isolates obtained from the diseased material are identified using a polyphasic approach based on Gram staining, oxidation-fermentation testing, partial 16S rRNA gene- and *gyrB*-sequencing. *P. ananatis* and *P. eucalypti* are regularly isolated in South Africa, while *P. vagans* and *P. dispersa* are more commonly isolated in Colombia and Thailand, respectively (Swart, 2009). In 2006/2007, bacteria were isolated from diseased *Eucalyptus* material in Colombia, Rwanda and South Africa which could not be assigned to any of the recognized *Pantoea* species. *GyrB* gene sequencing placed these isolates in the genus *Pantoea*, and indicated they constituted three novel species (Swart, 2009). In the present study, these isolates were further examined using a polyphasic approach to confirm they constitute three novel *Pantoea* species.

**Strains and DNA extraction**

Bacteria were isolated from diseased *Eucalyptus* material as previously described (Brady *et al*., 2009). Reference strains were obtained from the BCCM/LMG Bacteria Collection (http://www.belspo.be/bccm) and recovered on tryptic soya agar according to the provider's instructions. A list of strains used in this study is available in Supplementary Table A on IJSEM Online. Genomic DNA was extracted using the alkali method (Niemann *et al*., 1997) and stored at – 20 °C.

**16S rRNA gene sequencing**

Almost complete 16S rRNA gene sequences (1346 bp) were determined for two strains from each proposed novel species using the primers and conditions as previously described (Coenye *et al*., 1999). Consensus sequences were aligned using the ClustalW application in

BioEdit v 7.0.9.0 (Hall, 1999) and the overhangs trimmed. Phylogenetic trees were constructed using the maximum parsimony and neighbour joining methods in Mega 5.0 (Tamura *et al*., 2011) and Paup 4.0b10 (Swofford, 2000), respectively. The reliability of the clusters was evaluated by bootstrap analysis with 1000 replicates. As the topology of the resulting phylogenetic trees was similar, only the maximum parsimony tree is shown.

In the 16S rRNA gene maximum parsimony tree (Fig. 1) the novel species form three definite clusters corresponding to the country of isolation, each with 100 % bootstrap support. The isolates from Colombia and Rwanda are more closely related to each other than to the South African isolates and form a clade on a separate branch, while the South African isolates cluster with *P. dispersa* and *P. eucrina*. Several "core" *Pantoea* species form a well-supported clade with the type strain of *P.agglomerans* while the remainder cluster at a lower level. It has been demonstrated previously that *Pantoea* is a polyphyletic genus (Brady *et al*., 2010b), making it increasingly difficult to allocate novel species to this genus based solely on 16S rRNA gene sequencing. Numerous genera within the family *Enterobacteriaceae* are polyphyletic when analysis is based on 16S rRNA gene sequences alone, and whether this gene is an appropriate choice to construct phylogenies of closely related bacterial taxa has been questioned (Naum *et al*., 2008).

The 16S rRNA gene sequence pairwise similarity obtained is greater than 99 % amongst the Colombian isolates, greater than 99.4 % amongst the isolates from Rwanda and greater than 99.8 % between the South African isolates. The Colombian, Rwandan and South Africa isolates displayed more than 97.0 % 16S rRNA gene sequence pairwise similarity amongst each other and to various species of the family *Enterobacteriaceae*. Based on 16S rRNA gene sequencing, the closest phylogenetic relatives of the Colombian and Rwandan isolates

are *P. septica*, *P. eucrina*, *P. dispersa*, *P. cypripedii*, *Erwinia aphidicola*, *Kluyvera intermedia*, *Buttiauxella agrestis* and *Enterobacter ludwigii*. The isolates from South Africa are most closely related to *P. dispersa* and *P. eucrina*.

**Multilocus Sequence Analysis (MLSA)**

MLSA, based on partial sequences of *gyrB, rpoB, infB* and *atpD*, four protein-encoding genes, was performed on all strains belonging to the three novel species as described previously (Brady *et al*., 2008). Consensus sequences were aligned using the ClustalW application in BioEdit v 7.0.9.0 (Hall, 1999) and the overhangs trimmed. The best-fit evolutionary model was selected by Modeltest 3.7 (Posada & Crandall, 1998) and maximum likelihood and neighbour joining trees were constructed in Phyml (Guindon & Gascuel, 2003) and Paup 4.0b10 (Swofford, 2000) respectively, using the parameters determined by Modeltest. Bootstrap analysis with 1000 replicates was performed on each of the trees to gauge the reliability of the clusters. The topology of both trees was similar and therefore only the maximum likelihood tree is shown.

The peptide sequences were also determined for each gene and a concatenated peptide sequence tree was constructed in Phyml using the parameters described previously (Brady *et al*., 2008). In the maximum likelihood tree based on concatenated sequences of the four genes (Fig. 2), the isolates from Colombia, Rwanda and South Africa form three separate clusters (with 100 % bootstrap values) in the strongly-supported clade containing all recognized *Pantoea* species. The same topology was observed in the concatenated peptide sequence tree (data not shown) providing support at the protein level for the delineation of these isolates as three novel *Pantoea* species. The three novel species were found to share 20 of the 23 *atpD* signature nucleotides that can be used to differentiate *Pantoea* species from closely related

*Tatumella* and *Erwinia* species (Brady *et al*., 2010a). The MLSA data therefore placed the isolates in the genus *Pantoea* and suggested that they belong to three novel species. As observed in the 16S rRNA gene phylogenetic tree, the isolates from Colombia and Rwanda are more closely related to each other than to those from South Africa. Based on the MLSA data, the closest phylogenetic relatives of the three novel species are *P. eucrina, P. dispersa* and *P. cypripedii*.

**DNA-DNA hybridizations**

Two isolates were selected from each novel species for DNA-DNA hybridizations. Isolates from Colombia and Rwanda were hybridized amongst each other, and a representative isolate from each proposed species to the type strains of *P. septica*, *P. eucrina*, *P. dispersa*, *P. cypripedii*, *E. aphidicola*, *K. intermedia*, *B. agrestis* and *E. ludwigii*. The isolates from South Africa were also hybridized amongst each other, and a representative isolate to the type strains of *P. dispersa*, *P. eucrina* and *P. cypripedii*. Large scale DNA extraction was performed on the strains using a modified version (Cleenwerck *et al*., 2002) of the method described by Wilson (1987). DNA-DNA hybridizations (four replications) were performed at 45 °C using the microplate method (Ezaki *et al*., 1989) with some modifications (Cleenwerck *et al*., 2002). Reciprocal reactions (A x B and B x A) were performed for each DNA pair from all strains and their variation was within the limits for this method (Goris *et al*., 1998). Isolates of the novel species exhibited more than 98 % DNA-DNA relatedness when hybridized against each other, while less than 44 % DNA-DNA relatedness was observed between the Colombian and Rwandan isolates, and less than 35 % between these isolates and the type strains of the other species. The isolates from South Africa displayed less than 40 % DNA-DNA relatedness to *P. dispersa*, *P. eucrina* and *P. cypripedii*, their closest

phylogenetic relatives. The hybridization results prove that the isolates constitute three novel species and are summarized in Supplementary Table B on IJSEM Online.

**DNA G +C content**

The DNA G + C content of the novel species was measured by HPLC (Mesbah *et al*., 1989) and is as follows: LMG 26273$^T$ and LMG 26274, 53.2 and 53.0 mol %; LMG 26275$^T$ and LMG 26276, 51.9 and 52.0 mol % and LMG 26277$^T$ and LMG 26278, 55.5 and 55.6 mol %. These values fall within the G + C content range of the emended description of *Pantoea* (Brady *et al*., 2010b).

**Phenotypic assays and fatty acid analysis**

API 20E, API 50CHB/E (bioMérieux) and GN2 MicroPlate (Biolog) tests were performed, according to the manufacturer's instructions, on the isolates from Colombia, Rwanda and South Africa. Cell suspensions were prepared from cultures grown on tryptic soya agar at 28 °C for 12 hours. API and Biolog tests were read after 24 and 48 hours of incubation. Data were compared to those previously published for *Pantoea* species (Brady *et al*., 2009, 2010a,b) and generated under the same conditions. The novel species were found to share all phenotypic traits characteristic of *Pantoea* (Brady *et al*., 2010b, Grimont & Grimont, 2005, Mergaert *et al*., 1993). Results are listed in the species descriptions below. The three novel species can be differentiated from each other by their reactions to dulcitol, sucrose, adonitol, fucose, psicose and serine. The most useful characteristics for differentiating the novel species from each other and their closest phylogenetic relatives are listed in Table 1.

The whole-cell fatty acid methyl ester composition was determined for two isolates from each novel species as well as for the type strain of the type species of the genus,

*P. agglomerans* using the Microbial Identification System, Sherlock version 3.10 (MIDI) and the TSBA50 identification library version 5.0 according to the protocol previously published (Mergaert *et al.*, 1993). An Agilent Technologies 6890N gas chromatograph (Santa Clara, CA, USA) was used for separation of the fatty acid methyl esters. Cells were harvested from cultures grown on trypticase soy agar (BBL 11768) for 24 h at 28 °C. The novel species and *P. agglomerans* displayed similar fatty acid compositions, corresponding with those available in literature (Mergaert *et al*., 1993 and 1999). The major fatty acids include $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$, and summed features 2 (iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH) and 3 ($C_{16:1}$ $\omega7c$ and /or iso-$C_{15:0}$ 2-OH). The fatty acid profiles for the novel species are presented in the species descriptions below.

Based on the genotypic and phenotypic data generated in this study, it is clear that the isolates from diseased *Eucalyptus* seedlings in Colombia, Rwanda and South Africa constitute three novel species in the genus *Pantoea*. Therefore we propose to classify them as *Pantoea rodasii* sp. nov. (isolated from Colombia, type strain LMG $26273^T$ = BD $943^T$), *Pantoea rwandensis* sp. nov. (isolated from Rwanda, type strain LMG $26275^T$ = BD $944^T$) and *Pantoea wallisii* sp. nov. (isolated from South Africa, type strain LMG $26277^T$ = BD $946^T$).

**Description of *Pantoea rodasii* sp. nov.**

*Pantoea rodasii* (ro.da'si.i. N.L. masc. gen. n. *rodasii,* of Rodas, named after Carlos Rodas for his contribution to forest pathology in Colombia).

Cells are Gram-negative, short rods (1 x 1.5-3 μm) occurring singly or in pairs, weakly motile and non-spore-forming. Colonies are round, smooth and convex with entire margins on tryptone soya agar and light beige in colour after incubation of 24 h at 28 °C.

Facultatively anaerobic, oxidase negative and catalase positive. Acetoin and β-galactosidase are produced, but $H_2S$, urease and indole are not and citrate is not utilized. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are all negative. Acid is produced from the fermentation of glycerol, L-arabinose, D-ribose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, *N*-acetylglucosamine, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-melibiose, sucrose, D-trehalose, gentiobiose, D-fucose and D-arabitol (API 50CHB/E). The following carbon sources are utilized at 28 °C by the majority of strains tested including the type strain, after 24 h incubation: Tweens 40 and 80, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, erythritol, D-fructose, D-galactose, gentiobiose, D-glucose, inositol, D-lactose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D,L-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, glucuronamide, D-alanine, L-alanine, L-aspargine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-proline, D-serine, L-serine, urocanic acid, inosine, uridine, thymidine, glycerol, D,L,α-glycerol phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate (Biolog). Strains display the following fatty acid profile: $C_{12:0}$ (4.5 %), $C_{14:0}$ (6.7 %), $C_{16:0}$ (27.4 %), $C_{17:0}$ cyclo (8.8 %), $C_{18:1}\omega7c$ (11.0 %), summed feature 2 (iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH) (13.9 %) and summed feature 3 ($C_{16:1}$ ω7*c* and /or iso-$C_{15:0}$ 2-OH) (23.8 %). The DNA G + C content of the type strain is 53.2 mol %. The type strain is LMG 26273$^T$ (= BD 943$^T$ = BCC 581$^T$). Strains belonging to this species were isolated from lesions on *Eucalyptus* leaves exhibiting symptoms of bacterial blight and dieback in Colombia.

**Description of *Pantoea rwandensis* sp. nov.**

*Pantoea rwandensis* (rwan.den'sis. N.L. fem. adj. *rwandensis*, of or belonging to Rwanda, referring to the country of isolation).

Cells are Gram-negative, short rods (1 x 2-3 μm) occurring singly or in pairs, non-motile and non-spore-forming. Colonies are round, smooth and convex with entire margins on tryptone soya agar and beige in colour after incubation of 24 h at 28 °C. Facultatively anaerobic, oxidase negative and catalase positive. β-galactosidase is produced, but $H_2S$, urease and indole are not and citrate is not utilized. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are all negative. Acid is produced from the fermentation of glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, *N*-acetylglucosamine, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-melibiose, D-trehalose, gentiobiose, D-fucose, L-fucose and D-arabitol (API 50CHB/E). The following carbon sources are utilized at 28 °C by the majority of strains tested including the type strain, after 24 h incubation: tweens 40 and 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-cellobiose, erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, inositol, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, L-rhamnose, D-trehalose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D,L-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, glucuronamide, L-alanine, L-alanyl-glycine, L-aspargine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-proline, D-serine, L-serine, inosine, uridine, thymidine, glycerol, D,L,α-glycerol phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate (Biolog). Strains display the following fatty acid profile: $C_{12:0}$ (4.2 %), $C_{14:0}$ (6.9 %), $C_{16:0}$ (26.1 %),

$C_{17:0}$ cyclo (7.1 %), $C_{18:1}\omega7c$ (11.8 %), summed feature 2 (iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH) (14.3 %) and summed feature 3 ($C_{16:1}$ $\omega7c$ and /or iso-$C_{15:0}$ 2-OH) (26.5 %). The DNA G + C content of the type strain is 51.2 mol %. The type strain is LMG 26275$^T$ (= BD 944$^T$ = BCC 571$^T$). Strains belonging to this species were isolated from lesions on *Eucalyptus* leaves exhibiting symptoms of bacterial blight and dieback in Rwanda.

**Description of *Pantoea wallisii* sp. nov.**

*Pantoea wallisii* (wal.li'si.i. N.L. masc. gen. n. *wallisii,* of Wallis, named after F.M. Wallis for his contribution to the field of phytobacteriology in South Africa).

Cells are Gram-negative, short rods (1 x 1-2.5 μm) occurring singly or in pairs, motile and non-spore-forming. Colonies are round, smooth and convex with entire margins on tryptone soya agar and pale yellow in colour after incubation of 24 h at 28 °C. Facultatively anaerobic, oxidase negative and catalase positive. β-galactosidase is produced, but $H_2S$, urease and indole are not and citrate is utilized. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are all negative. Acid is produced from the fermentation of glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, *N*-acetylglucosamine, arbutin, salicin, D-cellobiose, D-maltose, D-melibiose, D-trehalose, gentiobiose, D-lyxose, D-fucose, L-fucose and D-arabitol (API 50CHB/E). The following carbon sources are utilized at 28 °C by the majority of strains tested including the type strain, after 24 h incubation: dextrin, tweens 40 and 80, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, inositol, maltose, D-mannitol, D-mannose, β-methyl-D-glucoside, L-rhamnose, D-trehalose, pyruvic acid methyl ester, succinic acid mono-methyl ester, acetic acid, cis-aconotic acid, citric acid, formic acid, D-

galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D,L-lactic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, glucuronamide, D-alanine, L-alanine, L-alanyl-glycine, L-aspargine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-serine, inosine, uridine, thymidine, glycerol, D,L,α-glycerol phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate (Biolog). Strains display the following fatty acid profile: $C_{12:0}$ (5.8 %), $C_{14:0}$ (3.5 %), $C_{16:0}$ (25.6 %), $C_{17:0}$ cyclo (7.9 %), $C_{18:1}\omega7c$ (18.8 %), summed feature 2 (iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH) (15.3 %) and summed feature 3 ($C_{16:1}$ ω7*c* and /or iso-$C_{15:0}$ 2-OH) (16.3 %). The DNA G + C content of the type strain is 55.5 mol %. The type strain is LMG 26277$^T$ (= BD 946$^T$ = BCC 682$^T$). Strains belonging to this species were isolated from lesions on *Eucalyptus* leaves exhibiting symptoms of bacterial blight and dieback in South Africa.

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**Table 1:** Phenotypic characteristics differentiating the novel *Pantoea* species, *P. rodasii* sp. nov., *P. rwandensis* sp. nov. and *P. wallisii* sp. nov., from each other, from *P. agglomerans* (the type species of the genus), from other *Pantoea* species associated with bacterial blight and dieback of *Eucalyptus* (*P. ananatis*, *P. deleyi*, *P. eucalypti* and *P. vagans*) and from their closest phylogenetic neighbours (based on 16S rRNA gene sequences and MLSA data using partial sequences of *gyrB*, *rpoB*, *infB* and *atpD*).

1 = *Pantoea rodasii* (*n*=4), 2 = *Pantoea rwandensis* (*n*=4), 3 = *Pantoea wallisii* (*n*=5), 4 = *Pantoea agglomerans*, 5 = *Pantoea ananatis*, 6 = *Pantoea deleyi*, 7 = *Pantoea dispersa*, 8 = *Pantoea eucalypti*, 9 = *Pantoea eucrina*, 10 = *Pantoea septica*, 11 = *Pantoea vagans*, 12 = *Pantoea cypripedii*, 13 = *Pantoea gaviniae*, 14 = *Pantoea calida* (*n*=number of strains tested)

+, 90-100 % strains positive in 1-2 days; (+), 90-100 % strains positive in 1-4 days; -, negative; d, 11-89 % strains positive in 1-4 days; (d), 11-89 % strains positive in 3-4 days; ND, not determined

Data were taken from the following sources: taxa 1, 2, 3 (this study), taxa 4-12 (Brady *et al*., 2009, 2010a,b; Grimont & Grimont, 2005), taxa 13, 14 (Popp *et al*., 2010). Only data generated under the same conditions are listed.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acid from: | | | | | | | | | | | | | | |
| glycerol | + | + | + | (d) | + | + | (d) | d | - | d | d | + | + | + |
| dulcitol | - | + | + | - | - | - | - | - | - | - | - | - | - | - |
| D-lactose | - | - | - | d | + | - | + | (+) | d | d | (+) | - | + | + |
| sucrose | + | - | - | + | + | + | + | + | + | d | + | + | + | ND |
| D-raffinose | - | - | - | (d) | + | - | - | - | - | - | - | - | + | ND |
| Utilization of: | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| adonitol | + | - | - | - | - | - | - | - | + | - | - | - | - | - |
| i-erythritol | + | + | - | - | - | - | + | - | d | - | - | - | - | - |
| L-fucose | - | + | + | - | - | - | - | - | - | d | - | - | - | - |
| lactose | d | - | - | - | + | - | - | + | - | d | - | + | + | + |
| lactulose | - | - | - | - | + | - | - | - | - | d | - | - | + | + |
| D-melibiose | d | + | - | - | + | - | - | - | - | d | - | + | + | + |
| D-psicose | + | + | - | + | + | + | (d) | (d) | ND | ND | - | + | ND | ND |
| sucrose | + | - | - | (+) | + | + | + | + | + | d | + | + | + | + |
| xylitol | - | - | - | - | - | - | - | - | + | d | - | - | - | - |
| quinic acid | + | + | d | - | (+) | - | - | - | - | - | - | + | - | - |
| D-serine | d | d | - | - | - | - | - | - | ND | ND | - | (+) | ND | ND |

**Supplementary Table A.** Strains used in this study

LMG = BCCM/LMG Bacteria Collection, Ghent University, Belgium, R = Research Collection, BCCM/LMG Bacteria Collection, Ghent University, Belgium, BD = Plant Pathogenic and Plant Protecting Bacteria (PPPPB) Culture Collection, ARC-PPRI, Pretoria, South Africa, BCC = Bacterial Culture Collection, Forestry and Agricultural Biotechnology Institute, Pretoria, South Africa

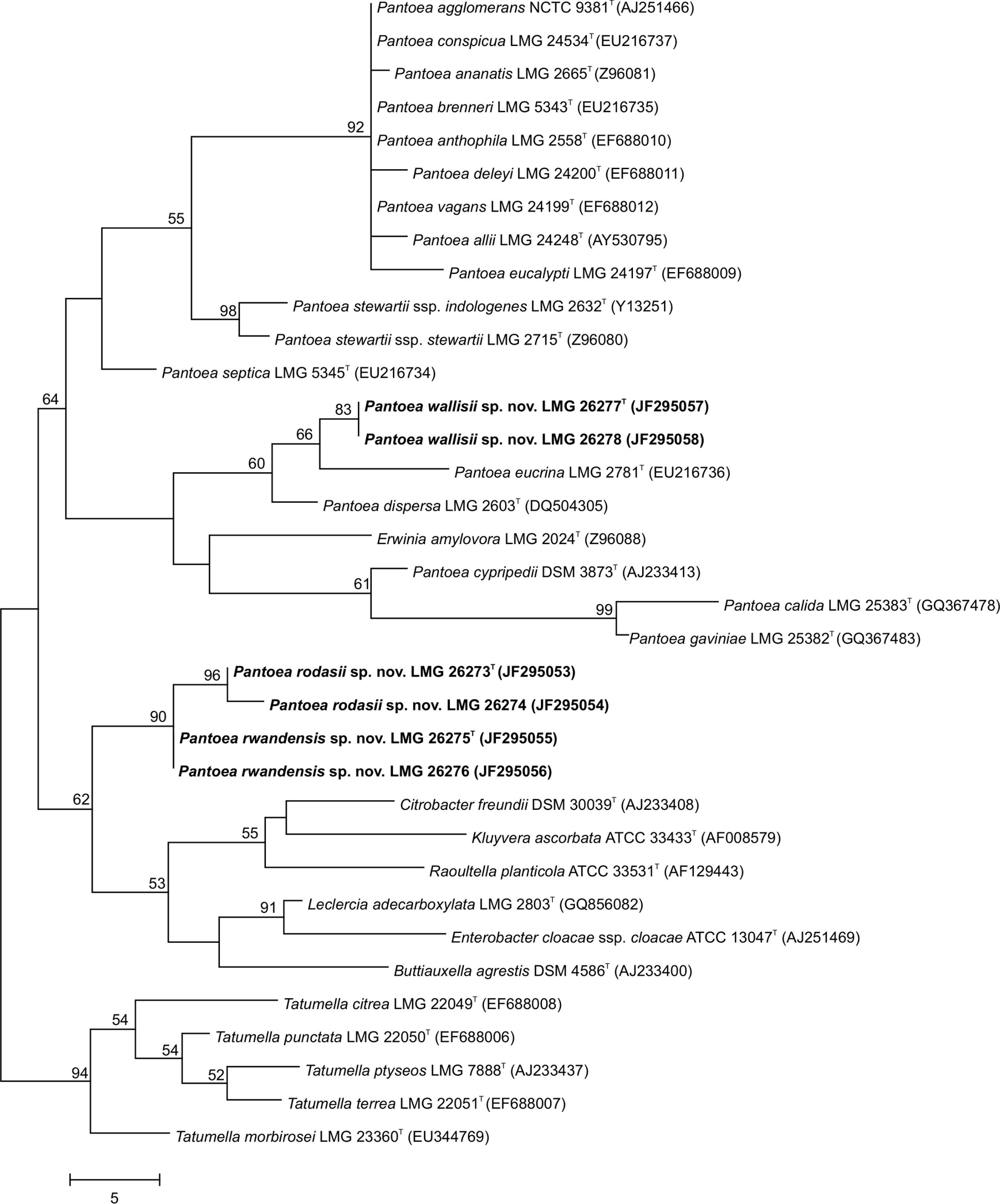
| **Species name** | **Strain No** | **Source** | **Location** |
|---|---|---|---|
| *Pantoea cypripedii* | LMG 2657$^T$ | Orchid | USA |
| *Pantoea dispersa* | LMG 2603$^T$ | Soil | Japan |
| *Pantoea eucrina* | LMG 2781$^T$ | Human | USA |
| *Pantoea septica* | LMG 5345$^T$ | Clinical | USA |
| *Pantoea rodasii* sp. nov. | LMG 26273$^T$ = BD 943$^T$ = BCC 581$^T$ | Eucalyptus | Colombia |
| | LMG 26274 = BCC 588 | Eucalyptus | Colombia |
| | R-43459 = BCC 582 | Eucalyptus | Colombia |
| | R-43462 = BCC 589 | Eucalyptus | Colombia |
| *Pantoea rwandensis* sp. nov. | LMG 26275$^T$ = BD 944$^T$ = BCC 571$^T$ | Eucalyptus | Rwanda |
| | LMG 26276 = BCC 568 | Eucalyptus | Rwanda |
| | R-43272 = BCC 569 | Eucalyptus | Rwanda |
| | R-43273 = BCC 570 | Eucalyptus | Rwanda |
| *Pantoea wallisii* sp. nov. | LMG 26277$^T$ = BD 946$^T$ = BCC 682$^T$ | Eucalyptus | South Africa |
| | LMG 26278 = BCC 692 | Eucalyptus | South Africa |
| *Buttiauxella agrestis* | LMG 7861$^T$ | Soil | Unknown |
| *Enterobacter ludwigii* | LMG 23768$^T$ | Human | Germany |
| *Erwinia aphidicola* | LMG 24877$^T$ | Aphid | Japan |
| *Kluyvera intermedia* | LMG 2785$^T$ | Surface water | France |

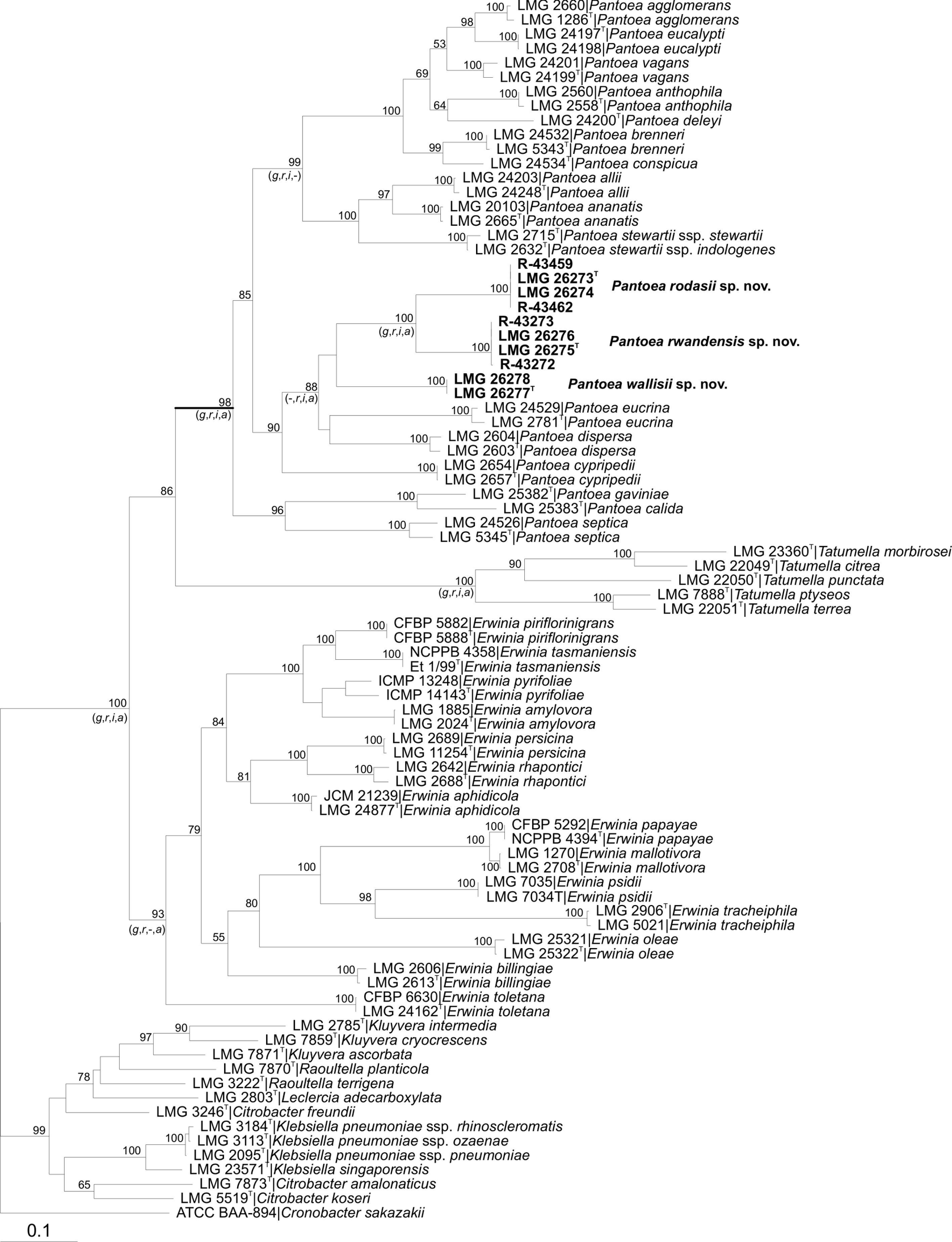
**Supplementary Table B.** DNA-DNA hybridization values amongst strains belonging to the three novel *Pantoea* species, *P. rodasii* sp. nov., *P. rwandensis* sp. nov. and *P. wallisii* sp. nov., and to selected type strains exhibiting more than 97 % 16S rRNA gene sequence similarity. Values are expressed as percentages (± difference between reciprocal values/2).

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| *Pantoea rodasii* sp. nov. | | | | | | | | |
| 1. LMG 26273$^T$ | 100 | | | | | | | |
| 2. R-43461 | 98 (± 9.0) | 100 | | | | | | |
| *Pantoea rwandensis* sp. nov. | | | | | | | | |
| 3. LMG 26275$^T$ | 41 (± 6.0) | 37 (± 0.5) | 100 | | | | | |
| 4. R-43271 | 44 (± 1.5) | 43 (± 3.0) | 102 (± 4.0) | 100 | | | | |
| *Pantoea wallisii* sp. nov. | | | | | | | | |
| 5. LMG 26277$^T$ | | | | | 100 | | | |
| 6. R-43474 | | | | | 100 (± 13.0) | 100 | | |
| 7. *Pantoea cypripedii* LMG 2657$^T$ | 26 (± 0) | | 24 (± 1.5) | | 26 (± 8.0) | 27 (± 4.5) | 100 | |
| 8. *Pantoea eucrina* LMG 2781$^T$ | 25 (± 2.5) | | 18 (± 1.0) | | 26 (± 6.0) | 28 (± 3.0) | 23 (± 3.5) | 100 |
| *Pantoea dispersa* LMG 2603$^T$ | 34 (± 2.5) | | 27 (± 6.5) | | 37 (± 12.5) | 40 (± 9.5) | | |
| *Pantoea septica* LMG 5345$^T$ | 22 (± 1.0) | | 19 (± 2.0) | | | | | |
| *Erwinia aphidicola* LMG 24877$^T$ | 24 (± 4.0) | | 19 (± 3.0) | | | | | |
| *Kluyvera intermedia* LMG 2785$^T$ | 13 (± 1.5) | | 14 (± 4.5) | | | | | |
| *Buttiauxella agrestis* LMG 7861$^T$ | 12 (± 0) | | 11 (± 0.5) | | | | | |
| *Enterobacter ludwigii* LMG 23768$^T$ | 17 (± 6.5) | | 15 (± 5.0) | | | | | |

**Figure 1:** Maximum parsimony tree based on almost-complete 16S rRNA gene sequences of members of the genus *Pantoea* and phylogenetically related species. Bootstrap values after 1000 replicates are expressed as percentages. Species belonging to the genus *Tatumella* were included as outgroups. The scale bar indicates the numbers of substitutions per site.

**Figure 2:** Maximum likelihood tree based on concatenated housekeeping gene sequences of *Pantoea* strains. Bootstrap values after 1000 replicates are expressed as percentages. Major clades also supported by the single gene phylogenies are indicated by *g* (*gyrB*), *r* (*rpoB*), *i* (*infB*) and *a* (*atpD*) in parentheses. *Cronobacter sakazakii* ATCC BAA-894 was included as an outgroup. Gene sequences for *C. sakazakii* were obtained from http://www.ncbi.nlm.nih.gov. The scale bar indicates the number of substitutions per site.


*Pantoea agglomerans* NCTC 9381$^{T}$ (AJ251466)
*Pantoea conspicua* LMG 24534$^{T}$ (EU216737)
*Pantoea ananatis* LMG 2665$^{T}$ (Z96081)
*Pantoea brenneri* LMG 5343$^{T}$ (EU216735)
92
*Pantoea anthophila* LMG 2558$^{T}$ (EF688010)
*Pantoea deleyi* LMG 24200$^{T}$ (EF688011)
*Pantoea vagans* LMG 24199$^{T}$ (EF688012)
55
*Pantoea allii* LMG 24248$^{T}$ (AY530795)
*Pantoea eucalypti* LMG 24197$^{T}$ (EF688009)
98
*Pantoea stewartii* ssp. *indologenes* LMG 2632$^{T}$ (Y13251)
*Pantoea stewartii* ssp. *stewartii* LMG 2715$^{T}$ (Z96080)
*Pantoea septica* LMG 5345$^{T}$ (EU216734)
64
83
**Pantoea wallisii sp. nov. LMG 26277$^{T}$ (JF295057)**
66
**Pantoea wallisii sp. nov. LMG 26278 (JF295058)**
60
*Pantoea eucrina* LMG 2781$^{T}$ (EU216736)
*Pantoea dispersa* LMG 2603$^{T}$ (DQ504305)
*Erwinia amylovora* LMG 2024$^{T}$ (Z96088)
*Pantoea cypripedii* DSM 3873$^{T}$ (AJ233413)
61
99
*Pantoea calida* LMG 25383$^{T}$ (GQ367478)
*Pantoea gaviniae* LMG 25382$^{T}$ (GQ367483)
96
**Pantoea rodasii sp. nov. LMG 26273$^{T}$ (JF295053)**
**Pantoea rodasii sp. nov. LMG 26274 (JF295054)**
90
**Pantoea rwandensis sp. nov. LMG 26275$^{T}$ (JF295055)**
**Pantoea rwandensis sp. nov. LMG 26276 (JF295056)**
62
*Citrobacter freundii* DSM 30039$^{T}$ (AJ233408)
55
*Kluyvera ascorbata* ATCC 33433$^{T}$ (AF008579)
*Raoultella planticola* ATCC 33531$^{T}$ (AF129443)
53
91
*Leclercia adecarboxylata* LMG 2803$^{T}$ (GQ856082)
*Enterobacter cloacae* ssp. *cloacae* ATCC 13047$^{T}$ (AJ251469)
*Buttiauxella agrestis* DSM 4586$^{T}$ (AJ233400)
*Tatumella citrea* LMG 22049$^{T}$ (EF688008)
54
*Tatumella punctata* LMG 22050$^{T}$ (EF688006)
54
94
52
*Tatumella ptyseos* LMG 7888$^{T}$ (AJ233437)
*Tatumella terrea* LMG 22051$^{T}$ (EF688007)
*Tatumella morbirosei* LMG 23360$^{T}$ (EU344769)
5


LMG 2660|Pantoea agglomerans
LMG 1286T|Pantoea agglomerans
LMG 24197T|Pantoea eucalypti
LMG 24198|Pantoea eucalypti
LMG 24201|Pantoea vagans
LMG 24199T|Pantoea vagans
LMG 2560|Pantoea anthophila
LMG 2558T|Pantoea anthophila
LMG 24200T|Pantoea deleyi
LMG 24532|Pantoea brenneri
LMG 5343T|Pantoea brenneri
LMG 24534T|Pantoea conspicua
(g,r,i,-)
LMG 24203|Pantoea allii
LMG 24248T|Pantoea allii
LMG 20103|Pantoea ananatis
LMG 2665T|Pantoea ananatis
LMG 2715T|Pantoea stewartii ssp. stewartii
LMG 2632T|Pantoea stewartii ssp. indologenes
R-43459
LMG 26273T
LMG 26274
R-43462
Pantoea rodasii sp. nov.
(g,r,i,a)
R-43273
LMG 26276
LMG 26275T
R-43272
Pantoea rwandensis sp. nov.
LMG 26278
LMG 26277T
Pantoea wallisii sp. nov.
(-,r,i,a)
(g,r,i,a)
LMG 24529|Pantoea eucrina
LMG 2781T|Pantoea eucrina
LMG 2604|Pantoea dispersa
LMG 2603T|Pantoea dispersa
LMG 2654|Pantoea cypripedii
LMG 2657T|Pantoea cypripedii
LMG 25382T|Pantoea gaviniae
LMG 25383T|Pantoea calida
LMG 24526|Pantoea septica
LMG 5345T|Pantoea septica
LMG 23360T|Tatumella morbirosei
LMG 22049T|Tatumella citrea
LMG 22050T|Tatumella punctata
(g,r,i,a)
LMG 7888T|Tatumella ptyseos
LMG 22051T|Tatumella terrea
CFBP 5882|Erwinia piriflorinigrans
CFBP 5888T|Erwinia piriflorinigrans
NCPPB 4358|Erwinia tasmaniensis
Et 1/99T|Erwinia tasmaniensis
ICMP 13248|Erwinia pyrifoliae
ICMP 14143T|Erwinia pyrifoliae
LMG 1885|Erwinia amylovora
LMG 2024T|Erwinia amylovora
LMG 2689|Erwinia persicina
LMG 11254T|Erwinia persicina
LMG 2642|Erwinia rhapontici
LMG 2688T|Erwinia rhapontici
JCM 21239|Erwinia aphidicola
LMG 24877T|Erwinia aphidicola
(g,r,i,a)
CFBP 5292|Erwinia papayae
NCPPB 4394T|Erwinia papayae
LMG 1270|Erwinia mallotivora
LMG 2708T|Erwinia mallotivora
LMG 7035|Erwinia psidii
LMG 7034T|Erwinia psidii
LMG 2906T|Erwinia tracheiphila
LMG 5021|Erwinia tracheiphila
(g,r,-,a)
LMG 25321|Erwinia oleae
LMG 25322T|Erwinia oleae
LMG 2606|Erwinia billingiae
LMG 2613T|Erwinia billingiae
CFBP 6630|Erwinia toletana
LMG 24162T|Erwinia toletana
LMG 2785T|Kluyvera intermedia
LMG 7859T|Kluyvera cryocrescens
LMG 7871T|Kluyvera ascorbata
LMG 7870T|Raoultella planticola
LMG 3222T|Raoultella terrigena
LMG 2803T|Leclercia adecarboxylata
LMG 3246T|Citrobacter freundii
LMG 3184T|Klebsiella pneumoniae ssp. rhinoscleromatis
LMG 3113T|Klebsiella pneumoniae ssp. ozaenae
LMG 2095T|Klebsiella pneumoniae ssp. pneumoniae
LMG 23571T|Klebsiella singaporensis
LMG 7873T|Citrobacter amalonaticus
LMG 5519T|Citrobacter koseri
ATCC BAA-894|Cronobacter sakazakii
0.1