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Ralstonia taiwanensis sp. nov., isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient

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A polyphasic taxonomic study, including 16S rDNA sequence analysis, DNA–DNA hybridizations, DNA base ratio determinations, amplified 16S rDNA restriction analysis, whole-cell protein analyses and extensive biochemical characterization, was conducted to clarify the relationships of eight isolates from root nodules of *Mimosa* species and one isolate from sputum of a cystic fibrosis patient. All nine isolates were classified as a novel *Ralstonia* species, for which the name *Ralstonia taiwanensis* sp. nov. is proposed. The type strain is LMG 19424^T (= CCUG 44338^T). *R. taiwanensis* effectively nodulated the *Mimosa* species and is the first β-proteobacterium that is known to be capable of root nodule formation and nitrogen fixation.

Keywords: *Ralstonia taiwanensis* sp. nov., root nodules, *Mimosa*, taxonomy

INTRODUCTION

The genus *Ralstonia* was established in 1995 by Yabuuchi and others to accommodate species previously known as *Alcaligenes eutrophus*, *Pseudomonas solanacearum* and *Pseudomonas pickettii* (Yabuuchi *et al*., 1995). Since then, several additional species isolated from environmental or human clinical sources, or both, have been described: *Ralstonia gilardii* (Coenye *et al*., 1999); *Ralstonia paucula* (Vandamme *et al*., 1999); *Ralstonia basilensis* (Steinle *et al*., 1998); and *Ralstonia mannitolytica* (De Baere *et al*., 2001). These *Ralstonia* species occupy very diverse ecological niches. *Ralstonia eutropha* and *R. basilensis* are environmental micro-organisms, the former being isolated from sludge, soil and waste-water (Steinle *et al*., 1998). *Ralstonia solanacearum* is one of the most important bacterial phytopathogenic species, causing bacterial wilt on a wide range of crops (Palleroni & Doudoroff, 1971). *Ralstonia pickettii*, *R. mannitolytica*, *R. paucula* and *R. gilardii* strains have been isolated from various clinical sources and environmental samples (Riley & Weaver, 1975; Coenye *et al*., 1999; De Baere *et al*., 2001; Vandamme *et al*., 1999).

Abbreviation: ARDRA, amplified rDNA restriction analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains LMG 19424^T and LMG 19425 are AF300324 and AF300325, respectively.

The genus *Ralstonia* is thus a most unusual genus, unifying species that are opportunistic human pathogens able to survive in oligotrophic environments with economically important plant pathogens and organisms that are of considerable biotechnological interest because of their potential for biodegradation of recalcitrant components and xenobiotics.

In the present study, polyphasic taxonomic analysis was used to elucidate the taxonomic relationships of eight isolates from root nodules of *Mimosa* species and one isolate from the sputum of a cystic fibrosis patient. These isolates were shown to represent a novel *Ralstonia* species, for which the name *Ralstonia taiwanensis* sp. nov. is proposed.

METHODS

Bacterial strains. *R. taiwanensis* strains and the *Ralstonia* reference strains used are listed in Table 1. The root nodule strains of *Mimosa pudica* and *Mimosa diplotricha* were collected from three fields at Ping-Tung Country in the southern part of Taiwan. Root nodules were immersed in 95% ethanol for 10 s, sterilized in 0·1% (w/v) mercuric chloride for 10 min and then washed six times with sterile distilled water. With sterile glass rods, individual nodules were crushed and streaked onto yeast extract-mannitol agar (Vincent, 1970) and incubated at 28 °C. Single colonies were selected and re-streaked for purity.

Analysis of protein electrophoretic patterns. Strains were incubated for 48 h. Preparation of cellular protein extracts,

Table 1. List of strains studied

Abbreviations: API, Appareils et Procédés d'Identification, Marcy-l'Etoile, France; ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection University of Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; LMG, BCCM/LMG Bacteria Collection, Laboratorium voor Microbiologie Gent, Gent, Belgium; NCIMB, National Collection of Industrial and Marine Bacteria, National Collections of Industrial, Food and Marine Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant-pathogenic Bacteria, Harpenden Laboratory, UK.

Strain	Other designation	Depositor*	Source
Ralstonia basilensis LMG 18990^T	RK1^T	DSMZ	Freshwater sediment (Switzerland)
Ralstonia eutropha LMG 1199^T	CCUG 1776^T, ATCC 17697^T	ATCC	Soil (USA, 1957)
Ralstonia gilardii LMG 5886^T	API 141-2-84^T, CCUG 38401^T	D. Monget	Whirlpool
Ralstonia paucula LMG 3244^T	CDC E6793^T, CCUG 12507^T	R. Weaver	Human, respiratory tract (USA)
Ralstonia pickettii LMG 5942^T	CCUG 3318^T, Pickett K-288^T	M. Pickett	Patient after tracheotomy (USA)
Ralstonia mannitolytica LMG 6866^T	NCIB 10805^T	NCIMB	Not known
Ralstonia solanacearum LMG 2299^T	NCPPB 325^T, CCUG 14272^T	NCPPB	*Lycopersicon esculentum* (USA)
Ralstonia taiwanensis LMG 19424^T	R1^T, CCUG 44338^T		Root nodule of *Mimosa pudica* (Taiwan)
Ralstonia taiwanensis LMG 19425	G1		Root nodule of *Mimosa diplotricha* (Taiwan)
Ralstonia taiwanensis LMG 19426	Ra5		Root nodule of *Mimosa pudica* (Taiwan)
Ralstonia taiwanensis LMG 19427	Ra7		Root nodule of *Mimosa pudica* (Taiwan)
Ralstonia taiwanensis LMG 19428	Ra10		Root nodule of *Mimosa pudica* (Taiwan)
Ralstonia taiwanensis LMG 19429	Ra18		Root nodule of *Mimosa pudica* (Taiwan)
Ralstonia taiwanensis LMG 19430	Ga16		Root nodule of *Mimosa diplotricha* (Taiwan)
Ralstonia taiwanensis LMG 19431	Ga18		Root nodule of *Mimosa diplotricha* (Taiwan)
Ralstonia taiwanensis LMG 19464	AUX0106	J. J. LiPuma	Sputum, cystic fibrosis patient (USA)

* Own isolate if not shown.

PAGE, densitometric analysis, and normalization and interpolation of protein profiles were performed as described by Pot *et al.* (1994); numerical analysis was performed using the GELCOMPAR 4.2 software package (Applied Maths). The profiles were recorded and stored on a PC. The similarity between all pairs of traces was expressed by the Pearson product–moment correlation coefficient, converted for convenience to a percentage value.

DNA preparation. DNA was prepared as described by Pitcher *et al.* (1989).

Amplified rDNA restriction analysis (ARDRA). Primers fD1 and rD1 were used for amplification of bacterial 16S rRNA genes by PCR. These primers correspond to nucleotide positions 8–27 and 1524–1540 of the *Escherichia coli* 16S rRNA gene, respectively, and can be used for amplifying nearly full-length 16S rRNA genes (Weisburg *et al.*, 1991). Bacteria were grown on tryptic soy agar (Difco) at 37 °C for 24 h. Two to three colonies of each strain were harvested and washed twice with sterile distilled water. The cell pellets were dissolved in 100 µl sterile distilled water, boiled for 10 min and centrifuged briefly. A 50 µl PCR cocktail containing 10 mM Tris/HCl (pH 8·3), 50 mM KCl, 2 mM $MgCl_2$, 0·001 % gelatin, 100 µM each of dATP, dTTP, dGTP and dCTP, 15 pmol each primer, 2·5 µl supernatant of boiled bacterial cells and 1.0 unit *Taq* DNA polymerase (Perkin-Elmer) was subjected to 35 cycles using a GeneAmp PCR system 2400 thermocycler (Perkin-Elmer). The amplification cycles included: 1 cycle of 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C; and a final extension for 5 min at 72 °C. The PCR products were checked by electrophoresis in 1.0 % agarose. The remaining PCR products were purified using the High Pure PCR Product Purification kit (Boehringer Mannheim). Purified PCR products (15 µl) were digested with *Alu*I, *Cfo*I, *Hin*fI, *Msp*I and *Nde*II (Boehringer Mannheim) separately and resolved in 3 % Metaphore agarose (FMC) gels (15 cm long) at 6.7 V cm^{-1} for 4 h. For each strain, the normalized restriction patterns obtained as a combined profile were analysed using the Dice similarity coefficient and the UPGMA clustering algorithm with MVSP 3.1 software (Kovach Computing Services).

Determination of the DNA G+C composition. DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture was then separated by HPLC using a Waters Symmetry Shield C8 column thermostatted at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5 % acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference.

DNA–DNA hybridizations. DNA–DNA hybridizations were performed 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 and the reaction was carried out in 50 % formamide. Each value was the mean of at least two hybridization experiments.

16S rDNA sequencing. For sequence analysis, purified PCR products of 16S rRNA genes were cloned using the pGEM-T Easy Vectors system (Promega). Plasmid DNA was prepared with the Wizard Plus Minipreps DNA Purification kit (Promega). Sequence analysis was performed using an Applied Biosystems 377 DNA Sequencer and the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit. Using the PILEUP program of the Wisconsin Genetics Computer Group package, the sequences were aligned together with reference sequences obtained from GenBank. All the sequences used were almost full length and were derived from the type strain wherever possible. The neighbour-joining method and bootstrap analysis (confidence values estimated from 1000 replications of each sequence) in the CLUSTAL W 1.7 program (Thompson *et al.*,

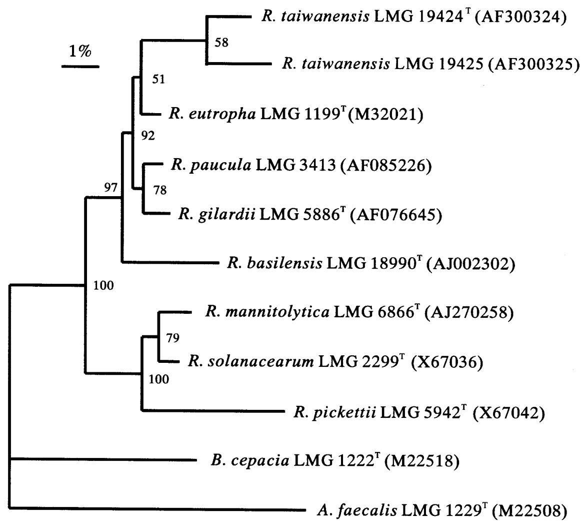

Fig. 1. Neighbour-joining phylogenetic tree of *R. taiwanensis* and related bacteria based on 16S rRNA sequence comparisons. Bootstrap values are indicated at nodes. Scale bar, 1% sequence dissimilarity (one substitution per 100 nt). Representative sequences in the dendrogram were obtained from GenBank.

1997) were used to produce a phylogenetic tree. The tree was drawn using the TREEVIEW program (Page, 1996). Sequence identities were calculated using the BIOEDIT program (Hall, 1999).

Biochemical analysis. Classical phenotypic tests were performed as described by De Vos *et al.* (1985). The API 20NE and API ZYM microtest systems were used according to the recommendations of the manufacturer (bioMérieux). For carbon substrate assimilation tests, BIOLOG GNII microtitre test plates were used. Early exponential phase cultures were used as inocula for the test plates (150 µl per well). Plates were incubated at 28 °C and examined after 24 and 48 h to allow for the development of a purple colour indicative of substrate oxidation.

The capacity to grow autotrophically using hydrogen as an energy source was tested as described previously (Schlegel *et al.*, 1961) using *Ralstonia* sp. strain CH34 as a positive control. Anaerobic jars (without catalyst) with hydrogen- and carbon dioxide-generating envelopes were used to create a hydrogen enriched microaerobic atmosphere.

Antimicrobial susceptibility testing. MIC values towards ampicillin, chloramphenicol, kanamycin, nalidixic acid, penicillin G, piperacillin, streptomycin and tetracycline were determined using the agar dilution method conforming to National Committee for Clinical Laboratory Standards (1995) guidelines. Strains were grown on Mueller–Hinton agar (Difco) for 16–20 h at 28 °C.

RESULTS

Phylogenetic analysis of 16S rDNA sequences

The 16S rDNA sequences of isolates LMG 19424^T and LMG 19425 were determined. Phylogenetic analysis revealed that both clustered within the *β-Proteobacteria* amongst *Ralstonia* strains. Fig. 1 shows the result of neighbour-joining cluster analysis of strains LMG 19424 ^{T} and LMG 19425 and related bacteria.

SDS-PAGE of whole-cell proteins

The whole-cell protein profiles of the eight root nodule isolates were compared with each other and with over 2000 profiles in a database comprising all presently known *Burkholderia*, *Ralstonia* and *Pandoraea* species and many unclassified Gram-negative non-fermenting bacteria (Coenye *et al.*, 1999, 2000; Vandamme *et al.*, 1997, 1999, 2000; P. Vandamme, unpublished data). The root nodule isolates had virtually identical whole-cell protein profiles and a high similarity level was found towards strain LMG 19464, an unclassified isolate from the sputum of a cystic fibrosis patient (Fig. 2). No high similarity levels (values above 80%) were detected towards reference strains of known species or towards any of the remaining unclassified

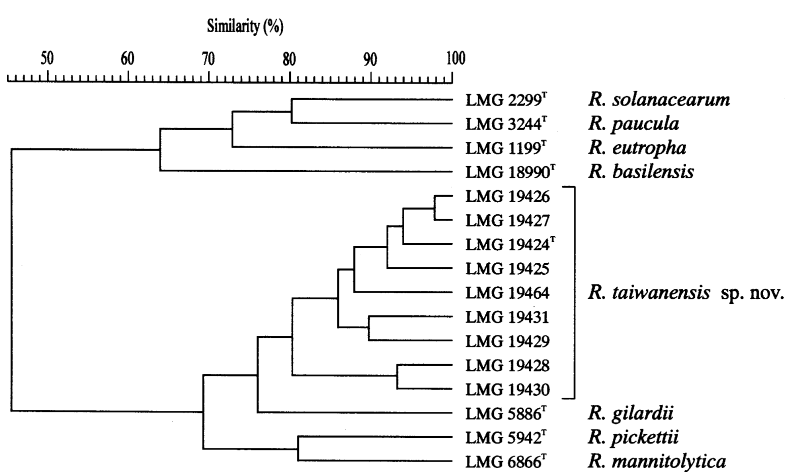

Fig. 2. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients between the whole-cell protein patterns of *R. taiwanensis* strains and *Ralstonia* reference strains studied. The correlation coefficient is expressed as percentage similarity for convenience.

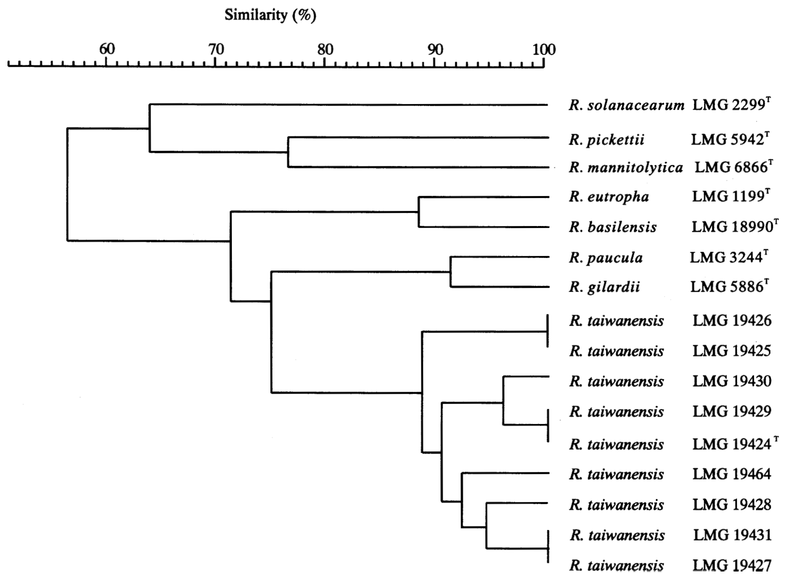

Fig. 3. Dendrogram derived from the unweighted pair group average linkage of Dice similarity coefficients between the combined ARDRA patterns of all strains studied. The correlation coefficient is expressed as percentage similarity for convenience.

Table 2. DNA G+C content and DNA–DNA binding values (%) of strains examined

Strain: 1, *R. taiwanensis* LMG 19424^T; 2, *R. taiwanensis* LMG 19425; 3, *R. taiwanensis* LMG 19428; 4, *R. taiwanensis* LMG 19431; 5, *R. taiwanensis* LMG 19464; 6, *R. paucula* LMG 3244^T; 7, *R. gilardii* LMG 5886^T; 8, *R. eutropha* LMG 1199^T; 9, *R. basilensis* LMG 18990^T.

Strain	G+C content (mol%)	Strain				
		1	2	3	4	5
1	67·3	100				
2	66·7	71	100			
3	66·7	77	70	100		
4	67·0	96	69	80	100	
5	67·7	65			71	100
6	63·7	26	25			
7	67·2	30	34			
8	66·8	46	44			
9	65·4	36	31			

isolates present in the database. Fig. 2 shows the result of the numerical analysis of the whole-cell protein profiles of the nine isolates and those of *Ralstonia* reference strains.

ARDRA analysis

The nine *R. taiwanensis* isolates were examined along with the type strains of the other *Ralstonia* species. In the numerical analysis of the combined ARDRA patterns (Fig. 3), all *R. taiwanensis* strains examined formed a single cluster. The type strains of all of the reference species occupied distinct positions.

DNA base ratio and DNA–DNA hybridization analysis

DNA was prepared from strains LMG 19424^T, LMG 19425, LMG 19428, LMG 19431 and LMG 19464. Their DNA G+C content was 66·7–67·7 mol% (Table 2). The DNA–DNA binding values among these five strains varied between 65 and 100% (Table 2). A mean binding value of 45% was calculated towards *R. eutropha* LMG 1199^T, their closest phylogenetic neighbour (Fig. 1); values of 36% and lower were calculated towards the type strains of other *Ralstonia* species (Table 2).

Antimicrobial susceptibility testing

The range of MIC values and MIC_{50} and MIC_{90} of the strains are given in Table 3.

Biochemical analysis

Classical biochemical tests, API 20NE tests and API ZYM tests were performed on the nine *R. taiwanensis*

Table 3. MIC values, MIC_{50} and MIC_{90} for the *Ralstonia taiwanensis* strains investigated

Strains investigated: LMG 19424[T], LMG 19425, LMG 19426, LMG 19427, LMG 19428, LMG 19430 and LMG 19431. The range of MIC values for all antibacterials was 0·25–128 μg ml⁻¹ (0·25–128 unit ml⁻¹ for penicillin G).

Antibacterial	MIC_{50} (μg ml^{-1})	MIC_{90} (μg ml^{-1})
Ampicillin	16	64
Chloramphenicol	8	16
Kanamycin	16	64
Nalidixic acid	4	16
Penicillin G	64	128
Piperacillin	8	16
Streptomycin	32	> 128
Tetracycline	0·5	1

isolates. The following characteristics were present in all strains: oxidase and catalase activity; nitrate reduction; aesculin hydrolysis; alkaline phosphatase, C4 esterase, C8 lipase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activity; growth at 30 and 37 °C; growth in 0·5 and 1·0% NaCl; and Tween 80 hydrolysis. The following characteristics were absent in all of the *R. taiwanensis* strains: growth on cetrimide agar; growth in the presence of 3% NaCl; amylase, urease, *β*-galactosidase and DNase activity; acid production from D-glucose, D-fructose, adonitol and maltose; production of acid and H_2S from triple-sugar iron agar; indole production; arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activity; and C14 lipase, trypsin, chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *α*-mannosidase, *α*-fucosidase and *N*-acetyl-*β*-glucosaminidase activity.

The oxidation of carbon substrates, using the BIOLOG GNII microtitre test system, was examined for nine *R. taiwanensis* strains. All strains investigated oxidized Tween 40, Tween 80, methyl pyruvate, monomethyl succinate, *cis*-aconitic acid, formic acid, D-gluconic acid, *β*-hydroxybutyric acid, *α*-ketovaleric acid, DL-lactic acid, propionic acid, sebacic acid, succinamic acid, succinic acid, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-ornithine, L-glutamic acid, L-leucine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, acetic acid, *α*-hydroxybutyric acid, *α*-ketobutyric acid, itaconic acid, bromosuccinic acid and alaninamide.

Table 4. Differential phenotypic characteristics of *R. taiwanensis* and other *Ralstonia* species

Strains: 1, *R. basilensis*; 2, *R. eutropha*; 3, *R. gilardii*; 4, *R. paucula*; 5, *R. pickettii*; 6, *R. solanacearum*; 7, *R. taiwanensis*. +, Character is present in all strains; −, character is absent in all strains; W, weakly positive reaction; V, strain-dependent result.

Characteristic	1	2	3	4	5	6	7
Catalase activity	+	+	+	+	−	+	+
Oxidase activity	+	+	−	+	−	+	+
Growth in the presence of penicillin (10 μg)	−	−	−	−	W	−	V
Hydrolysis of Tween 80	+	W	−	+	+	−	+
Growth at 42 °C	−	+	−	+	+	−	−
Oxidation/fermentation test for:							
D-Glucose	−	−	+	−	W	−	−
D-Fructose	−	−	+	−	+	−	−
D-Xylose	−	−	+	−	W	−	−
Nitrate reduction	−	+	−	−	+	+	+
Urease activity	+	−	−	+	V	−	−
Assimilation of:							
Glucose	−	−	−	−	+	+	−
Arabinose	−	−	−	−	+	−	−
N-Acetylglucosamine	−	+	−	−	+	−	−
Caprate	+	+	−	+	W	−	+
Citrate	+	+	−	+	+	+	V
Adipate	+	+	−	+	W	−	−
Phenylacetate	+	+	W	+	W	−	+
Alkaline phosphatase activity	+	+	+	+	W	W	+
Acid phosphatase activity	+	+	−	+	W	W	+
Esterase C4 activity	+	+	+	+	W	W	W
Cystine arylamidase activity	−	−	−	+	−	−	W
Phosphoamidase activity	+	+	W	+	W	W	+
Lipase C14 activity	−	−	−	+	+	−	−

None of the strains oxidized or assimilated α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, cellobiose, i-erythritol, gentiobiose, m-inositol, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-amino ethanol, 2,3-butanediol, glucose 6-phosphate, glucose 1-phosphate, DL-α-glycerol phosphate, hydroxy-L-proline, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose, L-fucose, D-galactose, α-D-glucose, D-mannitol, D-galactonic acid lactone, D-galacturonic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, γ-aminobutyric acid, D-psicose, D-serine or glucuronamide.

The following carbon substrates were oxidized at variable levels: γ-hydroxybutyric acid, ρ-hydroxy phenylacetic acid, L-histidine, L-phenylalanine, urocanic acid, citric acid, quinic acid, D-saccharic acid, D-glucosaminic acid, α-ketoglutaric acid, malonic acid, glycerol, D-glucuronic acid and DL-carnitine. None of the *R. taiwanensis* strains was capable of autotrophic growth.

Table 4 lists differential biochemical characteristics between *R. taiwanensis* and other *Ralstonia* species.

DISCUSSION

The present study reports on the polyphasic taxonomic characterization of eight root nodule isolates of *Mimosa* species and one human clinical isolate. Analysis of the nearly complete 16S rDNA sequences of two root nodule isolates (LMG 19424^T and LMG 19425) revealed sequence similarity values of 90·7–98·8 % towards 16S rDNA sequences of *Ralstonia* species; the similarity levels towards *Burkholderia* or *Alcaligenes* species, the closest relatives of the genus *Ralstonia*, were below 85 %. The DNA G+C content of about 67 mol% (Table 2) is within the range for the genus *Ralstonia*, which is between 64 and 69 mol% (Vandamme *et al.*, 1999; Coenye *et al.*, 1999; Yabuuchi *et al.*, 1995). DNA–DNA hybridizations among four of the root nodule isolates and the cystic fibrosis isolate revealed high values (Table 2) supporting the value of whole-cell protein electrophoresis as a first line screening method to select for species-level identity. DNA–DNA hybridizations towards the type strains of *R. eutropha*, *R. gilardii*, *R. paucula* and *R. basilensis*, their closest phylogenetic neighbours, revealed low to moderate DNA–DNA binding levels (Table 2) demonstrating unambiguously that this taxon represents a novel *Ralstonia* species.

These isolates all effectively nodulated both *Mimosa* species and the presence of *nif* genes in the genome of *R. taiwanensis* isolates has been demonstrated (W.-M. Chen, L. Moulin, T.-M. Lee, M. Gillis, P. Vandamme and C. Boivin-Masson, unpublished results). Rhizobia are the traditional soil bacteria capable of forming root or stem nodules on various leguminous plants where they undertake symbiotic fixation of atmospheric nitrogen. They are currently divided into six genera with approximately 30 species, including *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* (de Lajudie *et al.*, 1998; Nick *et al.*, 1999). Phylogenetically, these bacteria all belong to the α-*Proteobacteria* (Young, 1996). The present report is the first describing isolation and characterization of a β-proteobacterium capable of root nodule formation and nitrogen fixation in *Mimosa* species that belong to the Mimosoideae subfamily of the Leguminosae.

Both whole-cell protein and ARDRA analyses indicated that the nine strains described in the present study form a homogeneous group that could easily be differentiated from other *Ralstonia* species (Figs 2 and 3). In addition, classical biochemical tests allowed differentiation of the nine strains from the other *Ralstonia* species (Table 4). The absence of autotrophic growth and of growth at 42 °C readily differentiates *R. taiwanensis* from its closest neighbour, *R. eutropha*.

Description of *Ralstonia taiwanensis* sp. nov.

Ralstonia taiwanensis (tai.wan.en′sis. N.L. fem. adj. *taiwanensis* of Taiwan, where the root nodule strains were isolated).

Cells are Gram-negative, non-spore-forming and rod-shaped. After 24 h growth on tryptic soy agar at 30 °C, the mean cell size is about 0·5–0·7 μm in width and 0·8–2·0 μm in length. Growth is observed at 28, 30 and 37 °C. Catalase- and oxidase-positive. Nitrate is reduced. Aesculin hydrolysed. Tolerant to penicillin (10 μg per disc) and streptomycin (10 μg per disc). No urease, β-galactosidase or DNase activity. No indole production. No autotrophic growth. Additional characteristics are listed above. The DNA G+C content is about 67 mol%. Isolated from root nodules of *Mimosa pudica* and *Mimosa diplotricha* and from sputum of a cystic fibrosis patient and all strains have been deposited in BCCM/LMG Bacteria Collection (Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium). The type strain is LMG 19424^T (= CCUG 44338^T), which was isolated from the root nodule of *Mimosa pudica*. Phenotypic characteristics of the type strain are the same as described for the species. Its DNA G+C content is 67·3 mol%.

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