

# *Wautersia* gen. nov., a novel genus accommodating the phylogenetic lineage including *Ralstonia eutropha* and related species, and proposal of *Ralstonia* [*Pseudomonas*] *syzygii* (Roberts *et al.* 1990) comb. nov.

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Comparative 16S rDNA sequence analysis indicates that two distinct sublineages, with a sequence dissimilarity of > 4% (bootstrap value, 100%), exist within the genus *Ralstonia*: the *Ralstonia eutropha* lineage, which comprises *Ralstonia basilensis*, *Ralstonia campinensis*, *R. eutropha*, *Ralstonia gilardii*, *Ralstonia metallidurans*, *Ralstonia oxalatica*, *Ralstonia paucula*, *Ralstonia respiraculi* and *Ralstonia taiwanensis*; and the *Ralstonia pickettii* lineage, which comprises *Ralstonia insidiosa*, *Ralstonia mannitolilytica*, *R. pickettii*, *Ralstonia solanacearum* and *Ralstonia syzygii* comb. nov. (previously *Pseudomonas syzygii*). This phylogenetic discrimination is supported by phenotypic differences. Members of the *R. eutropha* lineage have peritrichous flagella, do not produce acids from glucose and are susceptible to colistin, in contrast to members of the *R. pickettii* lineage, which have one or more polar flagella, produce acid from several carbohydrates and are colistin-resistant. Members of the *R. pickettii* lineage are viable for up to 6 days on tryptic soy agar at 25 °C, whereas members of the *R. eutropha* lineage are viable for longer than 9 days. It is proposed that species of the *R. eutropha* lineage should be classified in a novel genus, *Wautersia* gen. nov. Finally, based on the literature and new DNA–DNA hybridization data, it is proposed that *Pseudomonas syzygii* should be renamed *Ralstonia syzygii* comb. nov.

## INTRODUCTION

The genus *Ralstonia* (Yabuuchi *et al.*, 1995) was created to accommodate bacteria from ecologically diverse niches that were classified previously as *Burkholderia* (Yabuuchi *et al.*, 1992) and *Alcaligenes*. The type species of the genus – *Ralstonia pickettii* (type strain, ATCC 27511<sup>T</sup>) – was regarded originally as the only representative of clinical importance (Fass & Barnishan, 1976; Fujita *et al.*, 1981;

Kahan *et al.*, 1983; Gardner & Shulman, 1984; Verschraegen *et al.*, 1985; Roberts *et al.*, 1990a; Lacey & Want, 1991; Dimech *et al.*, 1993; Raveh *et al.*, 1993). Recently, several novel species [*Ralstonia basilensis* (Steinle *et al.*, 1998; Goris *et al.*, 2001), *Ralstonia campinensis* (Goris *et al.*, 2001), *Ralstonia gilardii* (Coenye *et al.*, 1999), *Ralstonia insidiosa* (Coenye *et al.*, 2003a), *Ralstonia metallidurans* (Goris *et al.*, 2001), *Ralstonia mannitolilytica* corrig. (De Baere *et al.*, 2001; original spelling, *Ralstonia mannitolitytica*), *Ralstonia oxalatica* (Sahin *et al.*, 2000), *Ralstonia paucula* (Osterhout *et al.*, 1998; Vandamme *et al.*, 1999; Moissenet *et al.*, 1999), *Ralstonia respiraculi* (Coenye *et al.*, 2003b) and *Ralstonia taiwanensis* (Chen *et al.*, 2001)] have been described, some of which are of moderate clinical importance.

In a previous study (De Baere *et al.*, 2001), it was indicated that species of the genus *Ralstonia* can be separated clearly into two phenotypically and genotypically distinct groups. Here, it is proposed to consolidate these findings by allocating one group of species to a novel genus, *Wautersia*

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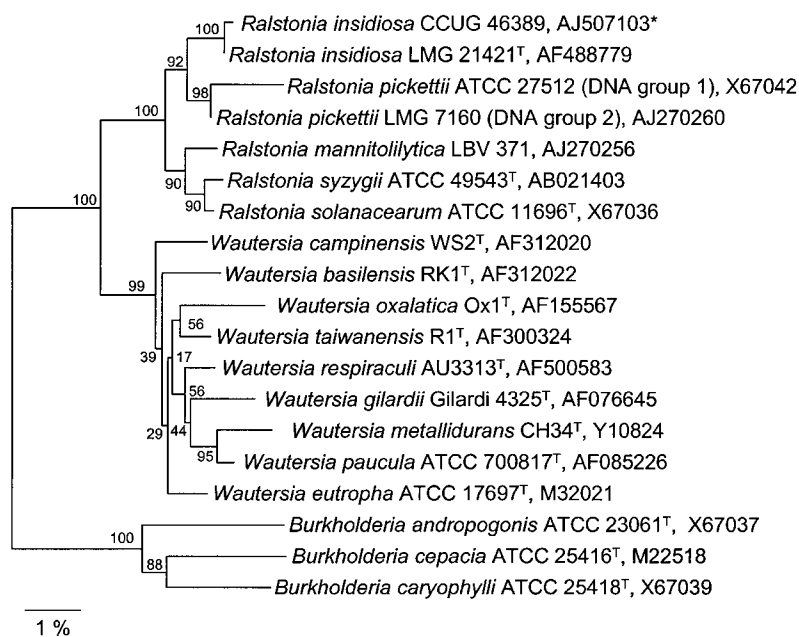
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Ralstonia insidiosa* strains CCUG 46389 and CCUG 46388 are AJ507103 and AJ539233.

The results of lactose and maltose acidification by *Ralstonia pickettii* strains, the 16S rRNA gene signature sequences of the *Ralstonia* species, detailed phenotypic data of different species and a similarity matrix of the 16S rDNA sequences are available as supplementary material (Tables A1, A2, A3 and A4, respectively) in IJSEM Online.

**Table 1.** List of *Ralstonia* and *Wautersia* species and strains tested during this and previous studies

Species	Type strain	Strains tested in this study	Reference
<i>Ralstonia insidiosa</i> (Coenye <i>et al.</i> 2003) biovar 1	LMG 21421 <sup>T</sup> =CCUG 46789 <sup>T</sup>	LMG 21421 <sup>T</sup> , CCUG 38965, CCUG 46212, CCUG 46213, CCUG 47187, CCUG 47416	Coenye <i>et al.</i> (2003a); this study
<i>Ralstonia insidiosa</i> (Coenye <i>et al.</i> 2003) biovar 2		CCUG 46387, CCUG 46388, CCUG 46389, CCUG 47426	Coenye <i>et al.</i> (2003a); this study
<i>Ralstonia mannitolilytica</i> (De Baere <i>et al.</i> 2001)	CCUG 38408 <sup>T</sup> =CCUG 45027 <sup>T</sup> =LMG 6866 <sup>T</sup> =NCIMB 10805 <sup>T</sup>	LMG 6866 <sup>T</sup> , LMG 19090, LMG 19091, LMG 19092	De Baere <i>et al.</i> (2001)
<i>Ralstonia pickettii</i> (Ralston <i>et al.</i> 1973) Yabuuchi <i>et al.</i> 1996 comb. nov.*	ATCC 27511 <sup>T</sup> =CCUG 3318 <sup>T</sup> =DSM 6297 <sup>T</sup> =LMG 5942 <sup>T</sup> =NCTC 11149 <sup>T</sup>	ATCC 27511 <sup>T</sup> , ATCC 27512, LMG 7014, LMG 7015, LMG 7160, LMG 19083, LMG 19084, LMG 19085, LMG 19086, LMG 19088	Yabuuchi <i>et al.</i> (1996)
<i>Ralstonia solanacearum</i> (Smith 1896) Yabuuchi <i>et al.</i> 1996 comb. nov.	A. Kelman 60-1 <sup>T</sup> =ATCC 11696 <sup>T</sup> =CCUG 14272 <sup>T</sup> =DSM 9544 <sup>T</sup> =LMG 2299 <sup>T</sup>	LMG 2299 <sup>T</sup> , LMG 2303	Yabuuchi <i>et al.</i> (1996)
<i>Ralstonia</i> [ <i>Pseudomonas</i> ] <i>syzygii</i> (Roberts <i>et al.</i> 1990) comb. nov.	R001 <sup>T</sup> =ATCC 49543 <sup>T</sup> =LMG 10661 <sup>T</sup>	LMG 6969	This study
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>basilensis</i> (Steinle <i>et al.</i> 1999) comb. nov.	RK1 <sup>T</sup> =DSM 11853 <sup>T</sup> =LMG 18990 <sup>T</sup> =LMG 19474 <sup>T</sup>	LMG 18990 <sup>T</sup>	Steinle <i>et al.</i> (1999)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>campinensis</i> (Goris <i>et al.</i> 2001) comb. nov.	WS2 <sup>T</sup> =CCUG 44526 <sup>T</sup> =LMG 19282 <sup>T</sup>		Goris <i>et al.</i> (2001)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>eutropha</i> (Davis 1969) Yabuuchi <i>et al.</i> 1996 comb. nov.	ATCC 17697 <sup>T</sup> =CCUG 1776 <sup>T</sup> =DSM 531 <sup>T</sup> =LMG 1199 <sup>T</sup>	LMG 1199 <sup>T</sup> , LMG 1194, LMG 1201	Yabuuchi <i>et al.</i> (1996)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>gilardii</i> (Coenye <i>et al.</i> 1999) comb. nov.	Gilardi 4325 <sup>T</sup> =ATCC 700815 <sup>T</sup> =CCUG 38401 <sup>T</sup> =LMG 5886 <sup>T</sup>	LMG 5886 <sup>T</sup> , LMG 3400, LMG 15537, NF 926, NF 933	Coenye <i>et al.</i> (1999)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>metallidurans</i> (Goris <i>et al.</i> 2001) comb. nov.	CH34 <sup>T</sup> =CIP 107179 <sup>T</sup> =DSM 2839 <sup>T</sup> =LMG 1195 <sup>T</sup>		Goris <i>et al.</i> (2001)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>oxalatica</i> (ex Khambata and Bhat 1953) Sahin <i>et al.</i> 2000 comb. nov.	Ox1 <sup>T</sup> =ATCC 11883 <sup>T</sup> =CCUG 2086 <sup>T</sup> =DSM 1105 <sup>T</sup> =LMG 2235 <sup>T</sup>		Sahin <i>et al.</i> (2000)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>paucula</i> (Vandamme <i>et al.</i> 1999) comb. nov.	ATCC 700817 <sup>T</sup> =CCUG 12507 <sup>T</sup> =LMG 3244 <sup>T</sup>	LMG 3244 <sup>T</sup> , LMG 3515	Vandamme <i>et al.</i> (1999)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>respiraculi</i> (Coenye <i>et al.</i> 2003) comb. nov.	AU3313 <sup>T</sup> =LMG 21510 <sup>T</sup> =CCUG 46809 <sup>T</sup>		Coenye <i>et al.</i> (2003b)
<i>Wautersia</i> [ <i>Ralstonia</i> ] <i>taiwanensis</i> (Chen <i>et al.</i> 2001) comb. nov.	R1 <sup>T</sup> =CCUG 44338 <sup>T</sup> =LMG 19424 <sup>T</sup>		Chen <i>et al.</i> (2001)

\*Two DNA groups are present (De Baere *et al.*, 2001; this study).



**Fig. 1.** Rooted phylogenetic tree based on 16S rRNA gene sequence homology for species of the genera *Ralstonia* and *Wautersia* and of some species of Gram-negative non-fermenters. Cluster analysis was based on the neighbour-joining method, with *Burkholderia cepacia* ATCC 25416<sup>T</sup> (M22518) as the outgroup. Numbers at branch-points are percentages of 100 bootstrap resamplings that support the tree topology. Asterisk indicates a sequence determined during this study. Bar, 1% estimated sequence divergence.

gen. nov., and by further elucidating the taxonomy of this group of organisms.

## METHODS

The type strains of species that are discussed here are listed in Table 1. Sequencing was carried out as described previously (Vanechoutte *et al.*, 2000) and similarity calculations and cluster analysis were carried out as described elsewhere (Nemec *et al.*, 2001). Restriction digestion of amplified rDNA with *Hae*III was carried out as described previously (Vanechoutte *et al.*, 1998). Phenotypic testing was carried out as described previously (De Baere *et al.*, 2001; Laffineur *et al.*, 2002). Fatty acid analysis was carried out as described previously (Wauters *et al.*, 1996). DNA–DNA hybridization was carried out as described previously (Ziemke *et al.*, 1998).

## RESULTS AND DISCUSSION

### Rationale for the creation of *Wautersia* gen. nov.

Sequence analysis of the 16S rRNA gene (Fig. 1) indicates that two distinct sublineages, with sequence dissimilarity of >4%, supported by a bootstrap value of 100%, are present within the genus *Ralstonia sensu lato*. The *Ralstonia eutropha* lineage comprises *R. basilensis*, *R. campinensis*, *R. eutropha*, *R. gilardii*, *R. metallidurans*, *R. oxalatica*, *R. paucula*, *R. respiraculi* and *R. taiwanensis*, and the *Ralstonia pickettii* lineage comprises *R. insidiosa*, *R. mannitolilytica*, *R. pickettii*, *Ralstonia solanacearum* and *Ralstonia syzygii* comb. nov. (previously *Pseudomonas syzygii*). This genotypic discrimination is supported by several clear phenotypic differences (Table 2). Species of the *R. eutropha* lineage have peritrichous flagella, do not produce acids from glucose and are susceptible to colistin. In contrast, species of the *R. pickettii* lineage are characterized by the presence of one or more polar flagella in motile species,

production of acid from several carbohydrates and colistin resistance. Both groups also differ in their viability on tryptic soy agar (TSA) at 25 °C (except for *R. syzygii*, which does not grow on TSA), with 6 days viability at most for the *R. pickettii* lineage and at least 9 days for the *R. eutropha* lineage. It is proposed that species in the *R. eutropha* lineage should be reclassified into a novel genus, *Wautersia* gen. nov., a name used throughout the remainder of this manuscript.

### The *R. pickettii* lineage

The *R. pickettii* lineage (genus *Ralstonia sensu stricto*) contains the species *R. insidiosa*, *R. mannitolilytica*, *R. pickettii*, *R. solanacearum* and *R. syzygii* comb. nov. A compilation of the phenotypic data gathered during this study and from the literature is given in Supplementary Table A3, which is available in IJSEM Online.

### *R. insidiosa*

During this study, three isolates that closely resembled *R. mannitolilytica* were found, except that they did not produce acid from mannitol or D-arabitol and they were

**Table 2.** Phenotypic characteristics that are applicable for differentiation of the genera *Ralstonia* and *Wautersia*

Characteristic	<i>Ralstonia</i>	<i>Wautersia</i>
Flagellation	Polar, 1–4	Peritrichous
Colistin (10 µg discs)	Resistant	Susceptible
Viability on TSA at 25 °C*	<6 days	>9 days
Acid production from carbohydrates	+	–

\*Not applicable to *R. syzygii*, which does not grow on TSA.

**Table 3.** DNA–DNA hybridization values (%) of closely related species obtained in this study

Species: 1, *R. solanacearum* LMG 2299<sup>T</sup>; 2, *R. syzygii* LMG 10661<sup>T</sup>; 3, *R. mannitolilytica* LMG 6866<sup>T</sup>; 4, *R. pickettii* LMG 5942<sup>T</sup> (DNA group 1); 5, *R. pickettii* LMG 7160 (DNA group 2); 6, *R. insidiosa* CCUG 46389. Where two values are given, these are the results of duplicate testing.

Species	1	2	3	4	5	6
1		33·7/45·6				
2	59·0/22·8					
3				45·4	59·7/39·8	35·9
4			44·1		77·5/71·1	43·4
5			23·7/25·5	51·7/44·9		20·4
6			37·7	35·4	37·0	

clearly phenotypically different from both *R. pickettii* DNA groups. DNA–DNA hybridization carried out during this study (Table 3) showed clearly that this group of three phenotypically identical strains belong to an as-yet-undescribed species. After submission of the first draft of this manuscript, phenotypically similar strains with nearly identical 16S rDNA sequences (see Fig. 1) were described as *R. insidiosa* (Coenye *et al.*, 2003a). The strains of *R. insidiosa* studied here, some of which are highly clinically relevant, had one to three polar flagella, were glucolytic and were resistant to colistin; they therefore fit within the genus *Ralstonia sensu stricto*. The strains studied in this report produced acid from glucose and were nitrate-negative, in contrast to the original description of *R. insidiosa*. Two biovars could be distinguished within *R. insidiosa*. Alkalinization/assimilation of *N*-acetylglucosamine on Simmons' agar is negative for *R. pickettii* and positive for *R. mannitolilytica*; this trait can also be used to differentiate between the two *R. insidiosa* biovars. One group (biovar 1: strains LMG 21421<sup>T</sup>, CCUG 38965, CCUG 46212, CCUG 46213, CCUG 47187 and CCUG 47416) was negative for acid production, assimilation of *N*-acetylglucosamine, gelatin hydrolysis and alkalinization of mucate on Simmons' base agar, whereas strains of the other group [biovar 2: strains CCUG 46387 (NF 663), CCUG 46388 (NF 882), CCUG 46389 (NF 928) and CCUG 47426] were positive for these characteristics. The first group was adipate-positive on API 20NE (bioMérieux), whereas the second group was negative for this characteristic. Reciprocal DNA–DNA hybridization values for strains LMG 21421<sup>T</sup> and CCUG 46389 were 87·9 and 87·4%, indicating that both biovars belong to the same species.

Remarkably, the *R. insidiosa* strains did not assimilate L-arabinose or D-xylose on minimal medium, although these carbohydrates are acidified on O/F (oxidation/fermentation) medium. This is not the case for *R. pickettii* and *R. mannitolilytica*, both of which assimilate and acidify L-arabinose and D-xylose. All three species acidify, but do not assimilate, maltose.

### *R. mannitolilytica*

Several clear phenotypic differences exist between *R. mannitolilytica* (De Baere *et al.*, 2001; Vaneechoutte *et al.*, 2001) and other *Ralstonia* species. *R. mannitolilytica* can be differentiated from other *Ralstonia* species, except for *Ralstonia* sp. strains LMG 19089 (RAL04/MC5) and LMG 19087 (RAL07/YL13), by its assimilation/acidification of mannitol and D-arabitol. *R. mannitolilytica* strains also differ from *R. pickettii* and *R. solanacearum* by their resistance towards desferrioxamine and from *R. pickettii* by their lack of alkalinization of tartrate and of nitrate reductase.

### *R. pickettii*

Historically, *R. pickettii* was created for a group of clinical isolates (Ralston *et al.*, 1973) and also comprised strains in CDC group Va-2 (Tatum *et al.*, 1974; Riley & Weaver, 1975). CDC groups Va-2 and Va-1 were regarded as two different biovars of *R. pickettii* (Pickett & Greenwood, 1980; referred to as *Pseudomonas pickettii*). In a previous study (De Baere *et al.*, 2001), it has been shown that two DNA groups could be distinguished, based on both 16S rDNA sequencing and DNA–DNA hybridization.

The DNA groups differed at positions 256 and 266 (*Escherichia coli* numbering; Woese *et al.*, 1983), with strains of DNA group 1 having the nucleotides A and T at those positions, whereas strains of DNA group 2 have G and C (see Supplementary Table A2 in IJSEM Online). By using restriction of the amplified 16S rRNA gene with *Hae*III, these DNA groups could be distinguished quickly (see column A in Supplementary Table A1 in IJSEM Online). Although it has been reported that correspondence was observed between genotypic groups and biovars, these findings were not reproduced in this study. In this study, extensive attempts to confirm the differential acidification of glucose and maltose in isolates of *R. pickettii* biovars Va-1 and Va-2 failed and turned out to be quantitative rather than qualitative (Supplementary Table A1, available in IJSEM Online). Aside from the classical O/F medium (Hugh & Leifson, 1953), phenol red low-peptone agar was used in slants, as described for acidification of ethylene glycol (Wauters *et al.*, 1998), replacing ethylene glycol by 1% (w/v) lactose or maltose. Low-peptone medium is usually more sensitive to acidification than classical O/F medium. Reproducibility of the tests was only moderate; this can possibly be explained in part by the low nutritional content of O/F medium, which limits the growth of isolates of a species such as *R. pickettii*. Differences in inoculum freshness and density may, therefore, strongly influence the speed of physiological reactivity. In our hands, it proved impossible to delineate the biovars unambiguously on the basis of lactose and maltose acidification, due to the high variability observed and the absence of a clear gap between slow and rapid reactivity.

Despite further extensive screening of biochemical characteristics, no clear-cut, unambiguous differentiation was

possible between the two DNA groups. Therefore, it is suggested that the biovar designations should no longer be used. There is no association between DNA groups and the former biovar designations, in contrast to our previous results (De Baere *et al.*, 2001). In this study, DNA–DNA hybridization was repeated by using the method of Ziemke *et al.* (1998) and a lack of reciprocity for the data was found: when DNA group 1 strain LMG 7160 was labelled, the values obtained for replicate testing of DNA–DNA hybridization with a strain of DNA group 2 were 77.1 and 77.5%, whereas reciprocal hybridization with DNA group 2 strain CCUG 6389<sup>T</sup> as the labelled strain resulted in values of 44.9 and 51.7% (Table 3).

### ***R. solanacearum***

*R. solanacearum* strains tested in this study could be differentiated from other *Ralstonia* species by acidification/assimilation of sucrose, lack of pyrrolidonyl arylamidase and assimilation of caprate, malonate, propionate, suberate, acetate and lactate. *R. solanacearum* has been described as non-motile (Coenye *et al.*, 1999; De Baere *et al.*, 2001) and the two strains studied here were non-motile and no flagella were observed; however, other sources mention the presence of one to four polar flagella (Krieg & Holt, 1984; Tans-Kersten *et al.*, 2001) and of flagellin genes and the occasional observation of high motility in culture (Tans-Kersten *et al.*, 2001). Tans-Kersten *et al.* (2001) showed that only 1–10% of the cell population is flagellated at times, which possibly explains several studies that have reported the absence of flagellation. It is concluded that *R. solanacearum* strains have polar flagella, as observed in strains of other *Ralstonia* species.

### ***R. syzygii***

*R. syzygii* comb. nov. has been recognized previously by others as a genuine *Ralstonia* species, rather than being a member of the genus *Pseudomonas* (Seal *et al.*, 1993; Taghavi *et al.*, 1996; Anzai *et al.*, 2000). In a previous study (De Baere *et al.*, 2001), it was shown that the 16S rRNA gene sequences obtained for *R. syzygii* strains LMG 6969, LMG 6970, LMG 10661<sup>T</sup> and LMG 10662 clustered with the *R. solanacearum* GenBank sequence (accession no. X67036) with >99% similarity. In this study, DNA–DNA hybridization indicated that *R. solanacearum* and *R. syzygii* are clearly two separate species (Table 3). Based on these combined data, it is proposed that *Pseudomonas syzygii* is renamed *Ralstonia syzygii* comb. nov.

### **Emended description of *Ralstonia insidiosa* Coenye *et al.* 2003**

*Ralstonia insidiosa* (in.si.di.o'sa. L. fem. adj. *insidiosa* deceitful, dangerous, referring to the fact that these seemingly harmless environmental organisms can be isolated from, and possibly cause infections in, humans).

The description is based on the data of Coenye *et al.* (2003a) and this study. Cells are Gram-negative, non-sporulating,

aerobic, non-fermentative, motile rods with one to three polar flagella. Aerobic growth is observed at 28, 32 and 37 °C and on *Burkholderia cepacia*-selective agar. Catalase, oxidase, lipase, phosphatase, proline aminopeptidase, pyrrolidonyl aminopeptidase and  $\gamma$ -L-glutamyl aminopeptidase activities are present. However, alkaline phosphatase is negative when Rosco tablets are used. No lysine decarboxylase, arginine dihydrolase, gelatinase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, tryptophan aminopeptidase or *N*-benzyl-arginine aminopeptidase activities are detected. Indole is not produced. No acid is produced from sucrose or mannitol. The original description (Coenye *et al.*, 2003a) mentions a lack of acid production from glucose, but in this study, oxidative acid production was observed from glucose, L-arabinose and xylose. Acid production from lactose is variable. Glucose, gluconate, caprate, adipate, malate and citrate are assimilated, but L-arabinose, mannose, mannitol and maltose are not. Alkalinization occurs on minimal mineral agar with acetate, allantoin, lactate and malonate, but not with galacturonate, oxalate or maleate. Nitrate reduction is negative. Resistant to colistin and desferrioxamine. Two biovars can be distinguished: strains of biovar 1 are negative for acid production from and assimilation of *N*-acetylglucosamine, gelatin hydrolysis and mucate on Simmons' base agar and positive for adipate on API 20NE; biovar 2 strains have the opposite characteristics. DNA G + C content is 63.9–64.3 mol%.

The type strain is LMG 21421<sup>T</sup> (= CCUG 46789<sup>T</sup>), which was isolated from the sputum of a patient with acute lymphoblastic leukaemia in the USA in 2001. Its DNA G + C content is 64.3 mol%. Phenotypic characteristics are the same as described above for the species. The type strain has no urease activity, does not assimilate *N*-acetylglucosamine and assimilates phenylacetate. The GenBank accession number for the 16S rRNA gene sequence of the type strain is AF488779.

### **Description of *Ralstonia syzygii* (Roberts *et al.* 1990) comb. nov.**

Basonym: *Pseudomonas syzygii* Roberts *et al.* 1990.

*Ralstonia syzygii* (sy.zy'gi.i. N.L. n. *Syzygium* generic name of the clove tree; L. gen. n. *syzygii* of the genus *Syzygium*).

The description is based on the data of Roberts *et al.* (1990b), De Baere *et al.* (2001) and this study. Gram-negative, non-sporulating, non-capsulated, non-motile, straight rods with rounded ends (0.5–0.6 × 1.0–2.5  $\mu$ m) that occur singly, in pairs or occasionally in short chains. Aerobic. Growth is poor (colonies of <1 mm after 7 days) or absent on many common bacteriological media. Good growth (colonies up to 5 mm after 7–12 days) occurs on the following iron salts-containing media: buffered charcoal yeast extract agar, Periwinkle wilt medium and iron-supplemented casamino acids medium. Growth on complex media is accompanied by a rise in pH. Optimum

temperature for growth is around 28 °C. No growth occurs at 4 or 37 °C. Resistant to colistin and susceptible to desferrioxamine. All strains are oxidase- and catalase-positive, grow at pH 6.0–7.5 and utilize fumarate, DL-malate, pyruvate and succinate. All strains are negative for levan, arginine dihydrolase and lecithinase, do not hydrolyse aesculin or starch, do not grow in the presence of 1 % NaCl and do not utilize *p*-alanine, L-arginine, L-cysteine, glycine, L-leucine, L-lysine, L-methionine, L-phenylalanine, DL-serine, L-threonine, L-tryptophan, L-valine, L-arabinose, D-cellobiose, D-galactose, lactose, maltose, D-mannose, D-raffinose, D-rhamnose, D-ribose, D-xylose, acetate, DL-lactate, D-tartrate, *m*-erythritol, glycerol, mannitol or sorbitol. Most strains (>85 %) grow at pH 5.0 and utilize L-aspartate, L-glutamate, L-tyrosine, D-glucose and sucrose. Variable results are obtained for tyrosinase activity, growth on 0.5 % NaCl at pH 8.0, utilization of L-asparagine, L-histidine, fructose, citrate and 2-oxoglutarate and nitrate reduction. All strains possess alkaline and acid phosphatases, esterase, esterase lipase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but do not possess trypsin, chymotrypsin,  $\alpha$ - or  $\beta$ -galactosidases,  $\beta$ -glucuronidase,  $\alpha$ - or  $\beta$ -glucosidases, *N*-acetyl- $\beta$ -glucosamidase,  $\alpha$ -mannosidase or  $\alpha$ -fucosidase, as determined by API ZYM test strips. Major cellular fatty acids are C<sub>14:0</sub>, C<sub>14:0</sub> 3-OH, C<sub>16:1</sub> $\omega$ 9c, C<sub>16:0</sub>, C<sub>17:0</sub> cyclo, C<sub>18:1</sub> $\omega$ 11c, C<sub>19:0</sub> cyclo and C<sub>18:1</sub> 2-OH. DNA G+C content is 66–67 mol% (buoyant density method).

The type strain is R001<sup>T</sup> (=ATCC 49453<sup>T</sup>=LMG 10661<sup>T</sup>=NCPBB 3446<sup>T</sup>). Isolated as a phytopathogen from xylem tissues of the clove tree (*Syzygium aromaticum*) and other *Syzygium* spp., and from insect vectors (*Hindola* spp.) in Indonesia. The GenBank accession number of the 16S rRNA gene sequence of the type strain is AB021403.

### The *R. eutropha* lineage (*Wautersia* gen. nov.)

The *R. eutropha* lineage (*Wautersia* gen. nov.) contains the species *Wautersia basilensis*, *Wautersia campinensis*, *Wautersia eutropha*, *Wautersia gilardii*, *Wautersia metallidurans*, *Wautersia oxalatica*, *Wautersia paucula*, *Wautersia respiraculi* and *Wautersia taiwanensis*. Phenotypic data gathered during this study and from the literature are listed in Supplementary Table A3 (available in IJSEM Online).

### Description of *Wautersia* gen. nov.

*Wautersia* (Wau.ter'si.a. L. fem. n. *Wautersia* named in honour of the Belgian microbiologist Georges Wauters).

Gram-negative rods that are motile by means of peritrichous flagella. Aerobic. Forms smooth colonies that reach 1–2 mm within 48 h at 30 °C on blood agar. Positive for catalase and oxidase. Glucose is neither acidified nor assimilated. Susceptible to colistin. Cellular fatty acids are of the saturated and monounsaturated straight-chain types,

mainly C<sub>16:1</sub> $\omega$ 9c, C<sub>16:0</sub>, C<sub>18:1</sub> $\omega$ 11c and C<sub>14:0</sub>. The type species of the genus is *Wautersia eutropha*.

### *Wautersia basilensis* (Steinle *et al.* 1998) comb. nov.

*W. basilensis* (Steinle *et al.*, 1998) is the only *Wautersia* species that is susceptible to desferrioxamine. Like *W. paucula*, it does not alkalinize allantoin and, unlike most other *Wautersia* species, it does not alkalinize oxalate.

### *Wautersia eutropha* (Davis 1969) Yabuuchi *et al.* 1996 comb. nov.

*W. eutropha* (Yabuuchi *et al.*, 1995) can be differentiated from other *Wautersia* species by its ability to assimilate L-serine, *N*-acetylglucosamine and 2-ketogluconate and its inability to alkalinize mucate on Simmons' agar base.

### *Wautersia gilardii* (Coenye *et al.* 1999) comb. nov.

*W. gilardii* (Coenye *et al.*, 1999) can be distinguished from *W. paucula* by its lack of urease and Tween esterase activities, from *W. eutropha* by alkalinization of mucate and lack of assimilation of *N*-acetylglucosamine, L-serine and 2-ketogluconate, and from *W. basilensis* by resistance to desferrioxamine, alkalinization of allantoin and assimilation of phenylacetate.

### *Wautersia paucula* (Vandamme *et al.* 1999) comb. nov.

In a previous study (De Baere *et al.*, 2001), it was shown that the available 16S rDNA sequences of *Ralstonia* group CDC IVc-2 strains [GenBank accession nos AF098288 (Moissenet *et al.*, 1999) and AF067657 (Osterhout *et al.*, 1998)] were identical to that of *W. paucula* (Vandamme *et al.*, 1999; accession no. AF085226). *W. paucula* strains differ from both *W. eutropha* and *W. gilardii* by strong urease production and a lack of alkalinization of allantoin, from *W. gilardii* by marked Tween esterase activity and from *W. eutropha* by alkalinization of mucate and absence of assimilation of *N*-acetylglucosamine, L-serine and 2-ketogluconate. It differs from *W. basilensis* by resistance to desferrioxamine and Tween esterase activity.

### *Wautersia campinensis* (Goris *et al.* 2001) comb. nov., *Wautersia metallidurans* (Goris *et al.* 2001) comb. nov., *Wautersia oxalatica* (ex Khambata and Bhat 1953) Sahin *et al.* 2000 comb. nov., *Wautersia respiraculi* (Coenye *et al.* 2003) comb. nov. and *Wautersia taiwanensis* (Chen *et al.* 2001) comb. nov.

The newly described species *W. campinensis*, *W. metallidurans*, *W. oxalatica*, *W. respiraculi* and *W. taiwanensis* cluster in the genus *Wautersia*, according to their 16S rRNA gene sequences (Fig. 1). From the literature (Sahin *et al.*, 2000; Chen *et al.*, 2001; Goris *et al.*, 2001; Coenye *et al.*, 2003b), it

is clear that where characteristics useful for the differentiation of *Ralstonia* and *Wautersia* species were given, i.e. flagellation, sugar acidification and colistin susceptibility, these indicated that the species should be classified as *Wautersia* species. The following characteristics were tested in this study: flagellation, acid production from carbohydrates, colistin susceptibility and survival at room temperature on solid agar (except for *W. respiraculi*). It was confirmed that the characteristics of these species corresponded with those that were determined previously as being characteristic of the genus *Wautersia* (Table 2).

#### **Description of *Wautersia basilensis* (Steinle et al. 1998) comb. nov.**

Basonym: *Ralstonia basilensis* Steinle et al. 1998.

*Wautersia basilensis* (ba.si.len'sis. L. fem. adj. *basilensis* from *Basilea* (Basel, Switzerland), where the strain was isolated).

The description is based on the data of Steinle et al. (1998) and this study. Cells are short rods ( $0.8 \times 1.2\text{--}2.2 \mu\text{m}$ ) that occur singly, in pairs or in short chains, are motile by means of peritrichous flagella and form round (sometimes with a slightly scalloped margin), smooth, convex and transparent colonies of about 0.5 mm diameter after 24 h incubation on TSA at 30 °C. Oxidase- and catalase-positive. Aerobic growth occurs at 4, 20, 30 and 37 °C, but no growth is detected at 41 °C. Variable for nitrate reduction, but no nitrite reduction occurs. No indole is produced from tryptophan. Urease activity is delayed. No acid is produced from glucose. Enzyme activities detected are: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase (weak) and naphthol-AS-BI-phosphohydrolase. Enzyme activities that are not detected are: arginine dihydrolase,  $\alpha$ -glucosidase, protease,  $\beta$ -galactosidase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase. Assimilates D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. Does not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine or maltose. Susceptible to colistin and desferrioxamine. DNA G+C content is 65.0–65.5 mol%. Major fatty acid components are C<sub>14:0</sub> (4.5%), C<sub>16:0</sub> (20.6%), C<sub>17:0</sub> cyclo (1.3%), C<sub>18:1</sub> $\omega$ 7c (24.7%), C<sub>16:1</sub> 2-OH (1.8%), C<sub>18:1</sub> 2-OH (4.8%), summed feature 2 ( $9 \pm 0\%$ ) and summed feature 3 ( $32 \pm 9\%$ ).

The type strain is RK1<sup>T</sup> (=DSM 11853<sup>T</sup>=LMG 18990<sup>T</sup>=LMG 19474<sup>T</sup>=DSM 11853<sup>T</sup>), which was isolated from a freshwater pond sediment at Amponville, France, after enrichment in a fixed-bed reactor with 2,6-dichlorophenol as the sole carbon and energy source. Its DNA G+C content is 65.0 mol% and its phenotypic characteristics are as described above for the species. The GenBank accession number of the 16S rRNA gene sequence of the type strain is AF312022.

#### **Description of *Wautersia campinensis* (Goris et al. 2001) comb. nov.**

Basonym: *Ralstonia campinensis* Goris et al. 2001.

*Wautersia campinensis* (cam.pin.en'sis. L. fem. adj. *campinensis* of the Kempen or Campine, the geographical region of north-east Belgium where the strains were originally isolated).

The description is based on the data of Goris et al. (2001) and this study. Cells are short rods ( $0.8 \times 1.2\text{--}1.8 \mu\text{m}$ ) that occur singly, in pairs or in short chains, are motile by means of peritrichous flagella and form round (sometimes with a slightly scalloped margin), smooth, convex and transparent colonies of about 0.5 mm diameter after 24 h incubation on TSA at 30 °C. Oxidase- and catalase-positive. Aerobic growth occurs at 20, 30, 37 and 41 °C, but not at 4 °C. Nitrate is reduced, but nitrite is not. No indole is produced from tryptophan. No acid is produced from glucose. Enzyme activities detected are: urease, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Enzyme activities that are not detected are: arginine dihydrolase, protease,  $\beta$ -galactosidase, lipase (C14), cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase.  $\alpha$ -Glucosidase is variable. Assimilates D-gluconate, caprate, adipate, L-malate and phenylacetate. No assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose or citrate. Major fatty acid components are C<sub>14:0</sub> (5.0%), C<sub>16:0</sub> (24.6%), C<sub>17:0</sub> cyclo (6.1%), C<sub>18:0</sub> (1.5%), C<sub>18:1</sub> $\omega$ 7c (19.5%), C<sub>14:0</sub> 2-OH (2.1%), C<sub>18:1</sub> 2-OH (2.7%), summed feature 2 (8.9%) and summed feature 3 (28.4%).

The type strain is WS2<sup>T</sup> (=LMG 19282<sup>T</sup>=CCUG 44526<sup>T</sup>), which was isolated from a zinc-desertified area in Lommel, Belgium. Its DNA G+C content is 66.6 mol% and its phenotypic characteristics are as described above for the species. The GenBank accession number of the 16S rRNA gene sequence of the type strain is AF312020.

#### **Description of *Wautersia eutropha* (Davis 1969) Yabuuchi et al. 1996 comb. nov.**

Basonym: *Alcaligenes eutrophus* Davis 1969.

The description is based on the data of Yabuuchi et al. (1995) and De Baere et al. (2001). Gram-negative, non-sporulating rods that are motile by means of peritrichous flagella. Grows aerobically at 30 and 37 °C on TSA. Catalase- and oxidase-positive. Nitrate is reduced. Urease may be produced upon exhaustion of other nitrogen sources. Indole is not produced. Gelatin and aesculin are not hydrolysed. No decarboxylation of lysine and ornithine occurs. No arginine dihydrolase activity. No acidification or assimilation of glucose occurs. Susceptible to colistin and resistant to desferrioxamine. Alkaline phosphatase and

pyrrolidone peptidase are positive. The main cellular fatty acids are C<sub>18:1</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>17:0</sub> cyclo and C<sub>14:0</sub>.

The type strain is ATCC 17697<sup>T</sup> (=CCUG 1776<sup>T</sup>=DSM 531<sup>T</sup>=LMG 1199<sup>T</sup>). Its G+C content is 66.5 mol%. The GenBank accession number of the 16S rRNA gene sequence of the type strain is M32021.

**Description of *Wautersia gilardii* (Coenye et al. 1999) comb. nov.**

Basonym: *Ralstonia gilardii* Coenye et al. 1999.

*Wautersia gilardii* (gi.lar'di.i. N.L. gen. n. *gilardii* in honour of G. L. Gilardi, an American microbiologist).

The description is based on the data of Coenye et al. (1999), De Baere et al. (2001) and Wauters et al. (2001). Gram-negative, non-sporulating rods that are motile by means of peritrichous flagella. This was shown clearly by Wauters et al. (2001), in contrast to a previous report by Coenye et al. (1999) that reported polar flagellation. Aerobic growth occurs at 30, 37 and 42 °C. Catalase- and oxidase-positive. Nitrate reduction is variable. No denitrification occurs. No urease, β-galactosidase or DNase activities are detected. No liquefaction of gelatin or hydrolysis of aesculin occurs. Indole is not produced. No acidification or assimilation of carbohydrates occurs. Positive for alkaline phosphatase (Rosco) and weakly positive for pyrrolidonyl arylamidase. Susceptible to colistin and resistant to desferrioxamine. The main fatty acid components are: C<sub>14:0</sub>, C<sub>16:0</sub>, C<sub>17:0</sub> cyclo, C<sub>18:0</sub>, C<sub>16:1ω7c</sub>, C<sub>16:0</sub> 2-OH and C<sub>19:0ω8c</sub> cyclo. DNA G+C content is between 68 and 69 mol%.

The type strain is Gilardi 4325<sup>T</sup> (=ATCC 700815<sup>T</sup>=CCUG 38401<sup>T</sup>=LMG 5886<sup>T</sup>), which was isolated from a whirlpool. The DNA G+C content of the type strain is 68.3 mol%. The GenBank accession number of the 16S rRNA gene of the type strain is AF076645.

**Description of *Wautersia metallidurans* (Goris et al. 2001) comb. nov.**

Basonym: *Ralstonia metallidurans* Goris et al. 2001.

*Wautersia metallidurans* (me.tal.li.du'rans. L. n. *metallum* metal; L. pres. part. *durans* enduring; N.L. part. adj. *metallidurans* enduring metal, from the fact that these strains are able to survive high heavy-metal concentrations).

The description is based on the data of Goris et al. (2001) and this study. Cells are short rods (0.8 × 1.2–2.2 μm) that occur singly, in pairs or in short chains, are motile by means of peritrichous flagella and form round (sometimes with a slightly scalloped margin), smooth, flat, convex and transparent colonies of about 0.5 mm diameter after 24 h incubation on TSA at 30 °C. Oxidase- and catalase-positive. Aerobic growth occurs at 20, 30 and 37 °C, but no growth is detected at 4 or 41 °C. Reduction

of nitrate and nitrite is variable. No indole is produced from tryptophan. No acid is produced from glucose. Enzyme activities detected are: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Enzyme activities that are not detected: arginine dihydrolase, α-glucosidase, protease, β-galactosidase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Urease activity can be weak or absent. Assimilates D-gluconate, adipate and L-malate. Assimilation of caprate, citrate and phenylacetate is variable. No assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine or maltose occurs. DNA G+C content is 63.7–63.9 mol%. Major fatty acid components are C<sub>14:0</sub> (4.4%), C<sub>16:0</sub> (20.9%), C<sub>17:0</sub> cyclo (3.2%), C<sub>18:1ω7c</sub> (19.8%), C<sub>16:0</sub> 2-OH (3.3%), C<sub>18:1</sub> 2-OH (1.4%), summed feature 2 (11.3%) and summed feature 3 (34.2%).

The type strain is CH 34<sup>T</sup> (=CIP 107179<sup>T</sup>=DSM 2839<sup>T</sup>=LMG 1195<sup>T</sup>), which was isolated from the wastewater of a zinc factory at Liège, Belgium. Its DNA G+C content is 63.7 mol%. The GenBank accession number of the 16S rRNA gene of the type strain is Y10824.

**Description of *Wautersia oxalatica* (ex Khambata and Bhat 1953) Sahin et al. 2000, comb. nov.**

Basonym: *Pseudomonas oxalaticus* Khambata and Bhat 1953.

*Wautersia oxalatica* (o.xa.la'ti.ca. N.L. fem. adj. *oxalatica* pertaining to oxalate).

The description is based on the data of Sahin et al. (2000) and this study. Gram-negative, non-sporulating, aerobic, short rods (0.3–0.4 × 0.9–1.5 μm) that are motile with peritrichous flagella. Colonies are cream-coloured and no carotenoid pigment is produced. Catalase- and oxidase-positive. Optimum growth with oxalate is at pH 6.6 at 25 °C. In liquid culture, doubling time with oxalate is 4.5 h. Unable to grow autotrophically with hydrogen. Assimilation of oxalate follows the glycolate pathway. Organic acids, except tartrate and aconitate, are used as carbon sources. Nitrate is reduced to nitrite. Urea is hydrolysed, but casein, starch and gelatin are not. Indole and hydrogen sulphide are not produced. Grows well on lactate and formate, but not on glucose. Able to use phenol, ethanol and ethylene glycol as carbon sources. Susceptible to colistin.

The type strain is Ox 1<sup>T</sup> (=ATCC 11883<sup>T</sup>=CCUG 2086<sup>T</sup>=DSM 1105<sup>T</sup>=LMG 2235<sup>T</sup>), which was isolated from the alimentary tract of an Indian earthworm. Its DNA G+C content is 68 mol% (*T<sub>m</sub>* method) or 67% (buoyant density method). The GenBank accession number of the 16S rRNA gene sequence of the type strain is AF155567.



**Description of *Wautersia paucula* (Vandamme et al. 1999) comb. nov.**

Basonym: *Ralstonia paucula* Vandamme et al. 1999.

*Wautersia paucula* (pau'cu.la. L. fem. adj. *paucula* rare, very few, to indicate that these strains only cause human infections sporadically).

The description is based on the data of Vandamme et al. (1999) and De Baere et al. (2001). Gram-negative, non-sporulating and rod-shaped. Cells are about  $0.8 \times 1.2$ – $2.0 \mu\text{m}$  after 24 h growth on TSA at  $30^\circ\text{C}$ . Motile by means of peritrichous flagella. Strains produce convex, circular, non-pigmented colonies with an entire edge and a smooth surface. Catalase- and oxidase-positive. No haemolysis occurs on horse blood agar. Aerobic growth occurs at 30, 37 and  $42^\circ\text{C}$ . No acid is produced from D-glucose, maltose, adonitol, D-fructose or D-xylose. Grows in the presence of 0.5 and 1.5 % NaCl, but not in the presence of cetrимide, 10 % lactose, penicillin (10  $\mu\text{g}$  discs), or 3, 4.5 or 6 % NaCl. Susceptible to colistin. Grows on Drigalski agar. Hydrolyses Tween 80. No fluorescence occurs on King B medium. Negative for lysine and ornithine decarboxylases and arginine dihydrolase activities. No amylase activity is detected. No reduction of nitrate or nitrite. Hydrolysis of urea, but not of gelatin or aesculin, occurs. No  $\beta$ -galactosidase or DNase activities are detected. Indole is not produced. No production of hydrogen sulfide or acid occurs in triple-sugar iron agar. No tryptophanase activity is detected. Assimilates D-gluconate, caprate, adipate, L-malate, citrate, phenylacetate and DL-lactate, but not D-glucose, trehalose, L-arginine, DL-norleucine, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose or sucrose. Alkaline and acid phosphatase, esterase C4, ester lipase C8, lipase C14, leucine, cystine and pyrrolidonyl arylamidases and phosphoamidase are present. However, alkaline phosphatase is variable when Rosco tablets are used. Valine arylamidase, trypsin, chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ - and  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase,  $\beta$ -mannosidase and  $\beta$ -fucosidase activities are not detected. Isolated from a variety of human clinical sources including blood, wounds, sputum, urine, eye, throat and peritoneal fluid, as well as pool water, groundwater and bottled mineral water. DNA G+C content is 65–67 mol%.

The type strain is ATCC 700817<sup>T</sup> (=CCUG 12507<sup>T</sup>=LMG 3244<sup>T</sup>), which was isolated from a human respiratory tract in the USA. Its DNA G+C content is 67 mol%. The GenBank accession number of the 16S rRNA gene sequence of strain LMG 3413 is AF085226. The sequence of the type strain has apparently not been submitted to GenBank.

**Description of *Wautersia respiraculi* (Coenye et al. 2003) comb. nov.**

Basonym: *Ralstonia respiraculi* Coenye et al. 2003.

*Wautersia respiraculi* (re.spi.ra'cu.li. L. n. *respiraculum*

breathing, respiration; L. gen. n. *respiraculi* of the respiratory system).

The description is based on the data of Coenye et al. (2003b). Gram-negative, non-fermentative, non-sporulating, motile rods. Growth is observed at 28, 32 and  $37^\circ\text{C}$ . Catalase- and oxidase-positive. No lysine decarboxylase, urease,  $\beta$ -galactosidase or lipase activities are detected. No indole is produced. No acid is produced from glucose, sucrose or lactose in O/F medium. Assimilates gluconate, caprate, adipate and malate, but not glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, citrate or phenylacetate. The following fatty acids are present: C<sub>14:0</sub>, C<sub>14:0</sub> 3-OH, C<sub>16:1</sub> $\omega$ 7c, C<sub>16:0</sub>, C<sub>17:0</sub> cyclo, C<sub>16:0</sub> 2-OH, C<sub>18:1</sub> $\omega$ 7c and C<sub>18:1</sub> 2-OH.

The type strain is AU3313<sup>T</sup> (=CCUG 46809<sup>T</sup>=LMG 21510<sup>T</sup>), which was isolated from the sputum of a person with cystic fibrosis in the USA in 2001. Phenotypic characteristics are the same as described above for the species. In addition, the type strain has phosphatase and  $\alpha$ -glucosidase activities and reduces nitrate, but is lipase-negative. The GenBank accession number for the 16S rRNA gene sequence of the type strain is AF500583.

**Description of *Wautersia taiwanensis* (Chen et al. 2001) comb. nov.**

Basonym: *Ralstonia taiwanensis* Chen et al. 2001.

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

The description is based on the data of Chen et al. (2001) and this study. Gram-negative, non-sporulating and rod-shaped; cells are about  $0.5$ – $0.7 \times 0.8$ – $2.0 \mu\text{m}$  after 24 h growth on TSA at  $30^\circ\text{C}$ . Motile by means of peritrichous flagella. Aerobic growth is observed at 28, 30 and  $37^\circ\text{C}$ . Catalase- and oxidase-positive. Nitrate is reduced. Aesculin is hydrolysed. Susceptible to colistin. No urease,  $\beta$ -galactosidase or DNase activities are detected. No indole is produced. No acid is produced from glucose. No autotrophic growth occurs. 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.

The type strain is R1<sup>T</sup> (=CCUG 44338<sup>T</sup>=LMG 19424<sup>T</sup>), which was isolated from a root nodule of *Mimosa pudica*. Its DNA G+C content is 67.3 mol%. The GenBank accession number of the 16S rRNA gene of the type strain is AF300324.

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