Ralstonia respiraculi sp. nov., isolated from the respiratory tract of cystic fibrosis patients

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Five isolates recovered from the respiratory tract of cystic fibrosis patients were included in a polyphasic taxonomic study that employed 16S rDNA sequence analysis, cellular protein and fatty acid analysis and biochemical characterization. Four isolates were classified as a novel *Ralstonia* species, for which the name *Ralstonia respiraculi* sp. nov. is proposed; the other isolate was phylogenetically closely related to *R. respiraculi*, but is likely to represent another novel *Ralstonia* species. The type strain of *R. respiraculi* is AU3313^T (=LMG 21510^T=CCUG 46809^T).

Individuals with the inherited disease cystic fibrosis (CF) are susceptible to a plethora of potentially life-threatening respiratory infections. It has been suggested that this is due to the fact that the respiratory system of a CF patient is an ecological niche that is suitable for growth of a wide variety of bacteria (Coenye *et al.*, 2002a). While *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex organisms are typical CF pathogens (Gilligan, 1991), *Burkholderia gladioli*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, members of the *Enterobacteriaceae* and various *Ralstonia* species can also be isolated from respiratory secretions of CF patients (Burns *et al.*, 1998; Coenye *et al.*, 2002a, b).

At the time of writing, the genus *Ralstonia* contains twelve species with validly published names: *Ralstonia pickettii* (the type species), *Ralstonia solanacearum*, *Ralstonia eutropha* (Yabuuchi *et al.*, 1995), *Ralstonia basilensis* (Steinle *et al.*, 1998), *Ralstonia gilardii* (Coenye *et al.*, 1999), *Ralstonia paucula* (Vandamme *et al.*, 1999), *Ralstonia oxalatica* (Sahin *et al.*, 2000), *Ralstonia mannitolilytica* (De Baere *et al.*, 2001), *Ralstonia campinensis*, *Ralstonia metallidurans* (Goris *et al.*, 2001), *Ralstonia taiwanensis* (Chen *et al.*, 2001) and *Ralstonia insidiosa* (Coenye *et al.*, 2003). *R. pickettii*, *R. mannitolilytica*, *R. gilardii*, *R. taiwanensis* and *R. insidiosa* have been isolated from various clinical samples, including respiratory secretions of CF patients (Burns *et al.*, 1998; Chen *et al.*, 2001; Coenye *et al.*, 2002a, b). In addition, a number of unidentified *Ralstonia* sp. isolates have been recovered from CF patients (Coenye *et al.*, 2002a, b). Here, we report on the polyphasic taxonomic study of five such *Ralstonia* sp. isolates that were recovered from the respiratory tract of CF patients.

The five strains studied (AU0626, AU1618, AU3313^T, AU3801 and AU3369) were isolated from different CF patients who were receiving care in five CF treatment centres in three different US states. Dates of isolation were between December 1997 and January 2002. Reference strains of other *Ralstonia* species have been described previously (Coenye *et al.*, 1999, 2003; Vandamme *et al.*, 1999; Chen *et al.*, 2001; De Baere *et al.*, 2001; Goris *et al.*, 2001). Strains were grown aerobically on Mueller–Hinton broth (Becton Dickinson) supplemented with 1·8 % (w/v) agar and incubated overnight at 32 °C, unless otherwise mentioned. Preparation of DNA, amplification of the 16S rRNA gene by PCR and 16S rDNA sequencing was performed as described previously (Coenye *et al.*, 2002a). Preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot *et al.* (1994). Strains were grown for 48 h on Trypticase Soy Agar (BBL) and incubated at 37 °C. Densitometric analysis, normalization and interpolation of protein profiles and numerical analysis were performed by using GelCompar 4.2 software (Applied Maths). Cellular fatty acid analysis and conventional biochemical testing were performed as described by Coenye *et al.* (1999 and 2003, respectively). RapID NF Plus (Remel) and API 20NE (bioMérieux) commercial identification systems were used according to the recommendations of the manufacturers. Species-specific 16S rDNA-based PCR assays for the identification of *Burkholderia–Ralstonia–Pandoraea* sp.,

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Abbreviation: CF, cystic fibrosis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AU3313^T, AU3801, AU0626, AU1618 and AU3369 are AF500583, AF500584, AF500585, AF500586 and AF500587, respectively.

Protein profiles of the *R. respiraculi* strains, strain AU3369 and reference strains of other *Ralstonia* species are available as supplementary material in IJSEM Online.

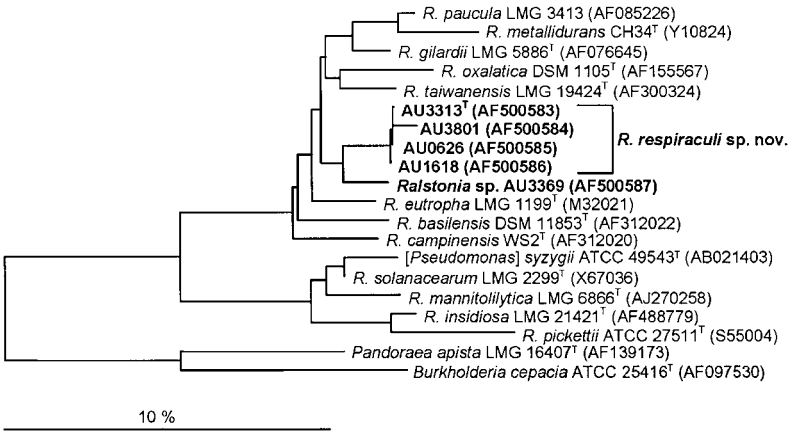

Fig. 1. Phylogenetic tree (based on 16S rDNA sequences) showing the position of *R. respiraculi* and *Ralstonia* sp. AU3369 within the genus *Ralstonia*. Bar, 10 % sequence dissimilarity.

R. pickettii, *R. mannitolilytica*, *R. insidiosa* and *A. xylosoxidans* were performed as described previously (LiPuma *et al.*, 1999; Coenye *et al.*, 2002b, 2003; Liu *et al.*, 2002).

The 16S rRNA genes of strains AU0626, AU1618, AU3313^T and AU3801 showed high sequence similarity to each other (mean similarity value, 98·8 %) and to the 16S rRNA gene of strain AU3369 (mean similarity value, 98·3 %). Comparison of these sequences with 16S rRNA gene sequences available in GenBank indicated that they belonged to the genus *Ralstonia* (Fig. 1). Sequence similarity values to reference strains of *R. eutropha*, *R. taiwanensis*, *R. paucula* and *R. campinensis* were between 97·2 and 96·1 %, whilst similarity to the 16S rRNA genes of other *Ralstonia* species was $<$95·4 % (Fig. 1). Visual comparison of the whole-cell protein profiles (see supplementary material in IJSEM Online) indicated that strains AU0626, AU1618, AU3313^T and AU3801 were characterized by highly similar protein patterns, whereas strain AU3369 and reference strains of other *Ralstonia* species showed clearly different protein patterns.

The cellular fatty acid compositions of strains AU0626, AU1618, AU3313^T and AU3801 were very similar and the following fatty acids were present in all four strains (mean $\pm$ SD): $C_{14:0}$ (4·49 $\pm$ 0·51 %), $C_{14:0}$ 3-OH (8·56 $\pm$ 0·75 %), $C_{16:1}\omega7c$ (31·23 $\pm$ 3·85 %), $C_{16:0}$ (23·56 $\pm$ 3·61 %), $C_{17:0}$ cyclo (7·42 $\pm$ 3·19 %), $C_{16:0}$ 2-OH (1·21 $\pm$ 0·24 %), $C_{18:1}\omega7c$ (20·38 $\pm$ 5·26 %) and $C_{18:1}$ 2-OH (1·85 $\pm$ 0·53 %). Trace amounts ($<$1·0 %) of $C_{14:0}$ 2-OH and $C_{18:0}$ were also present. The fatty acid composition of strain AU3369 was very similar to those of strains AU0626, AU1618, AU3313^T and AU3801 (data not shown).

All strains grew at 28, 32 and 37 °C. Growth on *B. cepacia*-selective agar (BCSA) was not observed. All strains showed oxidase, catalase, pyrrolidonyl aminopeptidase and γ-L-glutamyl aminopeptidase activities and assimilated gluconate, caprate, adipate and malate. Lysine decarboxylase, arginine dihydrolase, urease, lipase, β-glucosidase, gelatinase, β-galactosidase, tryptophan aminopeptidase, *N*-benzylarginine aminopeptidase, proline aminopeptidase, tryptophan aminopeptidase and *N*-acetylglucosaminidase activities were not observed. None of the strains assimilated glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, citrate or phenylacetate. Indole production and production of acid from glucose, sucrose or lactose were not observed. Nitrate reduction and the presence of lipase, phosphatase and α-glucosidase activity were strain-dependent characteristics. By using the API 20NE system, strains were either identified with a low score as *Alcaligenes faecalis*, *Comamonas testosteroni*, *Pseudomonas alcaligenes* or *Comamonas acidovorans* (for strains that reduced nitrate, profile 1000474), or were identified with a low score as *R. paucula*, *Alcaligenes faecalis*, *Comamonas testosteroni* or *Pseudomonas alcaligenes* (for strains that did not reduce nitrate, profile 0000474). No adequate identification was obtained by using the RapID NF Plus system. None of the five strains gave a positive result with the PCR assays developed for the identification of *R. pickettii*, *R. mannitolilytica*, *R. insidiosa* or *A. xylosoxidans*, but all gave positive results in the *Burkholderia–Ralstonia–Pandoraea* PCR test.

We performed a polyphasic taxonomic study to determine the taxonomic position of five strains isolated from the respiratory tract of CF patients in the USA. 16S rDNA sequence analysis indicated that these strains were closely related to each other and belonged to the genus *Ralstonia*. Their closest phylogenetic neighbours were *R. eutropha* and *R. taiwanensis*, but mean sequence similarity to the type strains of these species was $<$97·2 %. Biochemical characteristics and cellular fatty acid compositions of these isolates were very similar, but the one-dimensional protein profile of isolate AU3369 was clearly different from those of the other four isolates. The profiles of the five strains investigated were clearly different from those of all other *Ralstonia* species. Our data clearly indicate that isolates AU0626, AU1618, AU3313^T and AU3801 belong to a single novel *Ralstonia* species, for which we propose the name *Ralstonia respiraculi* sp. nov. Based on 16S rDNA sequence analysis and SDS-PAGE of whole-cell proteins, isolate AU3369 probably constitutes a distinct *Ralstonia* species. However, we do not propose a formal name for this taxon,

Table 1. Characteristics that are useful for the differentiation of *R. respiraculi* and strain AU3369 from other *Ralstonia* species

Species: 1, *R. respiraculi*; 2, *Ralstonia* sp. AU3369; 3, *R. eutropha*; 4, *R. campinensis*; 5, *R. basilensis*; 6, *R. metallidurans*; 7, *R. taiwanensis*; 8, *R. paucula*; 9, *R. gilardii*; 10, *R. pickettii*; 11, *R. mannitolilytica*; 12, *R. solanacearum*; 13, *R. insidiosa*; 14, *R. oxalatica*. Data for *R. oxalatica* are taken from Gillis *et al.* (1995); data for *R. insidiosa* are taken from Coenye *et al.* (2003); data for all other *Ralstonia* species are taken from Coenye *et al.* (1999), Vandamme *et al.* (1999), Chen *et al.* (2001), De Baere *et al.* (2001) and Goris *et al.* (2001). Reactions are scored as: +, >90 % of strains investigated react positively; −, <10 % of strains investigated react positively; V, 10–90 % of strains investigated react positively.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Urease activity	−	−	−	+	−	−	+	+	−	+	+	−	V	V
Assimilation of:														
N-Acetylglucosamine	−	−	+	−	−	−	−	−	−	+	+	−	−	V
Citrate	−	−	+	−	+	+	+	+	−	+	+	+	+	+
Phenylacetate	−	−	+	+	+	+	+	+	V	+	−	−	+	+

pending the recovery of similar isolates and availability of biochemical tests to differentiate it from *R. respiraculi*.

Biochemical characteristics that are useful for the differentiation of *R. respiraculi* and *Ralstonia* sp. AU3369 from other *Ralstonia* species are given in Table 1. Differentiation from *A. xylosoxidans* is possible by lack of acidification of glucose (von Graevenitz, 1995). In contrast to *B. cepacia* complex species (Henry *et al.*, 2001), *R. respiraculi* and strain AU3369 do not show β-galactosidase or lysine decarboxylase activities and do not grow on BCSA. In addition to these biochemical characteristics, differentiation from other *Ralstonia* species that may be encountered in CF specimens (*R. pickettii*, *R. mannitolilytica* and *R. insidiosa*) and from *A. xylosoxidans* is possible by using the PCR-based assays that were described previously for the identification of these organisms (Coenye *et al.*, 2002b, 2003; Liu *et al.*, 2002).

Description of *Ralstonia respiraculi* sp. nov.

Ralstonia respiraculi (re.spi.ra′cu.li. L. n. *respiraculum* breathing, respiration; L. gen. n. *respiraculi* of breathing, of the respiratory system).

Cells are Gram-negative, non-fermentative, non-sporulating, motile rods. Growth is observed at 28, 32 and 37 °C. No growth is observed on BCSA. Catalase and oxidase activities are present. No lysine decarboxylase, urease, β-galactosidase or lipase activities are present. No indole production occurs. No production of acid from glucose, sucrose or lactose occurs in oxidation–fermentation medium. Gluconate, caprate, adipate and malate are assimilated but glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, citrate and phenylacetate are not. Additional characteristics are given above. The following fatty acids are present: $C_{14:0}$, $C_{14:0}$ 3-OH, $C_{16:1}\omega7c$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{16:0}$ 2-OH, $C_{18:1}\omega7c$ and $C_{18:1}$ 2-OH. Characteristics that differentiate *R. respiraculi* from other *Ralstonia* species are summarized in Table 1.

The type strain, AU3313^T, was isolated from the sputum of a CF patient in the USA in 2001. Phenotypic characteristics are the same as described above for the species. In addition, the type strain shows phosphatase and α-glucosidase activities but no lipase activity, and reduces nitrate. *R. respiraculi* strains AU3313^T and AU1618 have been deposited in the BCCM/LMG (Laboratorium voor Microbiologie Gent, Belgium) and CCUG (University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden) culture collections as LMG 21510^T (=CCUG 46809^T) and LMG 21509 (=CCUG 46808), respectively.

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