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 (Gillin, 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 eutropa (Yabuuchi et al., 1995), Ralstonia baselensis (Steinle et al., 1998), Ralstonia gilardii (Coenye et al., 1999), Ralstonia paucula (Vandamme et al., 1999), Ralstonia oxalata (Sahin et al., 2000), Ralstonia mannitolilytica (De Baere et al., 2001), Ralstonia campensis, 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 37 °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., 

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

Tom Coenye,1 Peter Vandamme2 and John J. LiPuma1

1Department of Pediatrics and Communicable Diseases, University of Michigan, 1150 W. Med. Ctr Dr, MSRB III, Rm 8323, Ann Arbor, MI 48109-0646, USA

2Laboratorium voor Microbiologie, Universiteit Gent, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium

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).

---

**Published online ahead of print on 24 January 2003 as DOI 10.1099/ ijs.0.02440-0.**

**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 ISEM Online.
The 16S rRNA genes of strains AU0626, AU1618, AU3313T and AU3801 showed high similarity sequence 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 rDNA 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, AU3313T 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, AU3313T and AU3801 were very similar and the following fatty acids were present in all four strains (mean ± SD): C₁₄:₀ (4.49 ± 0.51%), C₁₆:₀ 3-OH (8.56 ± 0.75%), C₁₆:₁iso10c (31.23 ± 3.85%), C₁₆:₁o16c (23.56 ± 3.61%), C₁₇:₁ cyclo (7.42 ± 3.19%), C₁₆:₂ 2-OH (1.21 ± 0.24%), C₁₈:₁iso17c (20.38 ± 5.26%) and C₁₈:₁ 2-OH (1.85 ± 0.53%). Trace amounts (<1.0%) of C₁₄:₀ 2-OH and C₁₆:₂ were also present. The fatty acid composition of strain AU3369 was very similar to those of strains AU0626, AU1618, AU3313T 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 glucose, lactate, citrate, malate, succinate or fructose. Indole production and production of acid from glucose, sucrose or lactose were not observed. Nitrate reduction and the presence of lipase, phosphatase and a-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–Pandora* 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, AU3313T 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, *R. pickettii*, *R. mannitolilytica*, *R. insidiosa* and *A. xylosoxidans* were proposed as described previously (LiPuma et al., 1999; Coenye et al., 2002b, 2003; Liu et al., 2002).

![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.](image-url)
The type strain, AU3313\textsuperscript{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 χ-glucosidase activities but no lipase activity, and reduces nitrate. \textit{R. respiraculi} strains AU3313\textsuperscript{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\textsuperscript{T} (=CCUG 46809\textsuperscript{T}) and LMG 21509 (=CCUG 46808), respectively.

### Acknowledgements

This work was supported by a grant from the Cystic Fibrosis Foundation (United States) (to J.J.L.). T.C. is supported by the Caroll Haas Research Fund in Cystic Fibrosis. P.V. is indebted to the Foundation (United States) (to J.J.L.). T.C. is supported by the Fund for Scientific Research – Flanders for financial support.

### References


