**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 AU3313T (=LMG 21510T = CCUG 46809T).

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, Acinetobacter 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 gilardi (Coenye et al., 1999), Ralstonia paucula (Vandamme et al., 1999), Ralstonia oxalatia (Sahin et al., 2000), Ralstonia mannitolilytica (De Baere et al., 2001), Ralstonia campinensis, Ralstonia metalldurans (Goris et al., 2001), Ralstonia taiwanensis (Chen et al., 2001) and Ralstonia insidiosa (Coenye et al., 2003). R. pickettii, R. mannitolilytica, R. gilardi, 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, AU3313T, 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.,
R. pickettii, R. mannitolilytica, R. insidiosa and A. xylosoxidans were described previously (LiPuma et al., 1999; Coenye et al., 2002b, 2003; Liu et al., 2002).

The 16S rRNA genes of strains AU0626, AU1618, AU3313 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 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 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 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 %), C16:0 10:0 (31.23 ± 3.85 %), C16:0 23:6 ± 3.61 %), C17:0 cyclo (7.42 ± 3.19 %), C16:0 2-OH (1.21 ± 0.24 %), C18:1ω7c (20.38 ± 5.26 %) and C18:1ω2-OH (1.85 ± 0.53 %). Trace amounts (< 1.0 %) of C14:0 2-OH and C8:1ω8 were also present. The fatty acid composition of strain AU3369 was very similar to those of strains AU0626, AU1618, AU3313 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, 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, mannotol, 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 x-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 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,

![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, AU3313T, was isolated from the sputum of a CF patient in the USA in 2001. Phenotypic characteristics 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. xylosidans is possible by lack of acidification of glucose (von Graevenitz, 1995). In contrast to R. pickettii, 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. manititlilytica and R. insidiosa) and from A. xylosidans 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).

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

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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 AU3313T and AU1618 have been deposited in the BCCM/LMG (Laboratorium voor Microbiologische Gent, Belgium) and CCUG (University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden) culture collections as LMG 21510T (= CCUG 46809T) and LMG 21509 (= CCUG 46808), respectively.

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References


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, mannotol, N-acetylglucosamine, maltose, citrate and phenylacetate are not. Additional characteristics are given above. The following fatty acids are present: C14:0, C14:0 3-0H, C16:1ω7c, C16:0, C17:0 cyclo, C16:0 2-0H, C18:1ω7c and C18:1ω9c. Characteristics that differentiate R. respiraculi from other Ralstonia species are summarized in Table 1.


