

Characterization of Unusual Bacteria Isolated from Respiratory Secretions of Cystic Fibrosis Patients and Description of *Inquilinus limosus* gen. nov., sp. nov.

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Using a polyphasic approach (including cellular protein and fatty acid analysis, biochemical characterization, 16S ribosomal DNA sequencing, and DNA-DNA hybridizations), we characterized 51 bacterial isolates recovered from respiratory secretions of cystic fibrosis (CF) patients. Our analyses showed that 24 isolates belong to taxa that have so far not (or only rarely) been reported from CF patients. These taxa include *Acinetobacter* sp., *Bordetella hinzii*, *Burkholderia fungorum*, *Comamonas testosteroni*, *Chryseobacterium* sp., *Herbaspirillum* sp., *Moraxella osloensis*, *Pandoraea* genomospecies 4, *Ralstonia gilardii*, *Ralstonia mannitolilytica*, *Rhizobium radiobacter*, and *Xanthomonas* sp. In addition, one isolate most likely represents a novel *Ralstonia* species, whereas nine isolates belong to novel taxa within the α -Proteobacteria. Eight of these latter isolates are classified into the novel genus *Inquilinus* gen. nov. as *Inquilinus limosus* gen. nov., sp. nov., or as *Inquilinus* sp. The remaining 17 isolates are characterized as members of the family *Enterobacteriaceae*. The recovery of these species suggests that the CF lung is an ecological niche capable of supporting the growth of a wide variety of bacteria rarely seen in clinical samples. Elucidation of the factors that account for the association between these unusual species and the respiratory tract of CF patients may provide important insights into the pathophysiology of CF infection. Because accurate identification of these organisms in the clinical microbiology laboratory may be problematic, the present study highlights the utility of reference laboratories capable of identifying unusual species recovered from CF sputum.

Cystic fibrosis (CF), an autosomal-recessive hereditary disease caused by mutations in the CF transmembrane conductance regulator gene, is characterized by the production of abnormally thickened, viscous mucus and a disturbance in electrolyte transport across epithelial membranes (34). Defects in the CF transmembrane conductance regulator gene mainly affect the respiratory tract and the pancreas, and exacerbations of pulmonary infections cause significant morbidity and mortality in persons with CF (16). Typical CF pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Burkholderia cepacia* complex organisms (16), but other glucose nonfermenters such as *Ralstonia pickettii*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*, and *Burkholderia gladioli* can be recovered from CF sputum culture as well (3, 16). Correct identification of CF pathogens is important, since it underlies effective infection control measures and therapeutic intervention (24, 25, 36); however, several studies have shown that the identification of CF pathogens, including *B. cepacia* complex organisms, is far from straightforward (3, 8, 22, 28).

Here we report the characterization of 51 bacterial isolates recovered from respiratory secretions of CF patients. The majority of these isolates were identified by referring microbiology laboratories as *B. cepacia* complex, *Burkholderia* sp., or *R. pickettii* or could not be identified. Our initial assessment (us-

ing growth on selective media, biochemical tests, and PCR-based assays) indicated that they were in fact not members of these species. A polyphasic taxonomic study (including cellular protein and fatty acid analysis, 16S ribosomal DNA [rDNA] sequencing, DNA-DNA hybridizations, and phenotypical tests) showed that these isolates either belonged to novel taxa or to taxa that so far have not (or only rarely) been reported for isolates from CF patients; others were atypical (and hence difficult to identify) strains from taxa previously found in CF patients.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The 51 isolates investigated were obtained from the respiratory secretions of 47 CF patients attending 16 different treatment centers in the United States; all were sent to the *B. cepacia* Research Laboratory and Repository (University of Michigan, Ann Arbor, Mich.) for further analysis. Identifications by the referring laboratory (based on a wide variety of biochemical test kits) were as follows: *B. cepacia* complex (29 isolates), *Burkholderia* sp. (3 isolates), *R. pickettii* (2 isolates), *Acinetobacter* sp. (2 isolates), *Rhizobium radiobacter* (1 isolate), *Comamonas acidovorans* (1 isolate), *Pseudomonas mendocina* (1 isolate), and *Pseudomonas* sp. (1 isolate). Eleven isolates were unidentified. All isolates were grown aerobically on Mueller-Hinton broth (Becton Dickinson, Cockeysville, Md.) supplemented with 1.8% (wt/vol) agar and incubated at 32°C unless otherwise indicated. Preliminary investigations employing standard techniques and several previously described genus- and species-specific PCR-based assays (targeting 16S rRNA and *recA* genes [26, 27]) indicated that these organisms were not members of bacterial species typically recovered from CF sputum culture.

Phenotypic tests. For conventional biochemical testing, bacteria from fresh (24 to 48 h) Mueller-Hinton agar plates were inoculated into the test reagent. Oxidase and catalase testing of each isolate was performed with 1% tetramethyl *p*-phenylenediamine dihydrochloride and 3% hydrogen peroxide, respectively. Growth in 1, 6, and 10% NaCl was determined in Mueller-Hinton broth incubated at 37°C. Lysine decarboxylase, *o*-nitrophenyl- β -D-galactoside (ONPG),

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and oxidation-fermentation sugars (sucrose and lactose) were obtained from Remel (Lenexa, Kans.). These tests were incubated at 37°C and were examined daily for 7 days, except ONPG, which was examined at 24 and 48 h. The RapID NF Plus (Remel) and API20E (bioMerieux, Hazelwood, Mo.) commercial identification systems were used according to the recommendations of the manufacturers. Growth on *B. cepacia* selective agar (BCSA) (containing 600 U of polymyxin B, 10 µg of gentamicin, and 2.5 µg of vancomycin per ml) (18) was determined at 32°C.

SDS-PAGE of whole-cell proteins. Strains were grown aerobically on nutrient agar (Oxoid CM3) supplemented with 0.04% (wt/vol) KH₂PO₄ and 0.24% (wt/vol) Na₂HPO₄·12H₂O (pH 6.8) and incubated for 48 h at 28°C. Preparation of whole-cell proteins and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were performed as described previously (33). Densitometric analysis, normalization, and interpolation of the protein profiles and numerical analysis by using Pearson's product-moment correlation coefficient were performed with the GelCompar 4.2 software package (Applied Maths, Kortrijk, Belgium). Isolates were identified by comparison to a database containing profiles of all *Burkholderia*, *Ralstonia*, *Pandoraea*, *Alcaligenes*, and *Bordetella* species and various other gram-negative nonfermenters (5, 6, 7, 10, 38, 39).

FAME analysis. Strains were grown aerobically on Trypticase soy agar (Becton Dickinson). After an incubation period of 24 to 48 h at 28°C (35°C for isolates AU0476, AU0578, AU0599, AU0771, AU1109, AU1714, AU2065, and AU1979), a loopful of well-grown cells was harvested, and fatty acid methyl esters (FAMES) were prepared, separated, and identified by using the Microbial Identification System (MIDI, Inc., Newark, Del.) as described previously (40). Isolates were identified by comparison to commercially available databases (MIDI, Inc.).

16S rDNA sequencing. DNA was prepared by heating one or two colonies (picked from a plate grown overnight) at 95°C for 15 min in 20 µl of lysis buffer containing 0.25% (wt/vol) SDS and 0.05 M NaOH. After lysis, 180 µl of distilled water was added, and the DNA solutions were stored at -20°C. The nearly complete sequence (corresponding to positions 9 to 1500 in the *Escherichia coli* numbering system) of the 16S rRNA gene was amplified by PCR with the conserved primers UFPL (5'-AGTTTGATCTGCTCAG-3') and URPL (5'-GGTTACCTTGTTACGACTT-3'). The PCR product was purified by using the Promega Wizard PCR Prep kit (Promega, Madison, Wis.) according to the manufacturer's instructions. Sequence analysis was performed with an Applied Biosystems 3700 DNA sequencer and the protocols of the manufacturer (PE Applied Biosystems, Foster City, Calif.) by using the BigDye Terminator Cycle Sequencing Ready Reaction kit. The sequencing primers used were UFPL, URPL, 16SF1 (5'-GCCTTCGGGTTGTAAAGCAC-3'), 16SF2 (5'-CCTTACC TACCCTTGACA-3'), 16SB1 (5'-GCGCTCGTTGCGGGACT-3'), and 16SB2 (5'-GTATTACCGCGGCTGCTG-3'). Sequence assembly was performed by using EditSeq (DNASar, Inc., Madison, Wis.). The sequences were compared to sequences available in the GenBank database by using FASTA3 (<http://www.ebi.ac.uk/fasta33/>) (31), and the results obtained with FASTA3 were used to identify isolates to the genus or species level. Phylogenetic trees based on the neighbor-joining method were constructed by using the MegAlign (DNASar) software package.

Determination of the DNA base composition and DNA-DNA hybridizations. DNA was prepared as described previously (7). The DNA base composition was determined by high-pressure liquid chromatography, and DNA-DNA hybridizations were performed with photobiotin-labeled probes in microplate wells as described previously (7). The hybridization temperature was 55°C.

Nucleotide accession numbers. All 16S rDNA sequences determined in the present study were deposited in the GenBank database under the following accession numbers (strain): AY043368 (AU0783), AY043369 (AU1523), AY043370 (AU0939), AY043371 (AU2210A), AY043372 (AU2339), AY043374^T (AU0476^T), AY043375 (AU1979), AY043376 (AU1220), AY043377 (AU1775), AY043378 (AU0428), AY043379 (AU0255), AY043380 (AU0378), AY043381 (AU0626), AY043382 (AU2259), AY043383 (AU2502), AY043384 (AU2539), AY043385 (AU0664), AY043386 (AU0736), AY043387 (AU1209), AY043388 (AU1285), AY043389 (AU1388), AY043390 (AU14191), AY043391 (AU1496), and AY043392 (AU1713).

RESULTS

Of the 51 isolates investigated, 11 could be identified to the genus or species level by SDS-PAGE of whole-cell proteins. These were identified as *Ralstonia mannitolilytica* (five isolates), *Ralstonia gilardii* (one isolate), *Ralstonia* sp. (one iso-

TABLE 1. Isolates identified by SDS-PAGE of whole-cell proteins

Strain(s)	Identification	Growth on BCSA ^a
AU2290, AU2300, AU0428, AU0255, and AU0378	<i>Ralstonia mannitolilytica</i>	+
AU1589	<i>Ralstonia gilardii</i>	+
AU0626	<i>Ralstonia</i> sp.	-
AU2911	<i>Burkholderia fungorum</i>	-
AU1775 and AU2478	<i>Pandoraea</i> genomospecies 4	+
AU1784	<i>Bordetella hinzii</i>	-

^a +, Growth; -, no growth.

late), *Pandoraea* genomospecies 4 (two isolates), *Burkholderia fungorum* (one isolate), or *Bordetella hinzii* (one isolate). The isolates showed variable growth on BCSA (Table 1). The remaining 40 isolates could not be identified by SDS-PAGE of whole-cell proteins and were further analyzed by means of cellular fatty acid analysis and/or 16S rDNA sequence analysis. Among these were 14 isolates identified as *Acinetobacter* sp. (two isolates), *Comamonas testosteroni* (two isolates), *Chryseobacterium* sp. (two isolates), *Herbaspirillum* sp. (two isolates), *Moraxella osloensis* (one isolate), *Rhizobium radiobacter* (four isolates), or *Xanthomonas* sp. (one isolate). These strains also showed variable growth on BCSA (Table 2). Seventeen additional isolates were identified as belonging to the *Enterobacteriaceae* by cellular fatty acid analysis; these were further characterized by using the API 20E identification system and 16S rDNA sequencing. Among these 17 isolates, whole-cell protein electrophoresis differentiated three clusters with identical protein profiles and 4 isolates with unique protein profiles (Table 3). Several isolates (AU0476, AU0578, AU0599, AU0771, AU1109, AU1714, and AU2065) could not be classified into any existing taxon. These isolates formed a homogeneous group, as determined by SDS-PAGE (designated group 29), but could not be identified by cellular fatty acid analysis. The following fatty acids were present in all group 29 isolates investigated (AU0476^T, AU0599, AU1109, AU1714, and AU2065) (mean values ± the standard deviation): 16:0-2OH (5.1% ± 1.2%), 16:0-3OH (3.7% ± 0.9%), 18:1Ω7c (35.6% ± 1.2%), 17:0-2OH (5.0% ± 1.7%), 17:0-3OH (4.3% ± 1.3%), 19:0-cyclo ω8c (7.9% ± 2.5%), 18:1-2OH (15.5% ± 5.0%), and 18:0-3OH (9.0% ± 0.7%). In addition, small amounts of 10:0-3OH, SF2 (14:0-3OH, 16:1 iso I, or 12:0 ALDE), 16:0, 17:1ω7c, 17:0, and 18:0 could be detected in some but not all strains. The fatty acid composition of another isolate (AU1979) was similar to that of group 29 isolates, but its protein pattern was clearly different (data not shown). The 16S rDNA sequences of isolates AU1979 and group 29 representative AU0476 were determined and compared with each other and with available 16S rDNA sequences in the GenBank database. The sequences were similar to each other (>99.5% similarity) and to the previously determined 16S rDNA sequence of isolate DS15158 (GenBank accession no. AF85496) (99.4 to 99.9% similarity). Based on the phylogenetic analysis, these isolates clearly belong to the α-Proteobacteria, with their closest relatives being members of the genera *Azospirillum* (<88.6% similarity), *Dechlorospirillum* (<87.8% similarity), *Magnetospirillum* (<87.4% similarity), and *Rhizobium* (≈86.2% similarity) (Fig. 1).

TABLE 2. Isolates identified by cellular fatty acid analysis and/or 16S rDNA sequencing

Strain	Identification by FAME (score)	Phylogenetic affiliation based on 16S rDNA sequencing	Growth on BCSA ^d
AU0783	<i>Acinetobacter baumannii</i> (0.335)	Genus <i>Acinetobacter</i>	–
AU1523	<i>Acinetobacter baumannii</i> (0.616)	Genus <i>Acinetobacter</i>	+
AU1747 ^a	<i>Comamonas testosteroni</i> (0.194)		–
AU1748 ^a	<i>Comamonas testosteroni</i> (0.737)		–
AU0939 ^b	<i>Chryseobacterium meningosepticum</i> (0.544)	<i>Flavobacterium-Cytophaga-Bacteroides</i> group	+
AU0999 ^b	<i>Chryseobacterium meningosepticum</i> (0.341)		+
AU2210A	<i>Chromobacterium violaceum</i> (0.606)	Genus <i>Herbaspirillum</i>	+
AU2339	<i>Pseudomonas huttienensis</i> (0.558)	Genus <i>Herbaspirillum</i>	+
AU1220	<i>Moraxella catarrhalis</i> (0.323)	<i>Moraxella osloensis</i>	–
AU2259 ^c	<i>Rhizobium radiobacter</i> (0.432)	Genus <i>Rhizobium</i>	–
AU1577 ^c	<i>Rhizobium radiobacter</i> (0.444)		–
AU1253 ^c	No match		–
AU1254 ^c	<i>Rhizobium radiobacter</i> (0.795)		–
AU2502	<i>Xanthomonas hyacinthi</i> (0.314)	<i>Xanthomonas campestris</i> group	+

^a AU1747 and AU1748 have identical whole-cell protein profiles and therefore may represent a single clone.

^b AU0939 and AU0999 have similar whole-cell protein profiles and therefore may represent a single species.

^c AU2259, AU1577, AU1253, and AU1254 have similar whole-cell protein profiles and therefore may represent a single species.

^d –, No growth; +, growth.

DNA was prepared from group 29 isolates AU476 and AU2065 and strain AU1979, the G+C content of the genome of the isolates was determined, and these strains were included in the DNA-DNA hybridization experiments. Isolates AU476, AU2065, and AU1979 had G+C contents of 70.3, 70.7, and 70.9%, respectively. The hybridization experiments confirmed that group 29 strains AU476 and AU2065 formed one genomic group (DNA-DNA binding value of 93%). Hybridization values of AU1979 toward AU476 and AU2065 were lower (53 and 62%, respectively). We also determined the phenotypic characteristics of all group 29 isolates and of AU1979 (Table 4). Finally, the remaining strain AU2539 also could not be identified to the species level by means of fatty acid analysis. 16S rDNA sequence analysis revealed that it also belonged to the α -*Proteobacteria*, with the genera *Paracraurococcus* and *Craurococcus* as closest neighbors (with similarities lower than 93.3 and 91.2%, respectively) (Fig. 1). The biochemical characteristics of AU2539 were as follows: growth at 25, 28, 32, and 37°C; production of pink pigment on

Mueller-Hinton plates; no growth on BCSA; no lysine and ornithine decarboxylase, β -galactosidase, arginine dihydrolase, *N*-acetylglucosaminidase, α -glucosidase, β -glucosidase, pyrrolidonyl aminopeptidase, γ -L-glutamyl aminopeptidase, tryptophan aminopeptidase, or *N*-benzyl-arginine aminopeptidase activity present; oxidase, catalase, urease, gelatinase, proline aminopeptidase, lipase, and phosphatase activity present; no acid production from lactose or sucrose; no H₂S, indole, or acetoin production; no denitrification; and no utilization of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose, or citrate.

DISCUSSION

In the present study we used a polyphasic approach (including cellular fatty acid and protein analysis, 16S rDNA sequencing, DNA-DNA hybridizations, and biochemical characterization) to characterize 51 isolates recovered from the respiratory

TABLE 3. Enterobacterial isolates identified by cellular fatty acid analysis, 16S rDNA sequencing, and/or API 20E

Strain ^a	Identification by FAME (score)	Identification by API 20E	Phylogenetic affiliation based on 16S rDNA sequence
AU0664 ^a	Not done	Good identification as <i>Serratia marcescens</i>	<i>Serratia marcescens</i>
AU0736 ^a	<i>Cedecea davisae</i> (0.453)	Very good identification as <i>Serratia marcescens</i>	
AU0791 ^a	<i>Cedecea davisae</i> (0.548)	Good identification as <i>Serratia marcescens</i>	
AU1209 ^a	<i>Salmonella typhimurium</i> (0.467)	Good identification as <i>Vibrio vulnificus</i>	<i>Serratia marcescens</i>
AU1285 ^a	<i>Kluyvera ascorbata</i> (0.492)	Good identification as <i>Aeromonas hydrophila</i>	<i>Serratia marcescens</i>
AU2544 ^a	<i>Cedecea davisae</i> (0.491)	Good identification as <i>Aeromonas hydrophila</i>	
AU0774 ^b	<i>Cedecea davisae</i> (0.217)	Doubtful identification as <i>Vibrio vulnificus</i>	
AU1676 ^b	<i>Cedecea davisae</i> (0.467)	Very good identification as <i>Serratia marcescens</i>	
AU1677 ^b	<i>Cedecea davisae</i> (0.608)	Very good identification as <i>Serratia marcescens</i>	
AU1274 ^c	<i>Cedecea davisae</i> (0.331)	Unidentified (unacceptable profile)	
AU1392 ^c	<i>Salmonella typhimurium</i> (0.300)	Unidentified (unacceptable profile)	
AU1398 ^c	<i>Cedecea davisae</i> (0.237)	Unidentified (unacceptable profile)	
AU1388	<i>Yersinia pseudotuberculosis</i> (0.020)	Good identification as <i>Aeromonas hydrophila</i>	<i>Serratia marcescens</i>
AU1491	<i>Morganella morganii</i> (0.573)	Excellent identification as <i>Morganella morganii</i>	Genus <i>Morganella</i>
AU1496	<i>Klebsiella pneumoniae</i> (0.392)	Acceptable identification as <i>Escherichia hermannii</i>	<i>Klebsiella pneumoniae</i>
AU1713	<i>Enterobacter cancerogenus</i> (0.242)	Very good identification as <i>Enterobacter cloacae</i>	<i>Escherichia coli</i>

^a Isolates indicated by the same superscript letter belong to different protein electrophoretic subgroups (a, b, and c, respectively) of the same species; based on 16S rDNA sequencing and/or very good identification as *Serratia marcescens* by the API 20E system, all of these isolates were identified as *Serratia marcescens*.

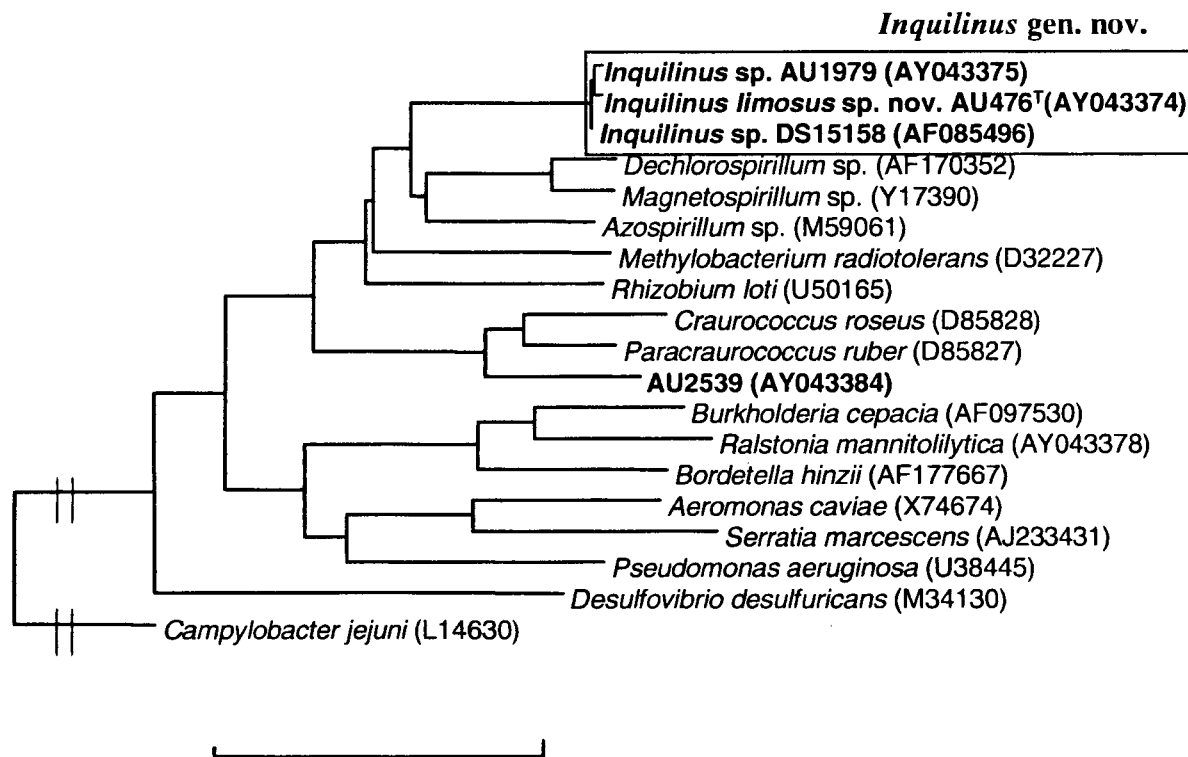


FIG. 1. Phylogenetic tree showing the position of the genus *Inquilinus* and isolate AU2539 within the *Proteobacteria*. The scalebar represents 10% sequence dissimilarity.

tracts of CF patients. Our initial analyses showed that these isolates did not belong to bacterial species typically recovered from CF patients. The more extensive analyses described here revealed that these isolates (i) belong to species previously not (or only rarely) recovered from CF patients, (ii) are atypical strains from taxa previously found in CF patients, or (iii) belong to novel taxa.

Unusual species in CF sputum. Isolates of *B. cepacia* complex, *B. gladioli*, *R. pickettii*, and several *Pandoraea* species are regularly recovered from CF patients (3, 5, 16). In the present study we identified several other species belonging to these genera in isolates obtained from CF cultures. These included several *R. mannitolilytica* isolates. Isolates from this recently described species have been recovered from blood, sputum,

TABLE 4. Phenotypic characteristics of *I. limosus* strains and *Inquilinus* sp. strain AU1979^a

Characteristic	<i>I. limosus</i>							<i>Inquilinus</i> sp. strain AU1979
	AU0476 ^T	AU0578	AU0599	AU0771	AU1109	AU1714	AU2065	
Presence of:								
Oxidase	+	+	+	+	+	+	-	-
Urease	-	-	-	-	-	-	+	-
β-Galactosidase	+	+	+	+	-	+	-	+
α-Glucosidase	-	+	+	-	+	-	+	+
γ-L-Glutamyl aminopeptidase	+	+	-	+	+	+	+	-
Gelatinase	-	-	-	-	+	+	+	-
Growth in 6% NaCl	-	+	-	-	+	-	+	-
Growth on BCSA	+	-	-	-	-	-	-	-
Production of acid from:								
Sucrose	-	+	+	-	-	-	-	-
Lactose	-	+	+	-	+	-	-	-

^a All strains tested were positive for the following characteristics: presence of catalase, β-glucosidase, phosphatase, lipase, *N*-acetyl glucosaminidase, proline aminopeptidase, pyrrolidonyl aminopeptidase, tryptophan aminopeptidase, and *N*-benzyl-arginine aminopeptidase activity; growth in 1% NaCl; growth at 25, 28, 32, 37, and 42°C; and acetoin production. All strains investigated were negative for the following characteristics: presence of lysine decarboxylase or arginine dihydrolase activity; denitrification; growth in 10% NaCl; H₂S or indole production; and utilization of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose, and citrate.

and other clinical samples; a number of hospital outbreaks involving this species have also been described (10). *R. mannitolilytica* may be difficult to differentiate from *B. cepacia* complex organisms, *R. pickettii*, and *Pseudomonas* species (10). In the present study all *R. mannitolilytica* isolates had been misidentified by the referring laboratories as *R. pickettii* or *Burkholderia* sp. *R. gilardii* has been isolated from the environment and various human clinical samples (6). Strain AU1589 represents the first reported *R. gilardii* isolate recovered from the respiratory tract of a CF patient. The referring microbiology laboratory identified this isolate as belonging to the *B. cepacia* complex. In addition to *R. mannitolilytica* and *R. gilardii*, another *Ralstonia* isolate, AU0626, was found. Although this strain could not be identified to the species level, SDS-PAGE of whole-cell proteins and the 16S rDNA sequence data clearly indicated that it belonged to the genus *Ralstonia*. Further taxonomic work is required to clarify whether this isolate represents a new *Ralstonia* species or is an atypical isolate from existing *Ralstonia* species.

The genus *Pandoraea* contains five named species and four unnamed genomospecies. *Pandoraea* strains have mainly been isolated from respiratory secretions of CF patients (5, 9). Isolates AU1775 and AU2478 were isolated from the same CF patient 10 months apart. Based on 16S rDNA sequence analysis, these isolates clearly belong to the genus *Pandoraea* (98.9 to 99.9% similarity to other *Pandoraea* species), and comparison of the one-dimensional protein profiles showed that they belonged to *Pandoraea* genomospecies 4 (data not shown). The only previously known isolate belonging to *Pandoraea* genomospecies 4 was also isolated from the sputum of a CF patient (9). Both AU1775 and AU2478 were identified by the referring laboratory as belonging to the *B. cepacia* complex. The fact that they were obtained from the same patient 10 months apart indicates the capacity for chronic colonization by this species.

B. fungorum is a recently described *Burkholderia* species that has been isolated from the environment, animals, and human clinical samples (7). Isolate AU2911 was identified as *B. fungorum* by SDS-PAGE of whole-cell proteins. Recently, several *B. fungorum* strains were isolated from CF patients (7). Several xenobiotic-compound-degrading strains with potential biotechnological applications as biodegradation agents (including *Burkholderia* sp. strain LB400) are closely related to *B. fungorum* (7, 13), and the finding that *B. fungorum* can be isolated from clinical samples (including respiratory secretions of CF patients) highlights the need for a general consensus on the potential large-scale use of members of the genus *Burkholderia* as biocontrol or biodegradation agents.

Bordetella hinzii strains have mainly been isolated from poultry with respiratory disease, although isolated cases of human infection have been reported (15, 20). Funke et al. (15) reported two strains obtained from a CF patient over a 3-year period; the clinical picture and laboratory data were clearly consistent with episodes of infectious pulmonary exacerbations and, despite aggressive antimicrobial therapy, the organism could not be eradicated from the patient. Isolate AU1784 was identified as *Bordetella hinzii* by SDS-PAGE of whole-cell proteins but could not be identified with several commercial biochemical systems and was misidentified as *Alcaligenes piechaudii* by fatty acid analysis (data not shown). This confirms previous data that this organism is difficult to identify (20).

Based on fatty acid composition and 16S rDNA sequence, isolates AU0783 and AU1523 were identified as belonging to the genus *Acinetobacter*. *Acinetobacter* spp. are widely distributed in nature and in the hospital environment and may also be recovered from the skin of healthy individuals. Misidentification of acinetobacters is relatively common due to the taxonomic complexity of the genus (which includes 9 named species and 14 unnamed genomic groups), the lack of discriminatory power of identification schemes based on phenotypic characteristics alone, and the frequent occurrence of isolates that do not seem to belong to any of the described genomic groups (29, 30). The 16S rDNA sequence of isolate AU0783 was most similar to sequences of *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex organisms. The 16S rDNA sequence of AU1523 is most similar to sequences of representatives of the recently described species *Acinetobacter ursingii*, a taxon considered to be of medical importance (29, 30). More data are required to establish whether these isolates belong to any of the described genomic groups within the genus *Acinetobacter* or whether these isolates truly represent novel taxa.

Isolates AU1747 and AU1748 were recovered from two siblings with CF and were both identified as *Comamonas testosteroni* by fatty acid analysis. Both isolates were obtained at the same time, and their whole-cell protein profiles are identical, suggesting patient-to-patient spread. *Comamonas* species are mainly environmental in origin and are generally not associated with human disease (41).

Isolates AU0939 and AU0999 were identified as belonging to the *Flavobacterium-Cytophaga-Bacteroides* phylum. The closest relatives were *Chryseobacterium* species (93.8 to 94.4% 16S rDNA sequence similarity). *Chryseobacterium* isolates are mainly isolated from the natural environment, but *Chryseobacterium meningosepticum* occurs in human clinical samples (including cases of meningitis, sepsis, pneumonia, and eye infections) (41). Although the relatively low similarity between the 16S rDNA sequence of AU0939 and representatives of *Chryseobacterium* species suggests that AU0939 and AU0999 constitute a novel taxon within this genus, a thorough taxonomic study would be required to clarify their taxonomic status.

16S rDNA sequence analysis of isolates AU2210A and AU2339 showed that they were most similar to *Pseudomonas huttiensis* (99.5%) and *Herbaspirillum frisingense* (99.6%), respectively. Recently, *P. huttiensis* has been shown to be misclassified, being more properly placed in the genus *Herbaspirillum* (1). *Herbaspirillum* spp. are mainly environmental nitrogen-fixing organisms and are frequently isolated from the roots and stems of various plants (21), although they also have been cultured from various clinical samples, including wounds and the eye (2).

Strain AU1220 was identified as *Moraxella osloensis* based on 16S rDNA sequence analysis (>99.3% similarity to other *M. osloensis* isolates; <93.3% similarity to other *Moraxella* species). This species has been associated with human infections, including meningitis, arthritis, and endocarditis. *M. osloensis* strains have also been isolated from hospital environments, as well as from the respiratory tracts of healthy adults (41).

Isolates AU2259, AU1577, AU1253, and AU1254 were

identified as *Rhizobium (Agrobacterium) radiobacter*. *Rhizobium radiobacter* infections have been associated with long-term indwelling catheters, and several cases also have been reported in immunocompromised individuals (12, 19). Our data suggest that *Rhizobium radiobacter* may have the potential to spread from patient to patient (AU1253 and AU1254 were simultaneously isolated from two siblings with CF).

Genuine *Xanthomonas* species (excluding *Stenotrophomonas maltophilia*) are notorious plant pathogens and have, to our knowledge, not been recovered from humans. Based on 16S rDNA sequence analysis and fatty acid analysis, isolate AU2502 clearly belongs to the genus *Xanthomonas* (the *Xanthomonas campestris* cluster) (17).

Atypical Enterobacteriaceae. Enteric bacilli are occasionally recovered from the respiratory secretions of CF patients, but they are usually transient colonizers and are in general not associated with severe disease (4, 16). Enterobacterial species recovered from CF respiratory tract cultures include *E. coli*, *Klebsiella pneumoniae*, and *Serratia marcescens* (3, 4). Identification of enterobacterial species is typically performed by using commercially available biochemical test kits, but there are several problems associated with their use (14). Slow-growing or fastidious strains require extended incubation for biochemical reactivity, and "pleiotropic" mutants isolated from patients who are taking antimicrobial agents often react atypically (14, 23). Occasionally, strains are isolated that grow rapidly but do not have biochemical reactions that fit the described taxa; these represent atypical strains or strains belonging to new taxa (14). In the present study we examined 17 isolates that were initially identified as enterobacteria by cellular fatty acid analysis. The majority had been identified as *Burkholderia* sp. by the referring laboratories. Identification of these isolates to the species level by cellular fatty acid analysis was only partially successful, confirming previous results that enterobacterial species cannot reliably be separated by using fatty acid analysis (M. Vancanneyt and P. Vandamme, unpublished data). Use of 16S rDNA sequence analysis in the identification of enterobacterial species is also problematic, since not all members of the *Enterobacteriaceae* have been subjected to extensive 16S rDNA sequence analysis (11, 37). Nevertheless, the combination of all available data strongly suggests that several of these isolates were *Serratia marcescens*, while one each was identified as *Morganella morganii*, *E. coli*, and *K. pneumoniae*.

Inquilinus gen. nov. The seven isolates that constituted protein electrophoretic group 29 and isolate AU1979 could not be identified by cellular protein or fatty acid analysis. Sequence analysis of the 16S rDNA gene from strains AU0476 and AU1979 revealed that these strains were closely related to the unidentified α -proteobacterium DS15158, which was previously isolated from a CF patient (32). The similarity values toward other taxa were much lower (Fig. 1). Our data clearly indicate that these eight isolates should be classified into a novel genus, for which we propose the name *Inquilinus* gen. nov. below. Based on data derived from SDS-PAGE of whole-cell proteins and DNA-DNA hybridizations, isolates AU0476, AU0578, AU0599, AU0771, AU1109, AU1714, and AU2065 clearly belong to a single species, for which we propose the name *I. limosus* gen. nov., sp. nov. below. Isolate AU1979 clearly belongs to a different species within the genus *Inquilinus*; however, we do not here propose a formal name for this

isolate, pending the study of additional similar isolates and the availability of discriminating phenotypic characteristics.

Isolate AU2539. This isolate was identified with a low probability score as *Methylobacterium mesophilicum* by cellular fatty acid analysis, suggesting that it belonged to the α -*Proteobacteria*. Analysis of the 16S rDNA sequence of this isolate confirmed this and showed that its closest neighbors are members of the genera *Paracraurococcus* and *Craurococcus*. These two genera were recently described for bacteriochlorophyll *a*-containing strains isolated from soil in Japan (35). From our data it is obvious that isolate AU2539 belongs to a novel genus within the α -*Proteobacteria*. However, we do not propose a formal name for this organism here, pending the availability of additional isolates.

Conclusions. The recovery of these unusual species from the respiratory secretions of CF patients clearly shows that the bacterial biodiversity in the airways of CF patients still is underestimated. This has several important implications. Although the clinical role of these and other unusual bacteria in CF lung disease is unclear, at least some of these organisms seem capable of prolonged colonization or spread among CF patients. An equally important issue is the challenge that these organisms pose to clinical microbiology laboratories: none of these organisms was initially identified correctly, with the majority either not identified or erroneously identified as *B. cepacia* complex or *R. pickettii*. The misidentification of isolates as *B. cepacia* complex can have a significant medical, social, and psychological impact (22, 24, 25, 28). Accurate species level identification of CF isolates is also important in order to identify outbreaks or to assess the clinical impact of unusual species. Since most of the taxa identified here have only been described recently or have not been found frequently in clinical samples, they are not included in most commercial databases; it is therefore not surprising that most were initially misidentified. Our observations suggest the need for increased awareness of this problem and highlight the usefulness of reference laboratories capable of providing polyphasic analyses of unusual isolates recovered from CF sputum. The data presented also suggest that the CF lung seems to be an ecological niche suitable for the growth of a wide variety of unusual bacteria not commonly associated with human disease. Factors that account for the association between typically nonpathogenic species and the respiratory tracts of CF patients are unknown; elucidation of these factors may provide important insights into the pathophysiology of CF infection.

Inquilinus* gen. nov. *Inquilinus (In.qui'li.nus. L. masc. n. *inquilinus*, an inhabitant of a place that is not its own). Cells are gram-negative, nonsporulating rods. Catalase activity is present. Growth is observed at 25, 28, 32, 37, and 42°C and in 1% NaCl. No growth occurs in 10% NaCl. There is no denitrification. There is no indole production. There is no lysine decarboxylase, ornithine decarboxylase, or arginine dihydrolase activity. There is no pigment production. Lipase, phosphatase, *N*-acetylglucosaminidase, β -glucosidase, proline aminopeptidase, pyrrolidonyl aminopeptidase, tryptophan aminopeptidase, and *N*-benzyl-arginine aminopeptidase activity are present. There is no utilization of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose, or citrate. Additional characteristics are shown in Table 4. The G+C content of the genome is be-

tween 70.3 and 70.9%. It is isolated from the respiratory secretions of CF patients. The type species is *Inquilinus limosus*.

Description of *Inquilinus limosus* gen. nov., sp. nov. *Inquilinus limosus* (li.mo'sus. L. masc. adj. *limosus*, full of slime, slimy). The description of *I. limosus* is the same as for the genus. The type strain, AU0476^T, was isolated from the respiratory secretions of a CF patient in the United States in 1998. The description of the type strain is the same as for the species. In addition, the type strain has the following characteristics: oxidase, β -galactosidase, and γ -L-glutamyl aminopeptidase activity are present; no urease, gelatinase, or α -glucosidase activity is present; there is no acid production from sucrose or lactose; there is no growth in 6% NaCl; there is growth on BCSA at 32°C. The G+C content of the type strain is 70.3%, and the GenBank accession number for its 16S rDNA sequence is AY043374. *I. limosus* AU0476^T was deposited in the BCCM/LMG Bacteria Collection (University of Ghent, Ghent, Belgium) as LMG 20952^T and in the CCUG Culture Collection (University of Goteborg, Goteborg, Sweden) as CCUG 45653^T. In addition, *Inquilinus* sp. strain AU1979 was deposited in the BCCM/LMG Bacteria Collection as LMG 20953.

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