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Phenotypic and Genetic Characterization of Clinical Isolates of CDC Coryneform Group A-3: Proposal of a New Species of *Cellulomonas*, *Cellulomonas denverensis* sp. nov.

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CDC coryneform group A-3 bacteria are rare human pathogens. In this study, six group A-3 isolates (two from blood, one from cerebrospinal fluid, and one each from homograft valve, lip wound, and pilonidal cyst) were compared to the type strains of phenotypically related organisms, *Cellulomonas fimi*, *Cellulomonas hominis*, *Oerskovia turbata*, and *Sanguibacter suarezii*, and characterized by phenotypic, chemotaxonomic, and genotypic studies. DNA-DNA reassociation analysis identified two genomic groups, and phylogenetic analysis of the 16S rRNA gene sequence identified the taxonomic positions of these groups to genus level. Two groups were defined, and both were more closely related to *Cellulomonas* species: one group of three strains, for which we propose the new species *Cellulomonas denverensis* sp. nov., with the type strain W6929 (ATCC BAA-788^T or DSM 15764^T), was related to *C. hominis* ATCC 51964^T (98.5% 16S rRNA gene sequence similarity), and the second group of three strains was related to *C. hominis* ATCC 51964^T (99.8 to 99.9% 16S rRNA gene sequence similarity). The definition of this new *Cellulomonas* species and the confirmation of three strains as *C. hominis* serve to further clarify the complex taxonomy of CDC coryneform group A-3 bacteria and will assist in our understanding of the epidemiology and clinical significance of these microorganisms.

Since 1965, the Centers for Disease Control and Prevention (CDC) Special Bacteriology Reference Laboratory (SRBL) has received eight unidentified gram-positive, rod-shaped bacteria that appeared on the basis of routine microbiologic tests to form a distinct group, which has been designated CDC coryneform group A-3. Group A-3 strains are motile, fermentative, pale yellow to yellow pigmented and have not been recognized as belonging to any established species. Six of these strains from human clinical sources were included in a 1977 study of Sottnek et al. (14). The increase in the interest in these bacteria as serious opportunistic pathogens of humans was stimulated when Hollis and Weaver (5) recognized and described many CDC coryneform groups among clinical isolates, including the coryneform group A-3. In a recent report, Funke et al. (4) assigned two of the coryneform group A-3 strains to the genus *Cellulomonas* as a new species, *Cellulomonas hominis*, on the basis of phenotypic characteristics and 16S rRNA gene sequencing. In this study, we present the phenotypic and genotypic comparison of the remaining six strains of coryneform group A-3 bacteria with *C. hominis*, as well as with the type strains of other related species and genera, *Cellulomonas fimi*, *Oerskovia turbata*, and *Sanguibacter suarezii*. From the results of our phenotypic and genotypic data, we propose a new clinically relevant species of *Cellulomonas*, *Cellulomonas denverensis* sp. nov., and establish its close association with *C. hominis*.

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MATERIALS AND METHODS

Strains. The clinical isolates used in this study were six strains of CDC group A-3 (Table 1). They were obtained from the collection of CDC's SBRL and Actinomycete Reference Laboratory. The reference strain of *C. hominis* (DMMZ CE39) was provided by Guido Funke, Department of Medical Microbiology and Hygiene, Gartner and Colleagues Laboratories, Weingarten, Germany. The type strains of *C. fimi* ATCC 484, *C. hominis* ATCC 51964, *Oerskovia turbata* ATCC 25835, and *Sanguibacter suarezii* ATCC 51766 were obtained from the American Type Culture Collection (Manassas, Va.). All strains were maintained at 4°C on Middlebrook 7H11 agar slants (Remel, Lenexa, Kans.) until use.

DNA reassociation studies. Harvesting and lysis of the bacterial cells were performed by previously described methods (7). DNA relatedness experiments utilized the hydroxyapatite method described previously (2). DNA was labeled in vitro with [^{32}P]dCTP by using a nick translation kit (Invitrogen, Carlsbad, Calif.). The temperature used for optimal hybridization was 70°C, and the percent divergence was calculated to the nearest 0.5% (2).

16S rRNA gene sequencing. The 16S rRNA genes of strains *C. denverensis* W6929^T (ATCC BAA-788^T), W6124, and W6117 and *C. hominis* ATCC 51964^T, DMMZ CE39, W7335, W7336 (ATCC BAA-786), and W7387 were analyzed as described by McNeil et al. (7). Related sequences were identified in a BLAST search against GenBank. Similarity searches were performed with Clustal W, and a distance matrix was created. In Treecon, the phylogenetic tree of aligned sequences was constructed with the neighbor-joining method and bootstrapped based on 1,000 replications (7).

CFA and quinone analysis. Bacteria were grown for 2 days on heart infusion agar with 5% rabbit blood at 37°C. Harvested cells were saponified, and the liberated fatty acids were methylated and analyzed by gas-liquid chromatography

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TABLE 1. Isolates used in this study[a]

Name/previous identification	ARL no.	SBS no.	Source	Geographic site/ reference(s) if applicable
C. denverensis (ATCC BAA-788^T, DSM 15764^T)/CDC group A-3	W6929^T (W2777^T)	C2508^T	Blood	Denver, Colo./7
C. denverensis/*O. turbata*	W6124 (W2733)	C6927	Homograft valve	Denver, Colo./7, 12
C. denverensis/CDC group A-3	W6117	A3936	Blood	Ohio
C. hominis (ATCC 51964^T, DSM 9581^T, DMMZ CE40^T)	W8284^T	E2655^T	Cerebrospinal fluid	Canada/4
C. hominis (DMMZ CE39)	W6263	D2991	Cerebrospinal fluid	Canada/4
C. hominis/CDC group A-3	W7335	C8262	Cerebrospinal fluid	Canada
C. hominis (ATCC BAA-786)/CDC group A-3	W7336	D3060	Pilonidal cyst	Texas
C. hominis/CDC group A-3	W7387	E4503	Lip wound	Conn.
C. fimi (ATCC 484^T, DSM 20113^T)	W7543^T	G8893^T	Soil	
S. suarezii (ATCC 51766^T, DSM 10543^T)	W7581^T		Bovine blood	Spain
O. turbata (ATCC 25835^T)	W6114^T	KC1355^T	Soil	Unknown

[a] ARL, Actinomycete Reference Laboratory; SBS, Special Bacteriology Section.

(16). Identification of fatty acids was performed using a commercially available software package (MIDI, Newark, Del.). Isoprenoid quinones were extracted from a 100-mg portion of lyophilized cells and analyzed by reverse-phase high-performance liquid chromatography (9). Cellular fatty acid (CFA) profiles were identified using a commercially available system (MIDI, Newark, Del.) utilized with a CDC library created using LGS software (16).

Whole-cell analyses. The methods used for whole-cell analyses for diaminopimelic acid and monosaccharides are those described by Berd (1).

G+C content in DNA. The G+C content was determined spectrophotometrically by thermal denaturation as previously described (6). *Escherichia coli* DNA was used as a control, and all samples were run at least three times.

Phenotypic characterization. All isolates were inoculated onto heart infusion agar with 5% rabbit blood (BBL, Becton Dickinson, Microbiology Systems, Cockeysville, Md.) and incubated at 25 and 35°C for 2 days for morphological studies. We used Gram staining to study microscopic morphology, and flagellum staining was used to demonstrate the presence and the site of attachment of flagella. The isolates were examined at low power (magnification, ×10) under a stereomicroscope for the presence of aerial and substrate hyphae. We conducted biochemical tests that are routinely used in the SBRL using previously described methods (16) except decomposition of casein at 25 and 35°C was done as described by Berd (1). Antimicrobial susceptibilities were determined by a previously described broth microdilution method with cation-supplemented Mueller-Hinton broth (10, 11). The antimicrobial agents tested were amikacin, amoxicillin-clavulanate, ampicillin, ceftriaxone, ciprofloxacin, clarithromycin, clindamycin, doxycycline, imipenem, minocycline, rifampin, trimethoprim-sulfamethoxazole, and vancomycin. The plates were incubated at 25 or 35°C for 48 h. Currently, there are no interpretive MIC breakpoints for isolates of this genus.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rRNA gene sequences of *C. denverensis* ATCC BAA-788^T, W6124, and W6117 are AY501362, AY655726, and AY655727, respectively. The GenBank accession numbers for the 16S rRNA gene sequences of *C. hominis* W7387, ATCC BAA-786, W7335, DMMZ CE39, and ATCC 51964^T are AY655732, AY655731, AY655730, AY655729, and AY655728, respectively.

RESULTS AND DISCUSSION

The six clinical strains (Table 1) studied were isolated from blood (n = 2), cerebrospinal fluid (n = 1), homograft valve, lip wound, and pilonidal cyst. These strains were received from reference laboratories in the United States and Canada. Except for *C. denverensis* ATCC BAA-788^T, a blood isolate from a patient with endocarditis, and W6124, from a homograft valve associated with the same patient, each at the University of Colorado Medical Center (7, 12), little clinical information was available to determine the clinical significance of the remaining strains.

A species is defined as a group of strains that exhibits levels of DNA relatedness of 70% or more at the optimal reassociation temperature and whose related sequences exhibit 5% or less divergence (15). A strain is assigned to a particular species when the relatedness of its DNA to labeled DNA from the type strain of that species fulfills the species definition. The DNA relatedness results for the six coryneform A-3 isolates summarized in Table 2 were consistent with the results of 16S rRNA gene analysis (Fig. 1). Three strains were found to belong to a new species, *Cellulomonas denverensis*, and three strains were confirmed as *C. hominis*.

TABLE 2. DNA relatedness of representative strains in this study

Source of unlabeled DNA	Relative binding ratio[a] (divergence[b]) for labeled DNA from:					
	C. denverensis ATCC BAA-788^T	*C. hominis* ATCC 51964^T	*C. hominis* (W7336) ATCC BAA-786	*C. fimi* ATCC 484^T	*S. suarezii* ATCC 51766^T	*O. turbata* ATCC 25835^T
C. denverensis ATCC BAA-788^T	100	ND[c]	33 (11.5)	9	13	5
C. denverensis W6124	100 (1.0)	ND	ND	ND	ND	ND
C. denverensis W6117	83 (2.0)	ND	ND	ND	ND	ND
C. hominis ATCC 51964^T	28 (11.5)	100	98 (1.0)	ND	ND	ND
C. hominis DMMZ CE39	32 (11.0)	100 (0.5)	91 (0.5)	ND	ND	ND
C. hominis W7335	29 (11.0)	ND	92 (0.5)	ND	ND	ND
C. hominis (W7336) ATCC BAA-786	25 (11.0)	85 (1.0)	100	19	10	5
C. hominis W7387	24 (11.0)	ND	95 (1.0)	ND	ND	ND
C. fimi ATCC 484^T	ND	ND	ND	100	ND	ND
S. suarezii ATCC 51766^T	ND	ND	ND	ND	100	ND
O. turbata ATCC 25835^T	9	ND	ND	ND	6	100

[a] (Percentage of heterologous DNA bound to hydroxyapatite/percentage of homologous DNA bound by hydroxyapatite) × 100 at 70°C. Values are means from at least two hybridization reactions.
[b] Decrease in thermal stability (in degrees Celsius) of heterologous DNA duplexes compared with those of homologous DNA duplexes.
[c] ND, not determined.

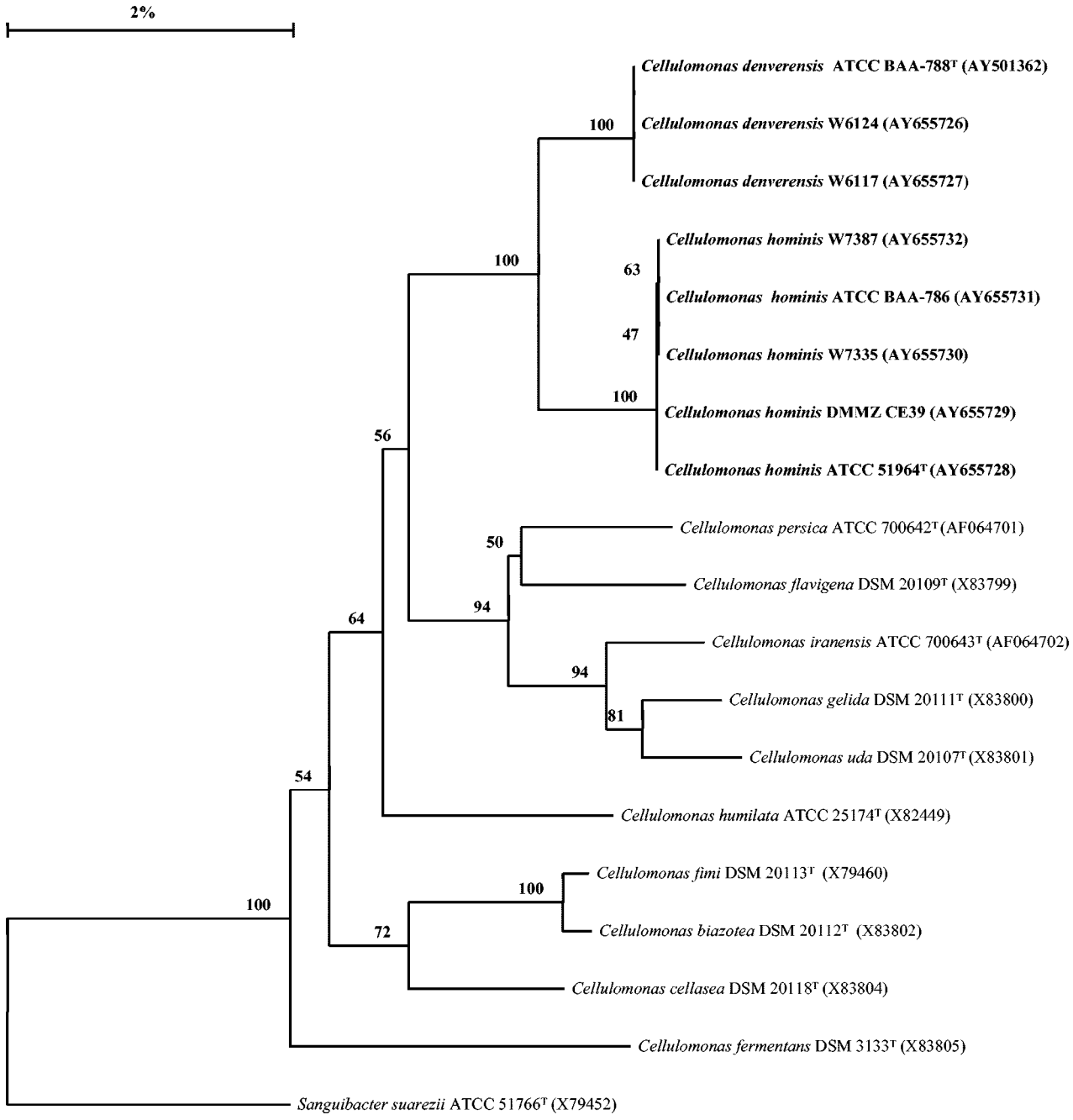

FIG. 1. Phylogenetic tree based on 16S rRNA sequences showing the position of *Cellulomonas denverensis* strains (ATCC BAA-788^T, W6124, and W6117) and *Cellulomonas hominis* strains (W7387, ATCC BAA-786, W7335, DMMZ CE39, and ATCC 51964^T). The tree was rooted by using *Sanguibacter suarezii* as the outgroup. The scale bar represents a 2% difference in sequence.

When we labeled the DNA of the reference and type strains of the nearest phylogenetic neighbors, *C. hominis* ATCC BAA-786 and *C. fimi* ATCC 484^T, we found the levels of relatedness to *C. denverensis* ATCC BAA-788^T to be low (33 and 9%, respectively) (Table 2). The labeled DNA of *C. denverensis* ATCC BAA-788^T was related to that of the other coryneform group A-3 isolates, with values ranging from 9 to 32%, with divergences ranging from 11 to 12%, confirming it as a new species. Labeled DNA of *C. denverensis* ATCC BAA-788^T was related 100%, with a divergence of 1.0% to W6124 (a homograft valve isolate associated with the same patient) (7), and was related 83%, with a divergence of 2.0%, to W6117. The high levels of DNA relatedness demonstrated that these isolates belong to the same species, and the low levels of 16S rRNA gene sequence similarity to other taxa support the distinctness of these isolates (Fig. 1).

The labeled DNA of *C. hominis* ATCC 51964^T (Table 2) was related 100%, with a divergence of 0.5%, to *C. hominis* DMMZ CE39 and was related 85%, with a divergence of 1.0%, to *C. hominis* ATCC BAA-786. In a reciprocal reac-

TABLE 3. Diagnostic chemotaxonomic properties of type strains and representative isolates in this study

Species	Whole-cell sugars	Predominant cellular fatty acids[a]	G+C content (mol%)	Menaquinones
Cellulomonas denverensis ATCC BAA-788^T	Mannose, rhamnose, ribose	14:0, i15:0, a15:0, i16:0, 16:0, a17:0	68.5	Major amounts of MK-9 and minor amounts of MK-7 and MK-8
Cellulomonas hominis ATCC 51964^T	Mannose, fucose, rhamnose	14:0, i15:0, a15:0, i16:0, 16:0, a17:0	70.0 (76.0)[b]	Major amounts of MK-9 and minor amounts of MK-7 and MK-8
Cellulomonas fimi ATCC 484^T	Glucose, fucose, rhamnose, ribose	14:0, i15:0, a15:0, i16:0, 16:0, a17:0	71.0	Major amounts of MK-9 and minor amounts of MK-7 and MK-8
Sanguibacter suarezii ATCC 51766^T	Galactose, mannose, ribose	14:0, i15:0, a15:0, i16:0, 16:0, a17:0	68.0	Major amounts of MK-9 and minor amounts of MK-7 and MK-8
Oerskovia turbata ATCC 25835^T	Galactose	14:0, i15:0, a15:0, i16:0, 16:0, a17:0	70.0	Major amounts of MK-9 and minor amounts of MK-7 and MK-8

[a] A number before a colon indicates the number of carbons; the number after the colon is the number of double bonds; i, iso, indicates a methyl branch at the iso position; a, anteiso, indicates a methyl branch at the anteiso position.
[b] Data different from Funke et al. (4) with the high-performance liquid chromatography method outlined by Mesbah et al. (8).

tion, *C. hominis* ATCC BAA-786 was 98% related to *C. hominis* ATCC 51964^T, with a divergence of 1.0%. We obtained high DNA-DNA relatedness values (92 and 95%) for the two remaining strains of *C. hominis* when compared with labeled *C. hominis* ATCC BAA-786. These relatedness experiments were consistent with the results of 16S rRNA gene sequence analysis since the percentages of similarity were close (ranging from 99.8 to 99.9%) (Fig. 1). In addition, the labeled ATCC BAA-786 did not show high reassociation levels with the type strain of *C. denverensis* (Table 2). These results indicate that these strains constitute a separate taxon.

Chemotaxonomic characteristics. Predominant fatty acids of *Cellulomonas* species, along with *S. suarezii* and *O. turbata*, are listed in Table 3. In contrast to the genetic differences of the six coryneform group A-3 strains, chemotaxonomic characteristics, CFA and quinone analyses, were not useful in separating C. *denverensis* from related species. These organisms shared a common cellular fatty acid profile characterized by predominant amounts of 14:0, i15:0, a15:0, i16:0, 16:0, and a17:0. In addition, these organisms shared similar respiratory quinone profiles characterized by major amounts of menaquinone-9 (MK-9) and minor amounts of MK-7 and MK-8.

Although all group A-3 isolates shared common cell wall structures, including the lack of diaminopimelic acid in the whole-cell wall analysis, this group was heterogeneous with respect to sugar composition (Table 3). These data suggest that examining whole-cell sugar composition may be of practical value in species identification: *C. denverensis* possesses mannose, rhamnose, and ribose, and *C. hominis* possesses mannose, fucose, and rhamnose.

Results of G+C content for representative coryneform A-3 strains and related species ranged from 68.0% for *S. suarezii* ATCC 51766^T to 71.0 mol% for *C. fimi* ATCC 484^T (Table 3). These results were consistent with those obtained with the denaturation method in other studies (3, 13). However, results for G+C content found for *C. hominis* in our study were sig-

TABLE 4. Differential characteristics of study isolates compared with type strains of related organisms[a]

Characteristic	Result for:						
	C. denverensis ATCC BAA-788^T	*C. denverensis* (n = 2)	*C. hominis* ATCC 51964^T	*C. hominis* (n = 3)	*C. fimi* ATCC 484^T	*S. suarezii* ATCC 51766^T	*O. turbata* ATCC 25835
Pigment on HIA slants in 3 days	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow	Yellow
Macroscopic morphology	Convex, entire edged	Convex, entire edged	Convex, entire edged	Convex, entire edged	Convex, entire edged	Convex, entire edged, "fried egg"	Convex, entire edged
Substrate hyphae	−	0/2[b]	−	0/3	−	−	+
Growth at:							
25°C	−	0/2	−	0/3	+	+	+
35°C	+	2/2	+	3/3	+	−	+
45°C	+	2/2	+	3/3	−	−	−
Gelatin liquefaction (14 days)	−	0/2	−[c]	0/3	−	−	+
Acid fermentation of:							
L-Arabinose	−	2/2	−	0/3	−	−	−
Glycerol	+	1/2	+	3/3	−	−	−
Melezitose	−	0/2	−	0/3	+	−	−
Melibiose	−	1/2	−	3/3	+	+	−
Raffinose	−	0/2	−	0/3	+	+	−
L-Rhamnose	+	2/2	+	3/3	+	+	−
D-Sorbitol	+	2/2	−	0/3	−	+	−

[a] Symbols and abbreviations: HIA, heart infusion agar; −, negative; +, positive; ±, weak reaction.
[b] Number positive/number tested.
[c] Funke et al. (4) described *C. hominis* as gelatin hydrolysis positive using the film strip method.

nificantly lower, at 70.0 mol%, than the results reported by Funke et al. (4). This discrepancy may have resulted from differences in methodologies, G+C content detected by high-performance liquid chromatography (8) versus detection determined with the denaturation method described by Mandel et al. (6).

Phenotypic analysis. Microscopic morphological studies showed that all isolates studied were gram-positive, pleomorphic bacilli, lacking spores and capsules. The isolates, under low-power stereomicroscopic examination, showed pale yellow to yellow, small-diameter (approximately 1-mm) colonies after incubation on heart infusion agar with 5% (vol/vol) rabbit blood for 2 days at 35°C. No substrate hyphae were seen. All colonies were smooth, convex, and entire edged. All isolates studied were motile with lateral and polar flagella, reduced nitrate, and hydrolyzed esculin. All coryneform group A-3 isolates were negative for gelatin liquefaction in 14 days and urease production and produced acid fermentatively from cellobiose, D-galactose, D-glucose, lactose, maltose, mannose, salicin, sucrose, D-trehalose, and D-xylose. None produced acid from adonitol, dulcitol, i-erythritol, i-*myo*-inositol, and D-mannitol. The biochemical differences among these isolates and type strains of related organisms are summarized in Table 4.

The antimicrobial susceptibility results are given in Table 5. Clarithromycin, clindamycin, imipenem, minocycline, rifampin, and vancomycin appear to be active against the six coryneform group A-3 isolates and the reference strains of *C. hominis*, *C. fimi*, *S. suarezii*, and *O. turbata*. The MICs of ciprofloxacin were high against *C. denverensis*, and the MICs of amoxicillin-clavulanic acid, ampicillin, and ceftriaxone had intermediate values against isolates of *C. denverensis*. The MICs of amikacin were high for all isolates studied. The trimethoprim-sulfamethoxazole MICs ranged from 0.25/4.8 μg/ml for *O. turbata* ATCC 25835 to >8/152 μg/ml for all *C. hominis* isolates. The results of the MICs of the type and reference strains of *C. hominis*, as well as the type strains of *C. fimi* and *O. turbata*, with the agar dilution method have been reported (4). Except for resistance of *O. turbata* to rifampin, results were comparable to our results.

The new species, *Cellulomonas denverensis*, a group of three strains, was phenotypically and phylogenetically (98.5% by 16S rRNA gene sequencing) most similar to *C. hominis* but differed by fermentation of D-sorbitol.

***Cellulomonas denverensis* sp. nov.** (den.ver.en′sis, N.L. adj. *denverensis* of Denver, Colo., the city of origin of the type strain). Cells are short (1 μm), thin, gram-positive, nonsporulating rods that are motile by polar and lateral flagella. Colonies are circular, smooth, and convex, and are pale yellow in about 3 days. Cells are fermentative. Growth occurs at 35 and 45°C but not at 25°C. Cells are catalase positive. Esculin is decomposed, while casein, gelatin, and urea are not. Nitrate is reduced to nitrite. Acid is produced from cellobiose, D-galactose, D-glucose, lactose, maltose, mannose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose, and sometimes from L-arabinose (2/3), glycerol (2/3), and melibiose (1/3). No acid is produced from adonitol, dulcitol, i-erythritol, i-*myo*-inositol, D-mannitol, melezitose, or raffinose. The diagnostic whole-cell sugars are mannose, ribose, and rhamnose. G+C content is 68.5 mol%. The GenBank accession numbers of the 16S rRNA gene sequences of ATCC BAA-788^T, W6124, and

TABLE 5. MICs of 12 antimicrobial agents for *Cellulomonas denverensis*, *Cellulomonas hominis*, and type strains of related species

Strain	MIC (μg/ml) of:										
	Amikacin	Amoxicillin-clavulanate	Ampicillin	Ceftriaxone	Ciprofloxacin	Clarithromycin	Clindamycin	Imipenem	Minocycline	Rifampin	Trimethoprim-sulfamethoxazole	Vancomycin
C. denverensis ATCC BAA-788^T	>32	16/8	16	32	>8	0.5	4	1	≤0.13	≤0.25	4/76	≤0.5
C. denverensis W6124	>32	16/8	16	32	>8	0.5	2	1	≤0.13	≤0.25	4/76	≤0.5
C. denverensis W6117	>32	16/8	16	32	>8	0.5	2	1	≤0.13	≤0.25	2/38	≤0.5
C. hominis ATCC 51964^T	>32	4/2	8	4	2	0.5	0.25	2	≤0.13	≤0.25	>8/152	≤0.5
C. hominis DMMZ CE39	>32	4/2	8	4	2	2	0.25	2	≤0.13	≤0.25	>8/152	≤0.5
C. hominis W7335	>32	4/2	8	4	4	2	1.0	2	≤0.13	≤0.25	>8/152	≤0.5
C. hominis ATCC BAA-786 (W7336)	>32	4/2	4	4	2	2	1.0	2	≤0.13	≤0.25	>8/152	≤0.5
C. hominis W7387	>32	4/2	4	4	2	2	1.0	2	≤0.13	≤0.25	>8/152	≤0.5
C. fimi ATCC 484^T	>32	2/1	4	2	0.25	1	0.5	0.5	≤0.13	≤0.25	1/19	≤0.5
S. suarezii ATCC 51766^T	>32	4/2	4	8	>8	1	0.25	0.25	≤0.13	≤0.25	2.38	≤0.5
O. turbata ATCC 25835^T	>32	8/4	8	>64	4	1	0.12	2	0.5	≤0.25	0.25/4.8	≤0.5

W6117 are AY501362, AY655726, and AY655727, respectively. The type strain is W6929 (ATCC BAA-788 ^{T} or DSM 15764 ^{T}) from blood.

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