

Identification of Some Clinical Strains of CDC Coryneform Group A-3 and A-4 Bacteria as *Cellulomonas* Species and Proposal of *Cellulomonas hominis* sp. nov. for Some Group A-3 Strains

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CDC coryneform group A-3 and A-4 bacteria were defined by Hollis and Weaver in 1981, but their taxonomic position is still unclear. By using biochemical and chemotaxonomical methods, four clinical strains belonging to CDC coryneform groups A-3 ($n = 2$) and A-4 ($n = 2$) were studied and could be assigned to the genus *Cellulomonas*, resulting in the first description of *Cellulomonas* strains isolated from clinical specimens. CDC coryneform group A-3 and A-4 strains were compared with the type strains of the seven species constituting the genus *Cellulomonas* at present as well as with the closely related species *Oerskovia turbata*, *Oerskovia xanthineolytica*, and *Jonesia denitrificans*, but their biochemical patterns were not compatible with the patterns of any of those species. Almost the entire sequences of the 16S rRNA genes of one representative strain of both CDC taxa were determined, and comparative sequence analysis confirmed the placement of the CDC coryneform group A-3 and A-4 strains studied in the *Cellulomonas*-*Oerskovia* subbranch of the actinomycetes. Both CDC taxa exhibited >99% base pair homology within their 16S rDNAs. On the basis of phenotypic and molecular data, we formally propose a new species, *Cellulomonas hominis* sp. nov., for the CDC coryneform group A-3 bacteria examined. The type strain is DSM 9581. The precise taxonomic status of the CDC coryneform group A-4 strains studied remains to be established by quantitative DNA-DNA hybridizations.

In 1981, Hollis and Weaver (10) published their guide for the identification of gram-positive rods, which became an invaluable reference for the clinical microbiologist. Because many of these organisms formed phenotypically distinct clusters which could not be assigned to established genera or species, Hollis and Weaver defined a number of CDC taxa. As a result of later taxonomic investigations some of these CDC taxa were proposed as new species (e.g., *Corynebacterium jeikeium* for CDC group JK and *Corynebacterium urealyticum* for CDC group D-2). However, some of the CDC taxa are still awaiting their exact taxonomic description. Among these are CDC groups A-3, A-4, and A-5, which exhibit considerable heterogeneity, as evidenced by the information on their individual characteristics or biochemical reactions (e.g., pigment, motility, nitrate reduction, gelatin hydrolysis) reported by Hollis and Weaver (10). A recent study demonstrated that some CDC group A-4 and A-5 strains are, in fact, members of the genus *Microbacterium* (8). In this report we present phenotypic and phylogenetic evidence that some CDC group A-3 and A-4 strains are members of the genus *Cellulomonas*. To our knowledge, this is the first report on the isolation of strains belonging to the genus *Cellulomonas* from clinical samples, although the clinical significance of our isolates is unknown. To date, the genus *Cellulomonas* contains seven validly described species (*C. biazotea*, *C. cellasea*, *C. fermentans*, *C. fimi*, *C. flavigena*, *C. gelida*, and *C. uda*) (1, 23). In addition, the closely related species *Oerskovia turbata* and *Oerskovia xanthineolytica* (previously CDC coryneform groups A-1 and A-2 [21]) as well as

Jonesia denitrificans (formerly *Listeria denitrificans* [19]) were also included in our comparative studies. On the basis of the results of a comprehensive phylogenetic analysis and the phenotypic distinctiveness of the strains, we formally propose a new *Cellulomonas* species, *Cellulomonas hominis* sp. nov., for the CDC group A-3 strains examined.

MATERIALS AND METHODS

Strains, media, and growth conditions. The four clinical isolates tested (Table 1) were referred to the Department of Medical Microbiology, University of Zürich, for identification. The type strains used in the study for comparative investigations were obtained from the Culture Collection of the Institut Pasteur (Paris, France) and are listed in Table 1. All strains were cultured on Columbia agar (all media were from Becton Dickinson Microbiology Systems, Cockeysville, Md., unless specified otherwise) with 5% sheep blood at 37°C in a 5% CO₂ atmosphere.

Biochemical tests. Preparation of the traditional media used for biochemical characterization was done as described by Nash and Krenz (14). All biochemical tests were carried out at 37°C. Cellulase activity was observed in tubes containing cells in 2 ml of 0.85% NaCl (with a density of McFarland no. 6 standard) by adding a piece of copy paper (0.75 by 1.5 cm; Novanta, Zürich, Switzerland) as the only carbon source; even initial lysis of the piece of cellulose within 10 days was rated as positive. Motility was observed in a hanging drop of Trypticase soy broth after incubation at 30°C for 24 h. Nitrate reduction was tested in nitrate broth (Difco, Detroit, Mich.). Hydrolysis of urea was measured in Christensen's urea broth, and esculin hydrolysis was observed on modified esculin agar (14). Fermentation of carbohydrates (reading after 120 h) was tested with the API CH50 system (API bioMérieux, Marcy l'Etoile, France) except for dextrin fermentation (not included in the CH50 strip) which was tested in cystine Trypticase agar medium containing 1% dextrin (Sigma Chemical Co., St. Louis, Mo.). Gelatin hydrolysis was detected by immersing film strips (Diagnostics Pasteur, Marnes-la-Coquette, France) in bacterial suspensions and incubating them for up to 1 week. Hydrolysis of casein, tyrosine, and xanthine meant clearing of the medium around the colonies after incubation for up to 10 days. DNase production was tested with DNase test agar with methyl green (Difco). Further enzymatic activities were determined with the API ZYM strip (API bioMérieux) by following the manufacturer's guidelines.

Antimicrobial susceptibility patterns. Susceptibility to antimicrobial agents

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TABLE 1. Strains included in the study

Strain group and no. ^a	Identification	Source (patients' sex and age [yr]) ^b
Reference strains (all type strains)		
CIP 82.11 (ATCC 486)	<i>C. biazotea</i>	Soil
CIP 102113 (ATCC 21681)	" <i>C. cartae</i> "	Soil
CIP 102222 (ATCC 487)	<i>C. cellasea</i>	Soil
CIP 103404 (ATCC 12830)	" <i>C. cellulans</i> "	Chalk soil
CIP 103003 (DSM 3133)	<i>C. fermentans</i>	Dumping ground
CIP 102114 (ATCC 484)	<i>C. fimi</i>	Soil
CIP 82.10 (ATCC 482)	<i>C. flavigena</i>	Unknown
CIP 102221 (ATCC 488)	<i>C. gelida</i>	Soil
CIP 102089 (ATCC 491)	<i>C. uda</i>	Compost
CIP 100331 (ATCC 25835)	<i>O. turbata</i>	Soil
CIP 81.28 (ATCC 27402)	<i>O. xanthineolytica</i>	Aluminum hydroxide gel antacid
CIP 55.134 (ATCC 14870)	<i>J. denitrificans</i>	Cooked ox blood
Clinical strains		
DMMZ CE39	CDC coryneform group A-3, <i>C. hominis</i>	Cerebrospinal fluid (f, nk)
DMMZ CE40	CDC coryneform group A-3, <i>C. hominis</i>	Cerebrospinal fluid (m, nk)
DMMZ CE23	CDC coryneform group A-4	Blood culture (m, 24)
DMMZ CE24	CDC coryneform group A-4	Blood culture (f, 71)

^a CIP, Collection des Bactéries, Institut Pasteur, Paris, France; ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany; DMMZ, Department of Medical Microbiology, University of Zürich, Zürich, Switzerland.

^b f, female; nk, not known; m, male.

used in the treatment of infections caused by gram-positive rods (ciprofloxacin, clindamycin, erythromycin, gentamicin, penicillin G, rifampin, tetracycline, and vancomycin) was determined by following the guidelines for performance and interpretation of the National Committee for Clinical Laboratory Standards (15, 16). For interpretation of susceptibility to penicillin G the categories for staphylococci were applied. MICs were tested by the agar dilution procedure (Muel-ler-Hinton agar supplemented with 5% sheep blood).

CFA patterns. Cells were grown on Trypticase soy agar with 5% sheep blood for 48 h at 37°C with 5% CO₂. For analysis of the cellular fatty acids (CFAs) we used the Microbial Identification System (Microbial ID, Inc., Newark, Del.) as outlined previously (27).

Chemotaxonomic investigations. The method used for determination of the diamino acid of the cell wall peptidoglycan is given in a previous report (9). For the determination of the G+C content of the clinical strains the method outlined by Mesbah et al. (13) was used. Briefly, chromosomal DNA was degraded to nucleosides by P1 nuclease and alkaline phosphatase (Boehringer, Rotkreuz, Switzerland). Separation of the nucleosides was performed by high-performance liquid chromatography with a Waters 625 LC system (Millipore, Volketswil, Switzerland) equipped with a C₁₈ reversed-phase column. Nucleosides were detected with a Waters 486 Tunable Absorbance Detector at a wavelength of 254 nm, and the data were processed with a Waters 746 Data Module. For calculations of the G+C content we used the program written by K. D. Jahnke (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany).

Sequence determination of rRNA genes. Large fragments of the 16S rRNA genes of the test strains were amplified by PCR with universal primers pA (5'-AGAGTTTGATCCTGGCTCAG; positions 8 to 27, *Escherichia coli* numbering) and pH* (5'-AAGGAGGTGATCCAGCCGCA; positions 1541 to 1522) as described previously (11). PCR products were purified with a Prep-A-Gene kit (BioRad, Hercules, Calif.) according to the manufacturer's instructions and were sequenced with a *Taq* Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems Inc., Foster City, Calif.) and an automatic sequencer (model 373A; Applied Biosystems Inc.).

The 16S rRNA gene sequences and those of reference organisms that were determined were aligned by using the program PILEUP (5), and the alignments were corrected manually. A distance matrix was produced with the program DNADIST of the PHYLIP package (6), and a tree was constructed by the neighbor-joining method with the program NEIGHBOUR of the same package. The stability of groupings was assessed by bootstrapping by using the programs SEQBOOT, DNADIST, NEIGHBOUR, and CONSENSE (6).

Nucleotide sequence accession number. The nucleotide sequence of the 16S rRNA of strain DMMZ CE40 has been deposited in the EMBL Data Library under the accession number X 82598.

RESULTS AND DISCUSSION

The four clinical strains (Table 1) were isolated in pure culture from sterile body sites. We were not able to obtain any data from the patients' records in order to determine the clinical significance of the isolates.

The colony sizes of the clinical isolates and the *Cellulomonas* and *Oerskovia* reference strains were about 1 mm in diameter after 24 h of incubation at 37°C in 5% CO₂, whereas those of *J. denitrificans* isolates were only approximately 0.5 mm in diameter. Both CDC coryneform group A-3 and A-4 bacteria did not show any vegetative hyphae, whereas this phenomenon was observed for the two *Oerskovia* species within 2 days, and *J. denitrificans* left an impression on the agar plate, as reported previously (12, 20). The colonies of CDC group A-3 strains showed a white pigment that became yellowish within 3 days, whereas CDC group A-4 strains were yellow from the beginning. Gram stains of CDC coryneform group A-3 and A-4 strains incubated for 48 h at 37°C in 5% CO₂ showed relatively small, thin rods without irregular branching. The basic biochemical reactions of the four clinical strains and the reference strains are listed in Table 2. The major reaction applied in the routine laboratory to differentiate CDC group A-3 strains from CDC group A-4 strains is the ability of CDC group A-4 bacteria to ferment mannitol, whereas CDC group A-3 strains are negative for this reaction. In addition, the fermentation of β-methyl-xyloside differentiated between the two taxa (Table 2). Fermentation of the following carbohydrates was positive in all strains examined (including the 10 type strains): glycerol, L-arabinose, galactose, D-fructose, D-mannose, salicin, cellobiose, lactose, and β-gentiobiose. None of the 14 strains examined was able to ferment erythritol, adonitol, dulcitol, inositol, or 2-ketogluconate. Enzymatic activities of esterase (C₄), esterase lipase (C₈), leucine arylamidase, and α-glucosidase could be detected in all of the strains studied. Valine arylamidase, β-glucuronidase, and α-fucosidase activities were not found in any of the strains. As shown in Table 2, none of the biochemical profiles of the four clinical strains was compatible with the profiles of the seven defined *Cellulomonas* species, two *Oerskovia* species, or *J. denitrificans*. Surprisingly, we observed that the type strain of *O. turbata* was cellulolytic and was not able to hydrolyze casein, which is in contrast to the description given elsewhere (12).

The four clinical isolates and all reference strains tested were susceptible to tetracycline and vancomycin, and only the two *Oerskovia* strains and strain DMMZ CE24 were resistant

TABLE 2. Biochemical reactions of the strains studied^a

Biochemical reaction	<i>C. biozota</i>	<i>C. cellasa</i>	<i>C. fermentans</i>	<i>C. fimi</i>	<i>C. flavigena</i>	<i>C. gelida</i>	<i>C. uida</i>	<i>O. turbata</i>	<i>O. xanthineolytica</i>	<i>I. dentrificans</i>	DMMZ CE39 (CDC A-3), <i>C. hominis</i>	DMMZ CE40 (CDC A-3), <i>C. hominis</i>	DMMZ CE23 (CDC A-4)	DMMZ CE24 (CDC A-4)
Catalase	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Motility	+	-	-	+	+	+	-	+	+	+	+	+	+	+
Lysis of cellulose	+	+	+	+	+	+	+	+	-	+	+	+	-	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DNase	-	-	+	-	-	+	+	+	+	-	+	+	+	+
Alkaline phosphatase	s	m	-	m	-	+	+	w	+	+	+	+	+	+
Chymotrypsin	-	-	-	-	m	-	m	-	m	(w)	-	-	-	-
α -Mannosidase	-	-	-	-	-	w	m	-	-	-	(w)	w	w	s
Fermentation of:														
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	+	-	+	+	-	-	-	+	-	-	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dextrin	-	-	+	+	+	+	+	+	+	+	+	+	+	+
β -Methyl-xyloside	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	-	+	(+)	-	-	-	-	-	-	+	+	+	-
α -Methyl-mannoside	-	-	-	+	-	-	-	(+)	(+)	-	+	+	+	+
Gluconate	-	-	-	-	(+)	-	-	(+)	(+)	+	+	+	+	+

^a Casein and xanthine were hydrolyzed by *O. xanthineolytica*, and tyrosine was hydrolyzed by *C. flavigena* only. w, m, and s, approximately 5, 20, and >40 nmol of substrate hydrolyzed, respectively; parentheses indicate only a weak reaction.

TABLE 3. Antimicrobial susceptibility patterns of the strains studied

Strain	MIC ($\mu\text{g/ml}$)							
	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Penicillin G	Rifampin	Tetracycline	Vancomycin
<i>C. biazotea</i>	2	2	4	16	1	0.06	0.25	0.06
<i>C. cellasea</i>	0.25	0.06	≤ 0.03	1	0.06	≤ 0.03	≤ 0.03	0.06
<i>C. fermentans</i>	2	0.25	0.06	64	0.125	0.5	0.125	0.125
<i>C. fimi</i>	2	2	4	32	0.06	≤ 0.03	0.5	0.06
<i>C. flavigena</i>	1	0.5	0.25	8	0.125	0.125	0.25	0.25
<i>C. gelida</i>	8	1	0.5	>64	0.125	0.03	0.5	0.06
<i>C. uda</i>	2	2	0.5	64	0.125	≤ 0.03	0.25	0.06
<i>O. turbata</i>	8	4	2	8	4	4	ND ^a	0.5
<i>O. xanthineolytica</i>	2	4	4	2	2	8	ND	0.5
<i>J. denitrificans</i>	2	8	0.25	16	2	≤ 0.03	0.25	0.06
CDC A-3 (CE39), <i>C. hominis</i>	2	8	2	16	1	≤ 0.03	0.25	0.06
CDC A-3 (CE40), <i>C. hominis</i>	1	8	1	8	2	≤ 0.03	0.5	0.125
CDC A-4 (CE23)	2	8	2	32	4	0.06	1	0.125
CDC A-4 (CE24)	2	2	1	32	2	4	1	0.25

^a ND, not determined.

to rifampin (Table 3). The susceptibility pattern against the other five drugs tested was somewhat invariable; the limited potential (as revealed by in vitro testing only) of ciprofloxacin, clindamycin, erythromycin, gentamicin, and penicillin G against other actinomycetes has been reported previously (9).

12-Methyltetradecanoic acid ($C_{a15:0}$) was the major CFA in all strains examined (Table 4). The amount of hexadecanoic acid ($C_{16:0}$) was greater than that of 14-methylhexadecanoic acid ($C_{a17:0}$) in all strains examined except for the two *Oerskovia* strains. The latter two strains also exhibited relatively large amounts of 13-methyltetradecanoic acid ($C_{i15:0}$). Furthermore, significant amounts of tetradecanoic acid ($C_{14:0}$) and 12-methyltridecanoic acid ($C_{i14:0}$) were detected in all strains, whereas tridecanoic acid ($C_{13:0}$) was inconsistently present. These characteristics differentiate *Cellulomonas*, *Oerskovia*, and *Jonesia* strains from other related coryneform genera (2, 3, 4, 8, 27). These data for CDC group A-3 and A-4 strains are in accordance with the findings of Chou et al. (3) and von Graevenitz et al. (27), whereas Bernard et al. (2) found larger amounts of 14-methylpentadecanoic acid ($C_{i16:0}$) and $C_{a17:0}$. However, these discrepancies may be due to the limited number of strains tested in the various studies or heterogeneity within

CDC group A-3 and A-4 bacteria. Na'was et al. (17) presented CFA data for 8 CDC group A-4 strains only, although Hollis and Weaver (from the same laboratory) had tested 40 CDC A-4 strains for their tables (10). This may have been due to the recognition of heterogeneity within their CDC group A-4 strains by those investigators (17). However, our CDC group A-4 CFA data are qualitatively different from the data of Na'was et al. (17), who detected $C_{a17:0}$ at a range of 33 to 74% of total CFAs; in contrast, we detected $C_{a17:0}$ at a range of only 12 to 13% of total CFAs in the present study. It seems likely that some of the eight CDC group A-4 strains examined by Na'was et al. (17) may have corresponded to *Microbacterium* spp., in which $C_{a15:0}$ and $C_{a17:0}$ represent the major CFAs (4, 8).

Cell wall analysis revealed L-ornithine as the diamino acid in the four clinical isolates, thereby reinforcing their affinity with the genus *Cellulomonas* (24). In contrast, *Oerskovia* and *Jonesia* strains both contain cell walls based on L-lysine (12, 19). The G+C contents of the DNAs of the four clinical strains were within the range of 73 to 76%, which is consistent with the values for *Cellulomonas* (24) and *Oerskovia* (12) strains but significantly higher than that for *J. denitrificans* (57%) (19).

TABLE 4. CFA patterns of the strains studied

Strain	% Total fatty acid content ^a													
	$C_{13:0}$	$C_{i14:0}$	$C_{14:0}$	$C_{i15:0}$	$C_{a15:0}$	$C_{15:0}$	$C_{i16:0}$	$C_{16:0}$	$C_{i17:0}$	$C_{a17:0}$	$C_{17:0}$	Feat. ^b	$C_{18:1\omega 9t}$	$C_{18:0}$
<i>C. biazotea</i>	1	2	4	17	35	2	2	10	3	8	3	1	3	5
<i>C. cellasea</i>		1	2	4	55	2	2	10	1	9	1	1	3	3
<i>C. fermentans</i>		2	14	3	40	2	1	16		2	1	3	4	5
<i>C. fimi</i>		1	6	3	44		3	15		14		2	4	6
<i>C. flavigena</i>	1	2	12	2	34	4	1	23		2	1	2	5	4
<i>C. gelida</i>	2	2	4	4	43	8	3	17	1	7	2	1	3	1
<i>C. uda</i>	1	2	4	4	43	8	3	17	1	7	2	1	3	1
<i>O. turbata</i>		1	1	26	36	1	4	7	5	16	1		1	1
<i>O. xanthineolytica</i>		1	3	24	43	3	7	4		10	1		1	1
<i>J. denitrificans</i>		1	7	2	42	2	3	24		6	1	2	5	4
DMMZ CE 39 (CDC A-3), <i>C. hominis</i>	1	1	8	2	40	7	3	16		10	4	1	2	3
DMMZ CE 40 (CDC A-3), <i>C. hominis</i>	1	1	8	2	42	8	3	16		7	4	1	2	3
DMMZ CE 23 (CDC A-4)		1	7	2	48	3	2	18		13	2	1	1	3
DMMZ CE 24 (CDC A-4)		1	4	3	53	2	2	16		12	1	1	1	2

^a Amounts of less than 1% are not reported.

^b Comprises $C_{18:2\omega 6,9c}$ and $C_{a18:0}$.

TABLE 5. Percent 16S rRNA sequence similarities of *C. hominis* DMMZ CE40 and some other representative gram-positive bacteria with high G+C contents^a

Species	% Sequence similarity														
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1. <i>Cellulomonas hominis</i>	97.0	94.8	95.4	95.0	96.0	94.7	95.6	95.5	96.2	95.9	95.8	94.7	93.7	93.5	
2. <i>Oerskovia turbata</i>		95.0	95.5	95.0	95.8	95.1	94.4	95.3	95.6	95.7	95.5	95.9	95.3	94.0	
3. <i>Oerskovia xanthineolytica</i>			94.7	99.6	94.0	99.5	93.8	94.7	93.6	94.0	94.6	95.2	94.7	93.5	
4. <i>Cellulomonas biazotea</i>				94.5	97.7	94.4	95.5	99.6	96.3	95.7	95.6	94.6	94.4	93.8	
5. " <i>Cellulomonas cartae</i> "					93.9	99.7	93.8	94.5	93.7	94.3	95.0	95.3	94.6	93.5	
6. <i>Cellulomonas cellasea</i>						93.9	95.8	97.9	96.1	96.1	96.0	95.3	95.3	94.3	
7. " <i>Cellulomonas cellulans</i> "							93.6	94.4	93.3	94.1	94.9	95.3	94.8	93.5	
8. <i>Cellulomonas fermentans</i>								95.6	95.3	94.6	94.8	94.3	94.3	93.8	
9. <i>Cellulomonas fimi</i>									96.1	95.8	95.7	94.6	94.6	93.8	
10. <i>Cellulomonas flavigena</i>										97.4	96.9	93.5	93.4	92.5	
11. <i>Cellulomonas gelida</i>											99.0	93.1	93.1	92.7	
12. <i>Cellulomonas uda</i>												93.1	93.1	92.5	
13. <i>Sanguibacter keddieii</i>													98.1	95.4	
14. <i>Sanguibacter suarezii</i>														95.3	
15. <i>Terrabacter tumescens</i>															

^a Values are based on a pairwise comparison of ca. 1,393 nucleotides. The numbers in the column represent the species with the corresponding numbers on the left.

To establish the phylogenetic affinities of the clinical isolates, their partial 16S rRNA gene sequences were examined. The sequence of a large fragment (>1,400 nucleotides) from strains DMMZ CE40 (CDC A-3) and DMMZ CE24 (CDC A-4) was determined. These strains exhibited two closely re-

lated, although different (approximately 99.4% sequence similarity), 16S rRNA sequences. The sequences of short 16S rDNA fragments (approximately 500 bases, ranging from positions 40 to 520 and including the highly variable regions V1 to V3), from strains DMMZ CE39 (CDC A-3) and DMMZ CE23

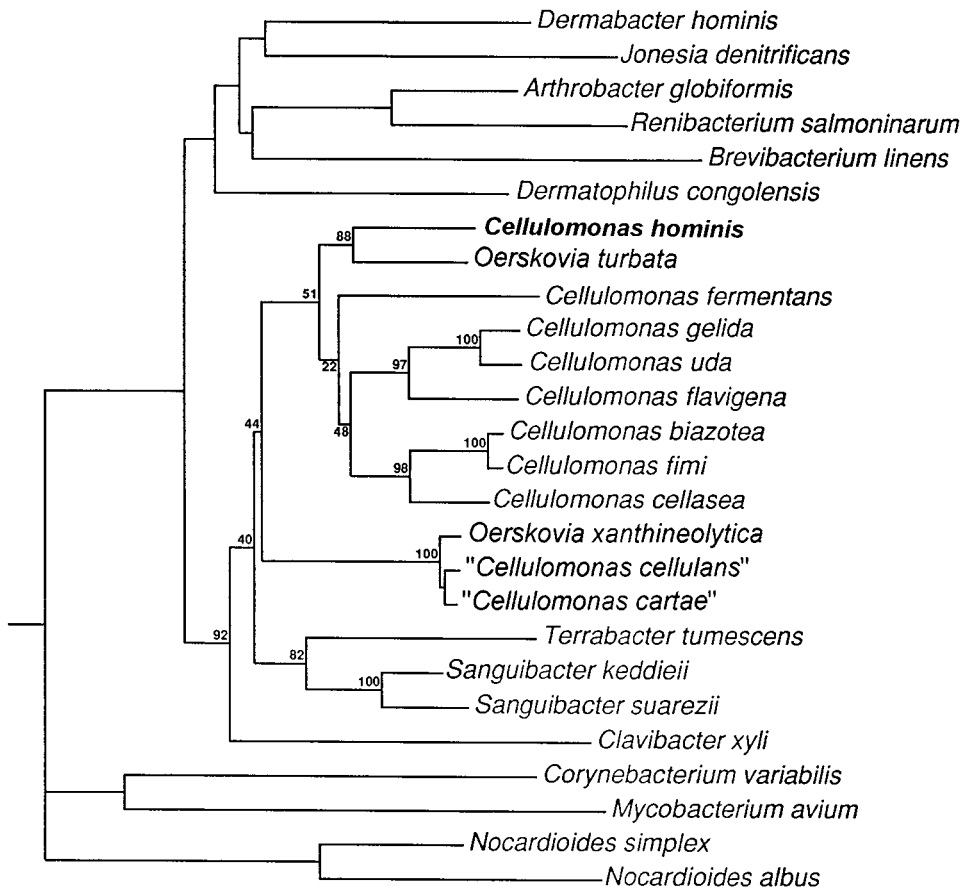


FIG. 1. Unrooted tree constructed by the neighbor-joining method showing the phylogenetic position of *C. hominis* DMMZ CE40 within the *Cellulomonas*-*Oerskovia* lineage of the gram-positive rods with high G+C contents. The numbers indicate bootstrap values.

(CDC A-4) were also determined and were shown to be identical to those of strains DMMZ CE40 and DMMZ CE24, respectively (i.e., the strains were genealogically homogeneous within their respective groups). To determine the generic positions of the clinical isolates, their sequences were compared with those of other gram-positive bacteria with high G+C contents (Table 5). The clinical strains displayed the highest sequence similarities (approximately 95 to 97%) with *Cellulomonas* spp. and *Oerskovia* spp. A tree depicting the phylogenetic position of strain DMMZ CE40 is shown in Fig. 1. From the phylogenetic analysis it is clear that the CDC group A-3 and group A-4 strains examined are members of the *Cellulomonas-Oerskovia* subbranch of the actinomycetes.

The taxonomic separateness of the genera *Cellulomonas* and *Oerskovia* has long been controversial (18, 26). Stackebrandt et al. (26) proposed the unification of *Cellulomonas* and *Oerskovia* (the former name having nomenclatural priority), although current opinion (based primarily on cell wall differences) is that these genera should retain their separate identities (25). It is, however, now evident that *Cellulomonas* spp. and *Oerskovia* spp. are phylogenetically intermixed and form a monophyletic group (7).

The observed 16S rRNA sequence divergences of approximately 3 to 5% between the clinical isolates and the currently recognized *Cellulomonas* species and *Oerskovia* species are indicative of a new species (22). Therefore, on the basis of both the phenotypic and phylogenetic characteristics, we formally propose that the CDC group A-3 strains examined be assigned to a new species, *Cellulomonas hominis* sp. nov. Although the CDC group A-4 strains are without doubt genealogically closely related to the CDC group A-3 strains, DNA-DNA hybridization studies (28) are necessary to ascertain whether they are members of this species or represent a separate species.

***Cellulomonas hominis* sp. nov.** *Cellulomonas hominis* (ho'mi.nis L. gen. n. *hominis*, of man, indicating that the two isolates included in the present study are derived from humans and not from the environment). The cells are short (1 μ m), thin gram-positive rods without irregular branching. No spores formed. The cells are motile. The colonies are circular, smooth, convex, and whitish initially but with yellowish pigmentation in about 3 days. The colony size is about 1 mm in diameter after 24 h of incubation at 37°C in 5% CO₂. The organism is catalase positive. Esculin and gelatin are hydrolyzed, but urea is not. Nitrate is reduced to nitrite. Acid is produced from L-arabinose, cellobiose, dextrin, D-fructose, galactose, β -gentiobiose, gluconate, glucose, glycerol, lactose, maltose, mannose, α -methyl-D-mannoside, rhamnose, salicin, sucrose, xylose, and β -methyl-xyloside. No acid is produced from adonitol, dulcitol, erythritol, inositol, 2-ketogluconate, or mannitol. The following enzyme activities are detected: DNase, esterase (C₄), esterase lipase (C₈), leucine arylamidase, α -glucosidase, and α -mannosidase. Valine arylamidase, chymotrypsin, β -glucuronidase, and α -fucosidase are not present. The DNA base composition ranges from 73 to 76 mol% G+C. The type strain DMMZ CE40 has been deposited in the Deutsche Sammlung für Mikroorganismen und Zellkulturen under accession number DSM 9581. This strain has the characteristics described for the species, and its G+C content is 76 mol%.

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