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Recognition of *Dermabacter hominis*, Formerly CDC Fermentative Coryneform Group 3 and Group 5, as a Potential Human Pathogen

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Thirty strains of fermentative coryneform-like bacteria designated CDC fermentative coryneform group 3 and coryneform group 5 were compared biochemically by cellular fatty acid analysis and by DNA relatedness with the type strain of *Dermabacter hominis*, ATCC 49369. DNA from 22 strains of both CDC groups showed 69 to 96% relatedness (hydroxyapatite method) to labeled DNA from ATCC 49369 and to DNA from CDC group 3 strain G4964, and the strains are considered to belong to *D. hominis*. The remaining eight strains were genetically but not phenotypically differentiable from *D. hominis*. They were genetically heterogenous, but hybridization results indicated that they probably belong to the genus *Dermabacter*. Thirteen of the 22 *D. hominis* strains and all 8 of the other *Dermabacter* strains had been isolated from blood, which indicates the pathogenic potential of this species and genus.

In 1988, Jones and Collins described four strains of gram-positive, asporogenous and nonmotile, rod-shaped bacteria belonging to a new genus, *Dermabacter hominis* (8). These strains had been isolated from the skin of healthy adults, and there was no evidence that the bacterium was related to human disease. Upon closer examination, *D. hominis* seemed to be phenotypically most similar to some strains received since the 1970s by the Special Bacteriology Reference Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. They had been provisionally grouped among other coryneform fermentative gram-positive rods as CDC coryneform group 3 and coryneform group 5. Separation of the two groups depended upon their ability to ferment xylose, with group 3 being xylose positive and group 5 (like the described species *D. hominis*) xylose negative. Biochemical characteristics of value in differentiating both groups from other similar bacteria are the fermentation of glucose, lactose, sucrose, and maltose; the hydrolysis of esculin; and the production of lysine and ornithine decarboxylases. They may or may not hydrolyze gelatin. Most of the strains had been isolated from blood or normally sterile body sites. In a recent report, a group 3 strain and a group 5 strain were assigned to the genus *Dermabacter* on the basis of 16S rRNA sequencing (6). In the present study, 30 strains of CDC groups 3 and 5 were further characterized by assaying for enzyme activities, and their relationship to *D. hominis* was examined by DNA-DNA hybridization.

MATERIALS AND METHODS

Strains and biochemical tests. The clinical isolates used in this study were 14 strains of CDC group 3 and 16 strains of CDC group 5 (Table 1). They were obtained from the collection of the Special Bacteriology Reference Laboratory at CDC, as were the human reference strain of *Actinomyces viscosus*, ATCC 27044, and five clinical isolates of coryneform fermentative CDC group A-3 (A3936, C2508, C8262, D2991, and D3060) that were used in the DNA relatedness studies. The type strain of *D. hominis*, ATCC 49369, was obtained from the American Type Culture Collection (Rockville, Md.). For biochemical tests, strains were grown aerobically at 35°C on heart infusion agar (HIA) with 5% rabbit blood (BBL Becton Dickinson Microbiology Systems, Cockeysville, Md.). Tests were done as described previously (5). Further enzyme activities were assayed by using the API ZYM System (API bioMérieux SA, La Balme-les-Grottes, France) following the guidelines of the manufacturer. Color reactions with values greater than or equal to 2 were recorded as positive.

CFA analysis. Bacteria were grown on three plates each of HIA with rabbit blood and were incubated for 2 days aerobically at 35°C. Previously described protocols (9) were followed for the determination of cellular fatty acids (CFAs).

Determination of *m*-DAP. Whole-cell hydrolysates of one CDC group 3 (G8586), one CDC group 5 (E6535), and two CDC group A-3 (C8262 and A3936) strains were prepared as described previously (1). The type strain of *D. hominis* was used as a control for the presence of *meso*-diaminopimelic acid (*m*-DAP).

DNA relatedness studies. For the preparation of unlabeled DNA, strains were grown in 3 to 5 liters of brain heart infusion broth (BHIB) and incubated at 37°C for 2 days. They were stored at 4°C until use. Lysis of cells was achieved by suspension in a lysing solution (approximately 20 ml/liter of BHIB) containing 0.2 M sucrose, 0.05 M Tris-HCl, and 4 mg of lysozyme per ml and by overnight incubation in a water bath at 37°C. Pronase Protease (final concentration, 50 μg/ml; Calbiochem, La Jolla, Calif.) and sodium dodecyl sulfate at a final concentration of 2% were added, and the solutions were transferred to a 60°C water bath for 1 h. The extraction and purification of DNA have been described previously by Brenner et al. (4), as has the DNA-DNA hybridization method with

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TABLE 1. Sources and clinical backgrounds of isolates

Strain (CDC group)	Source and diagnosis or underlying disease	Sex and age (yr)[a]	Geographic source and year received
E2835 (3)	Eye	M, 69	Maine, 1978
E5170 (3)	Blood	F, 41	Hawaii, 1979
E6535[b] (5)	Blood	M, 45	Ottawa, Canada, 1979
F3878 (3)	Blood; aplastic anemia	M, 30	Maryland, 1982
F5058 (3)	Blood; premature newborn	F, newborn	Hawaii, 1983
F5354 (3)	Lung; pneumonia	F, 55	California, 1984
F6232 (3)	Blood; colon cancer	F, 83	Pennsylvania, 1984
F6362 (3)	Blood; septicemia	F	South Carolina, 1984
F7461 (3)	Blood; septicemia	F, 84	Florida, 1985
F8268 (5)	Chest wall abscess	M, 2	Ohio, 1986
F9595 (5)	Peritoneal fluid; peritonitis	F, newborn	Texas, 1987
F9707 (5)	Blood; sepsis	M, 33	Texas, 1987
G972 (5)	Cerebrospinal fluid; meningitis	M, 44	California, 1988
G1344 (3)	Blood; infected ulcer stump, diabetes mellitus	M, 68	Pennsylvania, 1988
G1718 (3)	Blood; fever of unknown origin	F, 28	Texas, 1988
G2904[c] (5)	Blood	F	Ottawa, Canada, 1989
G2907[c] (5)	Blood	M, 67	Ottawa, Canada, 1989
G3197 (5)	Blood	F, newborn	Honolulu, 1989
G3313 (5)	Wound; foot lesion	M	Kansas, 1989
G3862 (5)	Blood; fever of unknown origin	F, 70	South Carolina, 1989
G4133 (5)	Liver abscess	M, 24	Ohio, 1989
G4964[c] (3)	Abscess and fever	F, 40	California, 1990
G5864 (5)	Septic arthritis		Goteborg, Sweden, 1991
G6181 (5)	Blood; pneumonia	F	Hawaii, 1991
G7119 (3)	Blood; shoulder		Leuven, Belgium, 1992
G7506 (5)	Blood; endocarditis	M, 29	Hawaii, 1992
G7815 (5)	Blood; bacteremia	F, 71	South Carolina, 1992
G8033 (3)	Blood; bacteremia	M, 30	Texas, 1992
G8081 (5)	Breast wound infection	F	Ohio, 1992
G8586 (3)	Blood; pneumonia	M, 59	Michigan, 1993

[a] M, male; F, female.
[b] E6535 = LCDC (Laboratory Centre for Disease Control, Ottawa, Ontario) 79-030 = ATCC 51325.
[c] G2904 = LCDC 88-0597 = ATCC 51326; G2907 = LCDC 89-0184; G4964 = ATCC 51458.

hydroxyapatite. Labeling of DNA was done with [^{32}P]dCTP by using a nick translation kit (Gibco BRL, Gaithersburg, Md.) as described by the manufacturer. Since optimal reassociation of DNA occurs at a temperature which is approximately 25°C lower than that of its melting point, all hybridization reactions were performed at 65°C (optimal reassociation) and 80°C (stringent reassociation). The divergence or the percent of unpaired bases within hybridized sequences is obtained by determining the decrease in thermal stability of a heterologous reassociated DNA duplex compared with that of the homologous duplex. Each degree Celsius reduction of the melting point (T_m) corresponds approximately to 1% divergence within related sequences (3). Divergence was calculated to the nearest 0.5%. All hybridization reactions were done at least twice.

The guanine-plus-cytosine (G+C) contents of four CDC group 3 and group 5 strains were determined at least four times spectrophotometrically by thermal denaturation. DNA from the type strain of *D. hominis*, ATCC 49369, was included as a control.

RESULTS

DNA relatedness studies. DNA from the type strain of *D. hominis* was labeled and tested for relatedness to CDC group 3 and group 5 strains, to CDC group A-3 strains, and to *A. viscosus* ATCC 27044 (Table 2). Twenty-two of 30 CDC group 3 and group 5 strains formed one hybridization group with *D. hominis*. Their average relatedness in reactions done at 65°C was 76%, and relatedness was not substantially reduced when reactions were done at 80°C. Average sequence divergence was 3.9% (with one strain, F6362, showing 5.5%). Only three strains (G3862, G7506, and G3197) showed divergence of less than 3%. Since divergence within strains in the *D. hominis* hybridization group was high, although within the limit seen between strains of a single species, DNA from CDC group 3 strain G4964 was labeled and used as a probe. The average DNA relatedness within this hybridization group (which included the same 22 strains) was 83% at 65°C, and the divergence values were lower (average of 2.6%, Table 2). Four strains (G1718, F9707, F8268, and G2904) showed relatedness and divergence values approximately identical to those of *D. hominis* ATCC 49369 and G4964. In only two strains (G3862 and E6535), DNA relatedness to ATCC 49369 was more than 10% higher than that observed to G4964 or divergence was less from ATCC 49369 than from G4964. All other strains (n = 12, F6362 included) showed closer relatedness to G4964 than to ATCC 49369.

The eight remaining CDC group 3 and group 5 strains were excluded from the *D. hominis* hybridization group by divergence values of 6% or more, coupled with relatedness values of 70% or less at the optimum reassociation temperature. Of these eight strains, G8033, G2907, G5864, and G4133 formed a single hybridization group, with an average relatedness of 77% and an average divergence of 3% to labeled DNA from G8033 (data not shown). The remaining four strains were not further characterized.

None of the labeled strains cited above showed significant relatedness to strains of CDC group A-3 or the human reference strain of *A. viscosus* (values from 8 to 26% at 65°C).

TABLE 2. Comparison of DNA relatedness and divergence of CDC group 3 and group 5 with the type strain of *D. hominis*

Source of unlabeled DNA	% Relatedness at reassociation temperature indicated and % divergence (D) from labeled DNA of:						G+C content (mol%)[a]
	D. hominis ATCC 49369			CDC group 3 strain G4964			
	65°C	D	80°C	65°C	D	80°C	
D. hominis ATCC 49369	100	0.0	100	78	4.0	77	62.0 ± 0.2
G3862	89	2.0	91	78	3.0	68	
G7506	85	2.5	79				
E5170	83	3.0	77	96	1.0	98	
E2835	84	4.0	81	90	1.5	81	
G8586	82	3.0	80	88	1.5	81	
G7815	82	3.0	75	82	1.5	83	
F3878	82	4.0		94	1.0	92	
F5354	81	4.0	80	93	1.0	94	
G1344	81	5.0	76	91	2.0	83	
G3313	81	5.0	70	89	3.0	75	
F6232	81	5.0		90	2.5	89	
F5058	80	3.0	80	85	1.5	84	
G1718	80	3.5	83	81	3.0	85	
G4964	79	3.0	70	100	0.0	100	61.8 ± 0.3
F7461	79	3.5	73	83	1.5	80	
G8081	79	4.5	75	87	2.0	72	
E6535	79	5.0	82	63	5.5	51	63.0 ± 0.3
G3197	77	2.5	76				
F9707	76	3.0	76	79	2.5	76	
F8268	76	5.0	79	73	4.0	53	
G2904	73	4.5	62	75	4.5	68	
F6362	69	5.5	71	74	4.0	66	
G6181	68	6.0	62	63	6.0	51	
G8033	59	6.5	47	55	6.0	41	61.1 ± 0.1
G2907	70	10.0	54				
G5864	52	8.0	32				
G4133	60	9.5	34				
F9595	66	12.5	36				
G7119	42	14.0	14				62.1 ± 0.1
G972	36	15.0	15				
CDC group A-3 strains							
C8262	26						
D2991	16						
C2508	13						
A3936	8						
D3060	8						
A. viscosus ATCC 27044	3						

[a] Mean ± standard deviation.

D. hominis has been reported to have a relatively high G+C content of 62 mol% (8), which was confirmed by our data. Four other strains tested were in the same range (61 to 63.3 mol%, Table 2).

CFAs. Twelve CDC group 3 strains, 7 group 5 strains, and *D. hominis* ATCC 49369 all had highly similar CFA profiles characterized by large amounts of branched-chain CFAs (anteisopentadecanoic acid [a15:0] and anteisoheptadecanoic acid [a17:0] [Table 3]).

***m*-DAP.** The two strains of CDC group A-3 did not contain *m*-DAP in whole-cell hydrolysates, whereas the CDC group 3 strain and the CDC group 5 strain tested did.

Phenotypic characterization. Both CDC groups 3 and 5 have been described extensively in an earlier publication (2). All group 3 and group 5 strains in the present study and the type

TABLE 3. CFA composition of CDC group 3 and CDC group 5 strains and the *D. hominis* type strain

Strain (no. of strains tested)	% CFA[a]												
	i14:0	14:0	i15:0	a15:0	i16:0	16:0	i17:0	a17:0	18:2	18:1w9c	18:1w7c	18:0	19cycC11-12
CDC group 3 (12)	1 (0–2)	4 (2–11)	8 (5–14)	24 (16–33)	8 (6–12)	8 (6–12)	t (0–1)	17 (9–24)	8 (4–13)	6 (4–12)	3 (0–8)	4 (2–8)	3 (1–7)
CDC group 5 (7)	1 (0–3)	3 (1–10)	8 (7–13)	25 (16–36)	9 (7–12)	8 (5–11)	t (0–1)	21 (10–27)	7 (0–10)	6 (4–9)	2 (0–5)	3 (0–6)	4 (1–9)
D. hominis ATCC 49369[T] (1)	1	1	8	23	10	7	1	29	5	5	0	3	5

[a] Values shown are percentages of total fatty acids and are arithmetic means rounded to the nearest whole percent: 0, 0.0% to 0.29%; t, 0.3% to 0.7%; the range is given in parentheses. For each CFA, the number to the left of the colon is the number of carbon atoms and the number to the right is the number of double bonds; i, methyl group at the penultimate (iso) carbon atom; a, methyl group at the antepenultimate (anteiso) carbon atom; c, *cis* isomer; w, double-bond position from the hydrocarbon end of the chain; cyc, cyclopropane ring.

strain of *D. hominis* corresponded to their original descriptions (2, 8), gram-positive, oxidase-negative (9% positive), catalase-positive, nonmotile, irregular rods, positive for the fermentation of glucose, lactose, sucrose, and maltose and variable for the fermentation of xylose. They all hydrolyzed esculin and decarboxylated ornithine and lysine. An additional characteristic not previously reported is the reduction of 0.01% nitrite within 48 h, but the reduction of a 0.1% nitrite concentration was not detected for up to 1 week. Strains varied in their ability to hydrolyze gelatin (35% positive). In the API ZYM System, all strains were positive for alkaline phosphatase, esterase-lipase C8, leucine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Most strains were positive in reactions for the presence of esterase C4 (89% positive) and α-galactosidase (85% positive).

DISCUSSION

We have shown that the genus *Dermabacter*, previously considered to be nonpathogenic, is a potential opportunistic pathogen, with isolates documenting that *Dermabacter* infections have occurred for almost 20 years. Although we have been unable to obtain satisfactory case histories for the patients, the fact that 20 of our cultures, and five cultures reported elsewhere (6), were isolated from human blood implies clinical significance.

We demonstrated that the species *D. hominis* contains both xylose-fermenting and non-xylose-fermenting strains, which had formerly been placed in coryneform CDC groups 3 and 5. Once again, this study shows that a single biochemical difference is not necessarily a good basis for creating separate species. Contrary to the original description (8), *D. hominis* includes strains both positive and negative for gelatin liquefaction. Our results confirm and extend a recent study which assigned both CDC groups to the genus *Dermabacter* on the basis of 16S rRNA gene analysis (6).

Biochemically, xylose-fermenting strains of *D. hominis* are most similar to the fermentative CDC group A-3, whereas non-xylose-fermenting strains resemble *A. viscosus* and *Rothia dentocariosa*. However, *D. hominis* differs from these species by its inability to reduce nitrate. It also differs from group A-3, *A. viscosus*, and *R. dentocariosa* by the presence of decarboxylases and in its CFA profile (2). Compared with *D. hominis*, CDC group A-3 strains and *R. dentocariosa* produce consistently larger amounts of a15:0 (>40%). Analysis by Bernard et al. (2) of CDC strains of *A. viscosus* from humans (included by Johnson et al. [6a] in *A. naeslundii* genospecies 2) showed that in contrast to *D. hominis*, *A. viscosus* produces large amounts of isopalmitic acid (16:0, >45%) and *cis*-9-octadecenoic acid (18:1w9c, >30%) and no a15:0. Also, in contrast to *D. hominis*, *Actinomyces* and *Rothia* strains and strains belonging to CDC group A-3 do not contain *m*-DAP in their cell walls (7).

The inclusion of CDC coryneform groups 3 and 5 in *D. hominis* was made primarily on the basis of DNA relatedness. Twenty-two of 30 CDC group 3 and group 5 strains constituted one group with DNA relatedness to the type strain of *D. hominis*. Except for one strain (F6362), their DNA relatedness at the optimal reassociation temperature was above 70% and the divergence in related sequences below 5%. Both values have been accepted as cutoffs for the definition of a single species, whereas lower values of approximately 40 to 65% relatedness are compatible with definition of different species in a single genus (10). Hybridization of DNA of members of the same species at a supraoptimal, stringent DNA reassociation temperature (80°C in this study) is expected to give values approximately identical to those obtained at an optimal DNA reassociation temperature (65°C in this study) or only slightly lower (≤15%), as was the case in this study. Interestingly, the degree of relatedness of most of the 22 strains was higher to labeled DNA of CDC group 3 strain G4964 than to the type strain of *D. hominis*. G4964 was similar to most other strains, showing a relatively low (although definitely species level) degree of relatedness and a relatively high degree of divergence from *D. hominis* ATCC 49369. This result implies that most pathogenic isolates of *D. hominis* are genetically closer to strain G4964 than to the presumably nonpathogenic type strain ATCC 49369, which was isolated as human skin flora (8). Strain G4964 was submitted to the American Type Culture Collection and is now listed as ATCC 51458.

The remaining eight strains, which did not hybridize with either G4964 or ATCC 49369 at the species level, could not be phenotypically differentiated by any of the tests used in this study. The G+C contents of two strains tested (G8033 and G7119, Table 2) were nearly identical to that of *D. hominis*. Four of the strains in this group formed an additional hybridization group with labeled DNA of strain G8033, indicating that this is a second species within the genus *Dermabacter* and that one or more additional species are represented by the four remaining ungrouped strains. These eight strains undoubtedly belong to the genus *Dermabacter*. At present, we prefer keeping them in *D. hominis* since they cannot be differentiated from each other or from this species.

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