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Attempts to identify coryneform isolates resembling Corynebacterium xerosis can lead clinical microbiologists to identification schemes with conflicting descriptions which result in confusing C. xerosis with Corynebacterium striatum. For the present study we purchased all available American Type Culture Collection and National Collection of Type Cultures reference cultures of C. xerosis (n = 10) and C. striatum (n = 4) and analyzed them as follows: (i) analysis of biochemical reactions in conventional tests and in the Rapid CORYNE system, (ii) whole-cell fatty acid analysis by using the gas-liquid chromatography research software of Microbial ID, Inc., and (iii) analysis of DNA homology in dot blot hybridizations. Three C. xerosis strains were indistinguishable from the C. striatum strains in whole-cell fatty acid analyses and DNA hybridizations and shared very similar biochemical reactions. The remaining seven strains of C. xerosis clustered into five groups on the basis of fatty acid patterns, DNA hybridizations, and biochemical tests. No reference strain of C. striatum fit the species description in Bergey's Manual of Systematic Bacteriology. The type strains of both C. striatum and C. xerosis fit their respective descriptions by the Centers for Disease Control and Prevention. This study suggests that the 10 commercially available reference strains of C. xerosis represent six different taxa which should be assigned to new species.

As the survival of severely compromised patients increases with advances in health care, clinical microbiologists more frequently encounter opportunistic coryneforms, commonly called diphtheroids, that cannot be identified by any of the available resources. Unfortunately, the inability of the microbiology laboratory to identify an isolate greatly reduces the clinician's recognition of its potential significance. For example, Corynebacterium jeikeium (CDC group JK) was not recognized as an important cause of sepsis in immunocompromised patients until it had been described in a 1979 publication (25). The Special Pathogens Laboratory at the Centers for Disease Control and Prevention (CDC) has addressed the problem of coryneform identification by describing more than 20 biochemically distinct tentative taxa to which they have assigned such names as group JK and group D-2 (14, 15). In spite of major contributions from the CDC, a recent study involving 21 biochemical tests found that only 60% of coryneform clinical isolates could be assigned to any species or CDC group (10). Unfortunately, identification schemes that are limited to validly published species enable reference laboratories to identify only one-third of their coryneform isolates (12, 13).

The fact that recognized species within the genus Corynebacterium may be difficult to identify with a high level of confidence can be at least partly attributed to numerous differences within reference strains of a single species (12). Diversity within the reference strains of Corynebacterium xerosis was described in 1970 when Yamada et al. (29) found that the C. xerosis type strain, ATCC 373, and strain ATCC 7711 had DNA containing 67 to 68 mol% G+C, which was markedly different from the 57 to 58 mol% found in other reference strains of this species (6, 19, 23, 29). The electrophoretic protein patterns of reference strains of *C. xerosis* also indicated that they were highly diverse organisms (16).

Confusion in the descriptions of *C. xerosis* and *Coryne-bacterium striatum* is found in a comparison of the two major resources available to clinical microbiologists for the identification of coryneform organisms (3, 14). In the CDC system, which includes 34 traits, *C. xerosis* differs from *C. striatum* only by the former's ability to produce acid from maltose and its lack of hemolysis on sheep blood agar. The maltose reactions listed in *Bergey's Manual of Systematic Bacteriology* (3) for *C. striatum* and *C. xerosis* are the opposite of those reported by CDC. The basis for the differences in the two descriptions of *C. striatum* is the fact that the CDC reactions are based on the *C. striatum* type strain ATCC 6940 (NCTC 764), whereas the description in *Bergey's Manual* (3) is from Munch-Petersen's report (22) of bovine strains that were not deposited in either the National Collection of Type Cultures (NCTC) or the American Type Culture Collection (ATCC) (3, 22).

The purpose of the present study was to compare all of the commercially available reference strains of C. *striatum* and C. *xerosis* in an attempt to resolve the discrepancies in the literature. The results from biochemical tests, whole-cell fatty acid analyses, and DNA-DNA dot blot hybridizations indicate that the 10 reference strains of C. *xerosis* comprise six different taxa, including one which is indistinguishable from the C. *striatum* reference strains.

MATERIALS AND METHODS

Strains. All strains used in the present study were freshly purchased from ATCC or NCTC. The following *C. striatum* strains were used: two cultures of the type strain (ATCC

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Strain ^a	DNA group ^b	Catalase	NO3	Urease	Gelatin	Motility	Esculin		Carboł	nydrate uti	Serum	Morphology			
								Glucose	Maltose	Sucrose	Mannitol	Xylose	stimulation	Colony ^c	Brothd
C. striatum															
A6940 ^T	Α	+	+	-	-	-	-	+	-	+	-	_	-	a-1	a-1
N764 ^T	Α	+	+	-	-	-	-	+	-	+	_	-	-	a-1	a-1
A43735	Α	+	+	-	_	-		+	-	+	-	-	_	a-1	a-1
A43751	Α	+	+	-	-	-	—	+	-	+	-	-	-	a-1	a-1
C. xerosis															
A7094	Α	+	$+^{e}$	-	-	-	_	+	-	+	_	-	_	a-1	a-1
A9016	Α	+	+	-	-	_	-	+	-	+	+	_	_	a-1	a-1
N9755	Α	+	+	_	-		_	+	-	+	_	-	-	a-2	a-1
A373 ^T	В	+	+	_	_	_	-	+	+	+	_	_	_	b-1	b
A7711	С	+	_	_	-	_	-	+	+ ^e	+	-	_	_	b-1	b
N7238	D	+	+	-	-	_	-	+	\pm^{f}	+8	_	_	+	d	
N7883	D	+	+	-	-	_	_	+	± ^f	+8	-	-	+	d	-
N7929	D	+	+	_	_	_	_	+	± ^f	+	-	_	+	d	_
N8481	Ε	+	+	+	-	_	_	+	+	+	_	_	+	d	-
N7243	F	+	-	_	-	-	-	+	+	+	-	-	_	b-2	a-2

TABLE 1. Conventional biochemical reactions and colony morphologies of C. striatum and C. xerosis reference strains

^a Reference strain numbers preceded by A and N were obtained from ATCC and NCTC, respectively. Superscript T's indicate type strains.

^b DNA groups are defined in the text, Fig. 2, and Table 4.

^c Morphology on Trypticase soy blood agar at 48 h. a-1, creamy gray-white colonies, 2 mm in diameter; a-2, colonies resemble a-1 colonies, but with tan pigment; b-1, dry, slightly yellow colonies, 0.2 to 1.0 mm in diameter; b-2, colonies resemble b-1 colonies, but with white color; d, translucent gray colonies, <0.2 mm in diameter.

^d Growth in heart infusion broth after 48 h. -, no growth; a-1, rapid dense growth throughout the tube; a-2, growth similar to that of a-1, but no turbidity at 24 h; b, large pellicle over a clear broth with settled clumps.

Delayed reaction.

^f Original reactions, in 0.3% rabbit serum, were interpreted as questionably positive, but repeat tests in 2% rabbit serum or Tween 80 had strong reactions at 48 h.

^g Weak reaction.

6940 and NCTC 764) and strains ATCC 43735 and ATCC 43751. The following *C. xerosis* strains were used: ATCC 373 (type strain), ATCC 7094, ATCC 9016, NCTC 9755, ATCC 7711, NCTC 7238, NCTC 7883, NCTC 7929, NCTC 8481, and NCTC 7243.

Media and culture conditions. For whole-cell fatty acid analyses, the organisms were grown on Trypticase soy agar supplemented with 5% sheep blood (Prepared Media Labs, Tualatin, Oreg.). To obtain sufficient biomass of earlystationary-phase cells, one to three plates were inoculated and incubated at 35°C in a 5% CO₂ atmosphere for up to 3 days, depending on each strain's growth rate. The procedure for the Rapid CORYNE system was that recommended by the manufacturer (bioMérieux Vitek, Inc., Hazelwood, Mo.), including the use of an inoculum from 24-h cultures harvested from two to four Columbia blood agar plates (11). For conventional biochemical tests, the cultures were harvested from overnight cultures on heart infusion agar supplemented with 5% sheep blood.

Biochemical tests. All reference strains were tested with conventional biochemicals by the recommended methods (5, 18). For lipid-dependent strains, 3-ml volumes of broth media were supplemented by the addition of 3 drops of 10% rabbit serum (final concentration, approximately 0.3%). Eugonic strains were incubated for 5 days, and lipid-dependent strains were incubated for 7 days. All strains were tested in the Rapid CORYNE strips according to the manufacturer's recommendations, as described by Gavin et al. (11).

Gas-liquid chromatography methods and instrumentation. Whole-cell fatty acid composition was determined by the Microbial Identification System, consisting of a Hewlett-Packard model 5890 gas chromatograph with auto injector (Hewlett-Packard 7673A) and integrator (Hewlett-Packard

3392) linked to a Hewlett-Packard series 300 computer. Software was purchased from Microbial ID, Inc., Newark, Del. Instrument configuration and organism growth and hydrolysis were those described in the Microbial Identification System protocol. The computer compared the retention times of the samples with that of the purchased standard solution (Microbial ID, Inc.) to achieve peak naming through computer-generated peak naming tables. Data were analyzed with the Library Generation Software of Microbial ID, Inc., which is a research program that provides two-dimensional plots of clusters or dendrograms of paired groups. The two-dimensional plots are based on principle component analysis. Principle Component 1 is the component responsible for the greatest degree of variability among the samples tested and is represented on the horizontal axis. Principle Component 2 is responsible for the second greatest degree of variability and is displayed on the vertical axis. The scale for both axes is the Euclidian distance. A group of strains which fit within an area of less than 70 Euclidian distances², measured by distances on the x and y axes, is considered to be sufficiently similar to potentially belong to the same species.

DNA hybridization. DNA was prepared from cells that were incubated by shaking at 37°C overnight in 200 ml of heart infusion broth supplemented with Tween 80 (final concentration, 0.2%), as described previously (4). The methods for nick translation, using $[\alpha^{-32}P]$ dATP and autoradiography, were those described by Buck and Groman (2). Dot blot hybridizations with whole chromosomal DNA were done by a modification of the method of Moseley et al. (21) by using 70% formamide to establish stringent conditions for *Corynebacterium* spp. with DNA containing 58 mol% G+C (17).

RESULTS

Morphology. The five colony morphologies observed in the 14 reference cultures are summarized in Table 1. All *C. striatum* strains and three *C. xerosis* strains that closely resembled *C. striatum* were 2 mm in diameter with a creamy texture. Colonies of *C. striatum* strains and two of the three *C. striatum*-like strains of *C. xerosis* (ATCC 7094 and ATCC 9016) were gray-white, and the third *C. xerosis* strain (NCTC 9755) was tan. *C. xerosis* ATCC 373 and NCTC 7711 produced slightly yellow, dry colonies that were 0.2 to 1.0 mm in diameter, and *C. xerosis* NCTC 7243 produced similar colonies that were white. Four *C. xerosis* strains (NCTC 7238, NCTC 7883, NCTC 7929, and NCTC 8481) produced tiny (<0.2 mm in diameter) translucent gray colonies which were typical of lipid-dependent coryneforms.

The growth patterns of the reference strains after growth in heart infusion broth cultures for 48 h ranged from profuse to none. Dense growth throughout the heart infusion broth occurred with all of the *C. striatum* strains and the three *C. striatum*-like strains of *C. xerosis*. *C. xerosis* NCTC 7243 also grew throughout the broth, but turbidity was not evident during the first day. Two *C. xerosis* strains, ATCC 373 and NCTC 7711, produced large pellicles over a clear broth that gradually accumulated settled clumps of cells. The four lipid-dependent strains of *C. xerosis* did not grow in heart infusion broth.

Biochemical tests. The biochemical reactions from conventional tests are shown in Table 1. All strains of *C. striatum* and *C. xerosis* produced catalase and acid from glucose and sucrose but did not attack gelatin, esculin, or xylose. Only one strain, *C. xerosis* ATCC 9016, produced acid from mannitol. Two *C. xerosis* strains (ATCC 7711 and NCTC 7243) did not reduce nitrate, and four *C. xerosis* strains (NCTC 7238, NCTC 7883, NCTC 7929, and NCTC 8481), including the only urease-producing strain, were lipid dependent. The maltose reactions of the four lipid-dependent strains could not be interpreted because the color change was so slight. Results from the Rapid CORYNE system are summarized as numerical profiles in Table 2. The reactions in conventional tests and the Rapid CORYNE system were in good agreement.

Whole-cell fatty acid analyses. The whole-cell fatty acid analyses of the C. striatum and C. xerosis reference strains are shown in Table 3. The major peaks were the unbranched fatty acids C_{16:0}, C_{18:1ω9}, and C_{18:0}. In all of the C. striatum and the three C. striatum-like strains of C. xerosis, C_{16:0} and $C_{18:1\omega9}$ accounted for over 87% of the fatty acids. Fatty acid patterns were analyzed with the Library Generation Software of Microbial ID, Inc., and the results are presented as the two-dimensional plot shown in Fig. 1. These results suggest that 3 of the 10 reference strains of C. xerosis are closely related to the C. striatum strains. This fairly tight cluster of seven strains sits within an area which is <70Euclidian distances², which is consistent with a single species. This C. striatum-like cluster has been designated group A to show correlation with the DNA results described below. Three of the four lipid-dependent C. xerosis strains (designated group D) appeared to be related at Euclidian distances that suggested a single species. Four strains of C. xerosis, including the type strain ATCC 373, did not cluster with any other strain.

DNA hybridization. Representative autoradiographs of DNA dot blots from the 14 reference cultures of *C. striatum* and *C. xerosis* are shown in Fig. 2. The type strain of *C. striatum* (ATCC 6940) hybridized with all reference strains

 TABLE 2. Rapid CORYNE strip profile of reference strains of

 C. striatum and C. xerosis^a

Species and strain ^b	DNA group	Profile no.
C. striatum		
ATCC 6940 ^T	Α	3100105
NCTC 764 ^T	Α	3100105
ATCC 43735	Α	3100305
ATCC 43751	Α	3100105
C. xerosis		
ATCC 7094	Α	2100105
ATCC 9016	Α	3100115
NCTC 9755	Α	3100305
ATCC 373 ^T	В	3110325
ATCC 7711	С	2110325
NCTC 7238	D	5100305
NCTC 7883	D	5100305
NCTC 7929	D	5100305
NCTC 8481	Ε	3001325
NCTC 7243	F	2100325

^{*a*} The Rapid CORYNE strip contained the following reactive ingredients in the ordered groups of three as follows: nitrate reduction, pyrazinamidase, and pyrrolidonyl arylamidase; alkaline phosphatase, β -glucuronidase, and β -galactosidase; α -glucosidase, *N*-acetyl- β -glucosaminidase, and esculin; urease, gelatin hydrolysis, and fermentation control; glucose, ribose, and xylose; mannitol, maltose, and lactose; sucrose and glycogen (catalase was the 21st test).

test). ^b Strains with superscript T's are type strains.

of C. striatum and the three C. striatum-like strains of C. xerosis. DNAs from the three lipid-dependent, urease-negative strains (NCTC 7238, NCTC 7883, and NCTC 7929) exhibited homology, whereas DNA from four C. xerosis strains (ATCC 373, ATCC 7711, NCTC 7243, and NCTC 8481) hybridized only with autologous DNA (data not shown). Under our hybridization conditions, which were designed for DNA with 58 mol% G+C, the two strains known to have 68 mol% G+C (ATCC 373 and ATCC 7711) exhibited weak hybridization with each other. However, under hybridization conditions designed for their higher G+C contents, DNAs from these two strains did not crosshybridize (data not shown).

On the basis of the DNA hybridization results, the 14 cultures of reference strains in the present study were arbitrarily assigned to DNA groups A through F. The summary of DNA hybridizations with DNA probes from strains representative of the six hybridization groups is presented in Table 4. As shown in Tables 1 through 3, the relationships between strains seen as a result of DNA hybridizations were in good agreement with the biochemical characteristics, colonial morphologies, and fatty acid compositions of the strains.

DISCUSSION

In the present study, we found that the type strain of C. *xerosis* is not closely related to any of the other nine reference strains of this species. Characterization of the 10 reference strains of C. *xerosis* by three independent methods, including biochemical tests, fatty acid analysis, and DNA hybridization, provided consistent evidence that this is a collection of diverse organisms comprising six different groups. It is notable that three C. *xerosis* strains were indistinguishable from the four reference cultures of C. *striatum*.

Strain ^a	DNA group	Total peak area (10 ³)	% Fatty acid ^b :																
			14:0	15:0	16:1ω9	16:1ω7	16:0	17:1ω5	17:0 i	17:0 a	17:1ω8	3 17:0	18:1ω9	18:1ω7	18:0	19:0 a	20:0	20:3ω6, 9, 12	features ^c
C. striatum																			
A6940 ^T	Α	207	2.1				53.4	0.9				0.6	37.8		4.5	0.6			
N764 ^T	Α	112	1.5				47.2						46.5		4.9				
A43735	Α	94	1.0				46.7						49.0		3.4				
A43751	Α	76	1.5				42.1						49.4		7.0				
C. xerosis																			
A7094	Α	234	1.1		1.9		46.1						47.7		3.2				
A9016	Α	277	0.8	2.2	0.9		51.0						37.1		5.9			2.1	
N9755	Α	101	1.3				49.7						44.4		4.5				
А373 ^т	В	191		2.2	0.1		14.7				19.3	16.0	38.4		7.7				6, 0.4
A7711	С	190			1.3		21.6				1.7	4.0	49.6		21.1		0.8		,
N7238	D	75					23.0			2.0		4.3	25.2	6.9	35.8				6, 2.8
N7883	D	99					25.6		0.8	2.1		3.7	26.2	5.8	33.3				6, 2.5
N7929	D	67	1.5				25.9			0.8		2.4	29.4	7.6	26.9				6, 5.5
N8481	Ε	46					17.4						43.2	7.1	24.1				6, 8.2
N7243	F	158	0.9	1.5		1.6	34.6				4.6	7.9	33.8		15.1				

TABLE 3. Microbial Identification System whole-cell fatty acid analysis of reference strains of C. striatum and C. xerosis

^a Reference strain numbers preceded by A and N were obtained from ATCC and NCTC, respectively. Superscript T's indicate type strains.

^b The double bond is located on the carbon number preceded by a ω ; i, methyl group at the penultimate (iso-) carbon atom; a, methyl group at the

antepenultimate (anteiso-) carbon atom.

^c Summed features usually comprise two or three compounds that eluted so closely to each other that they could not be reliable differentiated. Summed feature 6 is 18:2\omega6, 9, or 18:0 ante. Feature 8 is unknown.

The literature contains ample evidence of diversity within the reference strains of C. xerosis. The DNAs of both ATCC 373 and ATCC 7711 are known to have G+C contents of between 67 and 68 mol%, which is beyond the acceptable limits for members of the genus Corynebacterium and markedly different from the G+C contents of the DNAs of other reference C. xerosis strains, in which the G+C content is from 56 to 59 mol% (19, 23, 29). De Briel et al. (7) have recently reported differences in the C. xerosis reference strains ATCC 373 and ATCC 7711 on the basis of their mycolic acid patterns on high-pressure liquid chromatography (7). They also noted that the mycolic acids of two atypical strains of C. xerosis fit the pattern of C. striatum but not that of C. xerosis ATCC 373 or ATCC 7711. The most comprehensive evidence of diversity within C. xerosis was



FIG. 1. Two-dimensional plot of strains generated by principal component analysis of fatty acid profiles showing the distribution of reference strains of *C. striatum* (closed circles) and *C. xerosis* (open triangles). Letters indicate the DNA groups defined in Table 4.

found in Jackman's (16) numerical analysis of the electrophoretic protein patterns of eight reference strains of *C. xerosis*, which Jackman assigned to four distinct groups, including two additional subgroups. In general, the protein patterns from Jackman's studies indicated relationships among strains similar to those described in this report, with the following two exceptions: *C. xerosis* ATCC 373 and ATCC 7711 were grouped together, and the single *C. stria*-



FIG. 2. Chromosomal DNAs from the 14 reference strains of C. *striatum* (C.s.) and C. *xerosis* (C.x.) were spotted in the arrangement shown in the top of the figure and were hybridized with the three chromosomal DNA probes shown in the left column. Type strains are indicated by superscript T's.

	Hybridization with DNA probe, DNA group:											
Target DNA	C. striatum ^T A6940, group A	C. xerosis ^T A373, group B	C. xerosis A7711, group C	C. xerosis N7883, group D	C. xerosis N8481, group E	C. xerosis N7243, group F						
<i>C. striatum</i> A6940 ^T , N764 ^T , A43735, A43751,	+	_	-	_	_	-						
C. xerosis												
A7094, A9016, N9755	+	_	-	_	-	-						
A373 ^T	-	+	-	_	—							
A7711	-	-	+	-	-	-						
N7238, N7883, N7929	-		-	+	-	-						
N8481	-	-	-	-	+	-						
N7243	-	-	-	-	-	+						

^a Reference strain numbers preceded by A and N were obtained from ATCC and NCTC, respectively. Superscript T's indicate type strains.

tum strain (NCTC 764) included in Jackman's study did not cluster with any of the C. xerosis reference strains.

Some studies of C. xerosis reference strains have not reported the diversity described in this report. Bernard's (1) recent study of the fatty acid patterns of a large collection of coryneform taxa, including 22 strains of C. xerosis, did not note heterogeneity within this species, even though the study included two unrelated reference strains (ATCC 373 and NCTC 7238). A study by Hill et al. (12) suggested that the five NCTC reference strains of C. xerosis were a fairly homogeneous group, especially in their ability to reduce NO₃ and produce acid from maltose. The different conclusions between our study and those of Bernard (1) and Hill et al. (12) might be due to the methodologies or the strains that were used. Because we used freshly purchased cultures from ATCC and NCTC for the present study, we expect that our results reflect the reactions of the currently available reference strains in these collections.

Clinical microbiologists who rely on the CDC scheme for the identification of coryneforms can distinguish the type strains of C. striatum and C. xerosis on the basis of production of acid from maltose by C. xerosis (14, 18). The Rapid CORYNE system follows the CDC scheme and correctly identifies isolates that resemble the type strains of both of these species. However, attempts to confirm these identifications with Bergey's Manual (3) revealed disagreement with the CDC on C. xerosis's maltose reaction and C. striatum's reactions for maltose, sucrose, and nitrate. In the present study, we found that none of the C. striatum strains in either the NCTC or the ATCC collection fits the species description in Bergey's Manual (3). The reactions described in Bergey's Manual are based on the description by Munch-Petersen (22), whereas the CDC reactions are based on those of the type strain ATCC 6940 (14). A manuscript describing the history of the missing type strain of C. striatum has been submitted for publication.

In view of the major differences seen in the whole-cell fatty acids and the DNA-DNA hybridizations of the type strains for *C. striatum* ATCC 6940 and *C. xerosis* ATCC 373, it is remarkable that these two strains exhibited only two differences (acid from maltose and hemolysis) in the 34 tests used in the CDC laboratory (14). Similarly, only 3 of 21 tests in the Rapid CORYNE system distinguished these two type strains.

Some of the atypical reactions of *C. xerosis* found in the present study were observed by earlier workers. The lack of NO₃ reduction by *C. xerosis* ATCC 7243 has been reported

by Somerville (28). Marples (20) found urease-producing, lipid-stimulated diphtheroids during a survey of normal axillary skin and identified them as probable *C. xerosis* isolates, presumably because they produced acid from glucose and maltose. Most investigators have reported that *C. xerosis* is not lipid dependent (9, 24, 27, 28), although it has been noted that strain ATCC 373 is slightly stimulated by the addition of lipid (8). The lipophilic diphtheroids described by Smith (27) have been proposed as possibly being related to *C. xerosis* (26).

The urease reaction of *C. xerosis* ATCC 7711 was difficult to determine. A culture of this strain that had been stocked many years ago gave variable reactions in both Christensen's urea medium and the Rapid CORYNE strip, whereas an ATCC culture of this strain purchased in 1991 was urease negative by both methods. The ability of *C. xerosis* ATCC 7711 to split urea has been reported by Yamada (30).

The present study indicates that the 10 reference strains of *C. xerosis* are a very diverse group of organisms which appear to comprise six taxonomic groups, one of which is indistinguishable from the reference strains of *C. striatum*. The fact that the six *C. xerosis* groups described here were distinguishable on the basis of colonial morphologies, conventional biochemical reactions, whole-cell fatty acid profiles, and DNA homology suggests that studies in progress will support their assignment to a number of new species.

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