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Characterization of Some Bacterial Strains Isolated from Animal Clinical Materials and Identified as *Corynebacterium xerosis* by Molecular Biological Techniques[▽]

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Eighteen *Corynebacterium xerosis* strains isolated from different animal clinical specimens were subjected to phenotypic and molecular genetic studies. On the basis of the results of the biochemical characterization, the strains were tentatively identified as *C. xerosis*. Phylogenetic analysis based on comparative analysis of the sequences of 16S rRNA and *rpoB* genes revealed that the 18 strains were highly related to *C. xerosis*, *C. amycolatum*, *C. freneyi*, and *C. hansenii*. There was a good concordance between 16S rRNA and partial *rpoB* gene sequencing results, although partial *rpoB* gene sequencing allowed better differentiation of *C. xerosis*. Alternatively, *C. xerosis* was also differentiated from *C. freneyi* and *C. amycolatum* by restriction fragment length polymorphism analysis of the 16S-23S rRNA gene intergenic spacer region. Phenotypic characterization indicated that besides acid production from D-turanose and 5-ketogluconate, 90% of the strains were able to reduce nitrate. The absence of the fatty acids $C_{14:0}$, $C_{15:0}$, $C_{16:1}\omega7c$, and $C_{17:1}\omega8c$ can also facilitate the differentiation of *C. xerosis* from closely related species. The results of the present investigation demonstrated that for reliable identification of *C. xerosis* strains from clinical samples, a combination of phenotypic and molecular-biology-based identification techniques is necessary.

During the last decade, the genus *Corynebacterium* has undergone a significant expansion in the number of species described due to an increased concern about its potential pathogenic significance. About a quarter of the species currently recognized as belonging to the genus *Corynebacterium* (http://www.bacterio.net) have been isolated from animal sources, although only some of them can be considered well-established animal pathogens (5, 18). *Corynebacterium xerosis* is an unusual human pathogen (9) which has been recently isolated for the first time from animal clinical specimens (25). *C. xerosis* is phylogenetically closely related to *Corynebacterium freneyi*, *Corynebacterium amycolatum*, and *Corynebacterium hansenii* (19, 20, 26), but DNA-DNA hybridization experiments have demonstrated that they represent different species (19, 20). The unambiguous discrimination of these species based on phenotypic characteristics and comparative 16S rRNA gene analysis is problematic (7, 9, 20). Molecular-genetic methods, such as sequencing of the RNA polymerase beta subunit-encoding gene (*rpoB*), have been proposed as alternative or complementary molecular methods to 16S rRNA gene analysis for discerning phylogenetically closely related *Corynebacterium* species (13, 14). Also, restriction fragment length polymorphism (RFLP) analysis of the 16S-23S rRNA gene intergenic spacer region (IGS) has been proposed for the accurate differentiation of some of these species (7, 19, 20). However, these studies included a very limited number of *C. xerosis* strains because most of the strains previously classified as *C. xerosis* had been misidentified (9). Since the first description of *C. xerosis* from animals, additional strains have been recovered from clinical specimens of different animal species. In this work, we present the results of an extensive analysis of a significant number of *C. xerosis* strains from animals which could facilitate its identification and differentiation from other genotypically and phenotypically closely related species.

MATERIALS AND METHODS

Bacterial strains. Eighteen bacterial strains suspected to be *C. xerosis* were isolated from different veterinary clinical specimens. Two human *C. xerosis* strains, CCUG 45245 and CCUG 39723, five *C. freneyi* and eight *C. amycolatum* human strains, as well as the *C. xerosis* CCUG 56051^T, *C. freneyi* CCUG 45704^T, *C. hansenii* CCUG 53252^T, and *C. amycolatum* CCUG 35685^T type strains, obtained from the Culture Collection of the University of Göteborg (CCUG), were included for comparison. Additional information regarding the strains included in this study is listed in Table 1. All of the strains were cultured on Columbia agar plates (bioMérieux) and incubated at 37°C for 48 h.

Biochemical characterization. All strains were biochemically characterized using the commercial API Coryne, API ZYM, and API 50 CH systems (bioMérieux). The API Coryne and API ZYM strips were used according to the manufacturer's instructions. The API 50 CH strips were inoculated with a 2 McFarland standard suspension of bacterial cells in CHB/E medium (bioMérieux) as recommended by the manufacturer and incubated at 37°C for up to 5 days. Glucose fermentation at 42°C and growth at 20°C were performed as described previously (26).

Cellular fatty acid analysis. The cellular fatty acids of *C. xerosis* strains CCUG 56051^T, CCUG 39723, St85640, St53041, St51377/1, and St53244 were deter-

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TABLE 1. Details of the strains included in this study

Strain	Source	Clinical history	Colony color[b]	API Coryne numerical code
C. xerosis				
CCUG 56051^T	Human ear discharge		Y	3110325
St36404[a]	Goat liver	Suspected paratuberculosis	W-G	2110325
St33874[a]	Pig lung	Suspected erysipelas	Y	3110325
St34960[a]	Pig kidney	Respiratory problems	Y	3110325
St36130[a]	Pig skin	Subcutaneous abscess	Y	3110325
St38671[a]	Pig joint	Arthritis	W-G	3110325
St85640	Cow milk	Mastitis	Y	3110325
St53041	Pig fetus	Abortion	W-G	3110325
St51377/1	Pig blood	Sudden death	Y	3110325
St53244	Pig joint	Arthritic abscess	W-G	3110325
St51902	Pig spleen	Septicemia	W-G	3110325
St51463	Pig joint	Arthritis	W-G	3110325
St51377/2	Sheep lungs	Respiratory problems	Y	3110325
St51377/3	Pig. joint	Arthritis	Y	3110325
St46963	Pig kidney	Septicemia	W-G	3110325
St49327[a]	Pig liver	Septicemia	Y	3110325
St47126[a]	Sheep uterus	Abortion	Y	3110325
St49485[a]	Pig joint	Subcutaneous abscess	W-G	3110325
CCUG 45245	Not known	Not known	W-G	2110325
CCUG 39723	Human bone	Osteomyelitis	W-G	3110325
C. freneyi				
CCUG 45704^T	Human toe		W-G	3110325
CCUG 54468	Human duodenum	Duodenal biopsy specimen	W-G	3110325
CCUG 54466	Human vagina	Not specified	W-G	3100325
CCUG 54465	Human vagina	Not specified	W-G	2110325
CCUG 54469	Human vagina	Not specified	W-G	3110325
CCUG 54467	Human cervix	Not specified	W-G	3110325
M3	Sheep eye	Conjunctivitis	Y	2110325
C. amycolatum				
CCUG 35685^T	Human skin	Not specified	W-G	0101324
CCUG 58230	Human femur	Wound	W-G	2100324
CCUG 57527	Human blood	Not specified	W-G	2100325
CCUG 57358	Human abdomen	Not specified	W-G	3100325
CCUG 56284	Human skin	Wound	W-G	3100325
CCUG 46945	Human	Not specified	W-G	2100324
CCUG 34699	Human blood	Not specified	Y	3100324
CCUG 35623	Human blood	Not specified	W-G	2100325
CCUG 38870	Human skin	Not specified	W-G	3100324
C. hansenii CCUG 53252^T	Human	Liposarcoma	Y	2000325

[a] Strain previously described by Vela et al. (24).
[b] Y, yellowish; W-G, whitish-grayish.

mined by following the CCUG protocol (http://www.ccug.se). Briefly, isolates were grown aerobically on Columbia II agar base (BBL 4397596) with 5% horse blood for 16 to 48 h at 37°C. Cells were removed from the plate using a plastic inoculating loop, carefully scraped to avoid including medium in the sample. Fifty to 100 mg of cells was then transferred to glass tubes. Cells were saponified, and released fatty acids were methylated. Finally, fatty acid methyl esters were extracted. Analysis was carried out with a Hewlett Packard HP 5890 gas chromatograph.

DNA extraction. For DNA extraction, three colonies of each strain grown on Columbia agar were suspended in 100 μl of distilled water and boiled for 10 min at 100°C. After boiling, bacterial suspensions were centrifuged (7,600 × *g*, 2 min) and the supernatant was used as a source of template DNA for PCRs.

16S-23S rRNA gene PCR-RFLP analysis. The amplification of the 16S-23S rRNA gene IGS was carried out according to Aubel et al. (2) using primers G1 and L1 (12) with the following modifications. *In vitro* amplification was carried out with a reaction mixture of 100 μl containing template DNA (5 μl), 1 μM each primer (G1/L1), 200 μM each deoxynucleoside triphosphate (Biotools), and 2.5 U of Ultratools DNA polymerase (Biotools) and its amplification buffer. The amplifications were carried out in a Mastercycler gradient thermal cycler (Eppendorf) with an initial denaturation step of 94°C for 5 min; 30 serial cycles of denaturation at 94°C for 1 min, annealing at 47°C for 7 min, and extension at 72°C for 2 min; and a final extension step of 72°C for 10 min. Negative controls (no template DNA) were included in each batch of PCRs. PCR-generated products were detected by electrophoresis of 5 μl of each amplification mixture in 2% agarose gels supplemented with 1× SYBR Safe (Invitrogen, Eugene, OR). Amplified DNA was digested using the restriction enzyme CfoI (Fermentas Inc., Glen Burnie, MD). Approximately 15 μl of the PCR product was digested with 15 U of enzyme for 4 h at 37°C in a water bath. The electrophoretic patterns of digested products were photographed with a Fluor-S MultiImager (Bio-Rad Laboratories, Inc., Hercules, CA), and the data were analyzed using the Quantity One software package (Bio-Rad Laboratories, Inc.).

Sequencing of the 16S rRNA and *rpoB* genes. A nearly complete 16S rRNA gene fragment (>1,400 bp) was amplified as described previously (24). Amplification of the partial *rpoB* gene was performed as indicated by Khamis et al. (14), using previously described primers C2700F and C3130R (13). Multiple-sequence alignments and percent similarities of the *rpoB* and 16S rRNA genes of the various species were obtained with the CLUSTAL W program available from the EMBL-EBI web server (http://www.ebi.ac.uk/clustalw/). Phylogenetic trees were constructed according to three different algorithms: neighbor joining (21) using the programs SeqTools (http://www.seqtools.dk) and TREEVIEW (17),

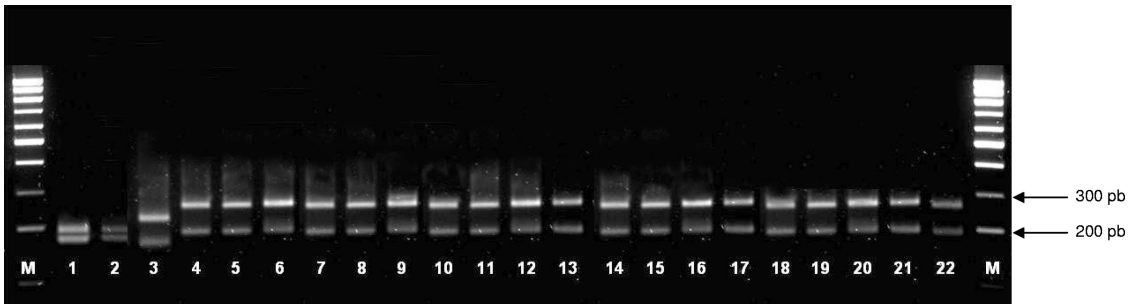

FIG. 1. Digestion of the PCR-amplified 16S-23S rRNA gene IGS of *Corynebacterium* species using the restriction endonuclease CfoI. Lanes 1 to 22: *C. freneyi* CCUG 45704^T, animal strain M3, *C. amycolatum* CCUG 35685^T, *C. hansenii* CCUG 53252^T, *C. xerosis* CCUG 56051^T, and animal strains St36404, St33874, St34960, St36130, St38671, St85640, St53041, St51377/1, St53244, St51902, St51463, St51377/2, St51377/3, St46963, St49327, St47126, and St49485, respectively. Lanes M, molecular weight markers (100-bp ladder).

maximum likelihood using the PHYML software (11), and maximum parsimony using the software package MEGA (molecular evolutionary genetics analysis) version 3.1 (16). Bootstrap replicates were performed to estimate the stability (1,000 replicates) of the branching nodes of the phylogenetic tree.

Nucleotide sequence and culture collection accession numbers. The 16S rRNA gene sequences of the strains isolated from veterinary clinical material included in this study have been deposited in the GenBank database under accession numbers FM213372 to FM213376, FN179318 to FN179334, and FN564566 to FN564568. The partial *rpoB* sequences of all of the strains characterized in this study have been deposited in the GenBank database under accessions numbers FN179293 to FN179317, FN564557 to FN564565, and FN552725. Strains St36130, St36404, and St47126 have been deposited in the Culture Collection of the University of Göteborg, Göteborg, Sweden, under collection numbers CCUG 53404 to CCUG 53406, and strains St85640, St53244, St53041, and St51377/1 have been deposited under collection numbers CCUG 57194 to CCUG 57197, respectively.

RESULTS

Genetic analysis. Three different patterns were observed after RFLP-IGS analysis. Most of the animal isolates (n = 17) suspected to be *C. xerosis*, as well as *C. xerosis* strains CCUG 56051^T, CCUG 45245, and CCUG 39723 and *C. hansenii* CCUG 53252^T, displayed identical fingerprinting patterns (Fig. 1, lanes 4 to 22), showing two bands of 280 and 200 bp. Animal strain M3 and the six *C. freneyi* strains presented the same fingerprinting pattern (Fig. 1, lanes 1 to 2), with bands of 200, 180, and 100 bp. Likewise, all of the *C. amycolatum* strains showed a pattern identical to that of *C. amycolatum* CCUG 35685^T (Fig. 1, lane 3), displaying RFLP fragments of 245 and 172 bp. Therefore, *C. xerosis*, *C. freneyi*, and *C. amycolatum* displayed different RFLP patterns, whereas *C. xerosis* and *C. hansenii* showed indistinguishable patterns.

Phylogenetic analysis based on the *rpoB* gene sequence revealed that the strains of *C. xerosis* cluster together to form a distinct subline within the genus *Corynebacterium* that was separate from that formed by *C. freneyi*, *C. amycolatum*, or *C. hansenii*, which was supported by high bootstrap resampling values (Fig. 2). In agreement with the RFLP-IGS results, animal strain M3 was more closely related to *C. freneyi* strains (Fig. 2). A similar tree topology was obtained after phylogenetic analysis based on 16S rRNA gene sequences (Fig. 3).

Comparative analysis of the partial *rpoB* sequences determined for the *C. xerosis* isolates revealed sequence similarities in the ranges of 100 to 99%, 97 to 93%, 86 to 84%, and 95 to 92% with the type strains *C. xerosis* CCUG 56051^T, *C. freneyi* CCUG 45704^T, *C. amycolatum* CCUG 35685^T, and *C. hansenii* CCUG 53252^T, respectively. Comparative analysis of the 16S rRNA gene sequences of the strains studied showed sequence similarities in the ranges of 100 to 99%, 99 to 98%, 98 to 96%, and 99 to 98% with the type strains *C. xerosis* CCUG 56051^T, *C. freneyi* CCUG 45704^T, *C. amycolatum* CCUG 35685^T, and *C. hansenii* CCUG 53252^T, respectively. These similarity values indicated that the strains studied are more close related to *C. xerosis*, *C. freneyi*, and *C. hansenii* than to *C. amycolatum*.

Phenotypic analysis. All of the strains genetically confirmed as *C. xerosis* gave whitish-grayish or yellowish pigmented colonies with a dry, rough, wrinkled surface (Table 1). Every one of the *C. freneyi* strains examined gave whitish-grayish and wrinkled-surface colonies, and animal strain M3, genetically confirmed as *C. freneyi*, displayed yellowish wrinkled colonies (Table 1). The *C. amycolatum* strains analyzed in the present study displayed whitish-grayish colonies, except strain CCUG 34699, which formed yellowish wrinkled colonies. *C. hansenii* CCUG 53252^T gave small, dry, rough, wrinkled-surface, yellowish pigmented colonies. Strains of the four *Corynebacterium* species were biochemically characterized using three commercial systems. With the API Coryne system, *C. xerosis* exhibited a quite homogeneous biochemical profile (Table 1) displaying the numerical profiles 3110325 (n = 18; 90.0%) and 2110325 (n = 2; 10.0%). These numerical profiles were also exhibited by four and two *C. freneyi* strains, respectively (Table 1). *C. amycolatum* exhibited great biochemical diversity, with five different profiles, none being displayed by *C. xerosis*. The numerical profile given by *C. hansenii* (2000325) was not shared by any *C. xerosis* strain either (Table 1). Biochemical characterization of the strains was extended by testing them for the abilities to ferment glucose at 42°C and grow at 20°C, as well as testing their enzymatic and carbohydrate metabolism, by using the API ZYM and API 50 CH systems (Table 2). Growth at 20°C was observed for *C. xerosis*, *C. freneyi*, and *C. hansenii* but not for *C. amycolatum*. On the other hand, *C. xerosis* and *C. hansenii* did not ferment glucose at 42°C, whereas *C. freneyi* and *C. amycolatum* were positive by this test (Table 2). *C. xerosis*, as well as the other three species included in this study, expressed esterase lipase (C_8) activity and were able to ferment glucose, ribose, and maltose, but none fermented xylose, mannitol, fructose, or glycogen. None produced pyrrolidonyl arylamidase, β-galactosidase, α-galactosidase, β-glucuronidase, β-glucosidase, α-manno-

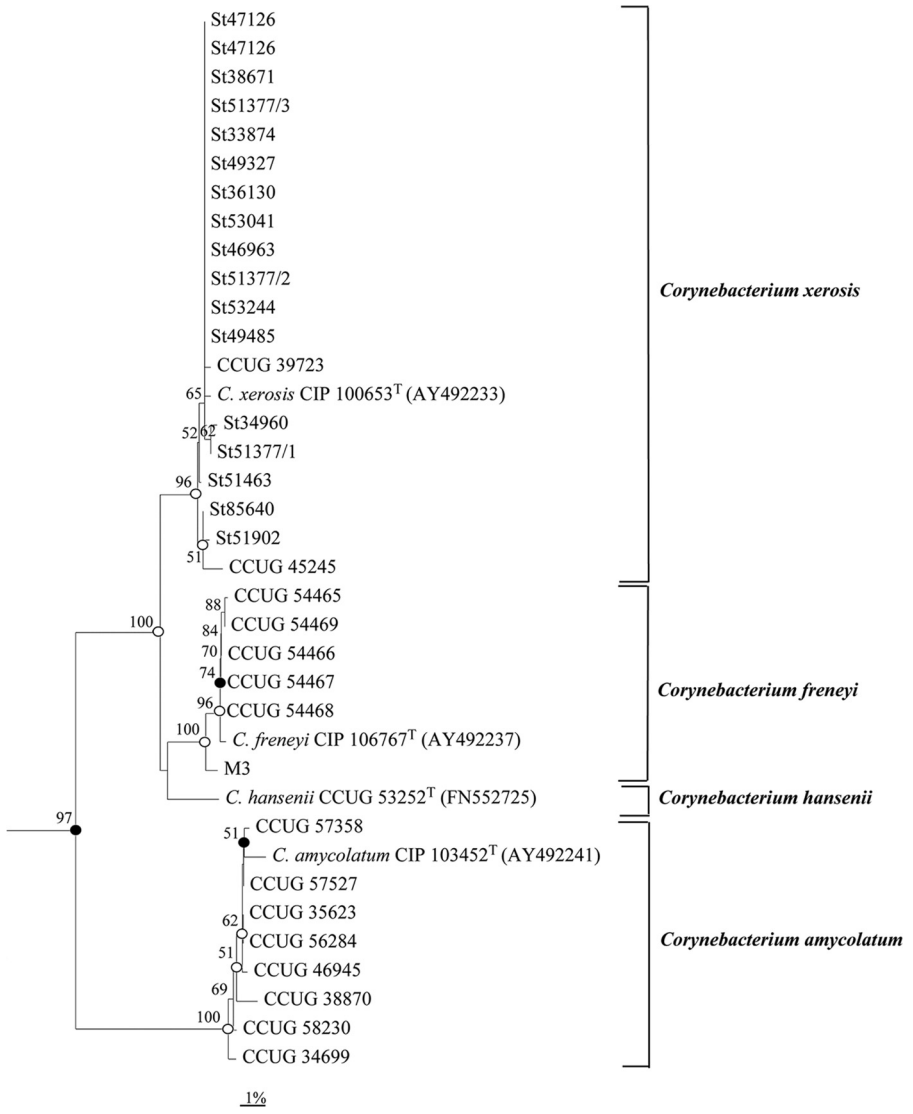

FIG. 2. Dendrogram based on *rpoB* gene sequence comparison obtained with the neighbor-joining algorithm showing the phylogenetic relationship between animal strains of *C. xerosis* and the close relatives *C. freneyi*, *C. amycolatum*, and *C. hansenii*. Bootstrap values (expressed as percentages of 1,000 replications) higher than 50% are given at the branching points. Filled circles indicate that the corresponding nodes (groupings) are also obtained on the parsimony tree. Open circles indicate that the corresponding nodes (groupings) are also obtained on the maximum-likelihood and parsimony trees. *Corynebacterium bovis* CIP 54.80^T (AY492236) was used as an outgroup. Bar, 1% sequence divergence.

sidase, α-fucosidase, *N*-acetyl-β-glucosaminidase, valine arylamidase, cystine arylamidase, trypsin, or chymotrypsin, and none hydrolyzed esculin or urea. The biochemical characteristics with different results among the four species analyzed in this study are shown in Table 2.

C. xerosis exhibited large to moderate amounts of the fatty acids $C_{16:0}$ (10.6 to 16.2%), $C_{18:0}$ (15.8 to 9.2%), $C_{18:1}\omega9c$ (58.8 to 49.7%), and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$ (7.4 to 19.5%). These fatty acids were also detected in similar relative percentages as predominant fatty acids in *C. freneyi*, *C. amycolatum*, and *C. hansenii*. The fatty acid C_{17} present in small amounts in *C. xerosis* (0.0 to 5.5%) was also detected in *C. freneyi* (6.7 to 13.3%), *C. amycolatum* (2.4 to 18.5%), and *C. hansenii* (0.4%). The fatty acids $C_{14:0}$, $C_{15:0}$, $C_{16:1}\omega7c$, and $C_{17:1}\omega8c$ were not detected in *C. xerosis*, but they were present in *C. freneyi* and *C. amycolatum*. In addition, the fatty acid $C_{16:1}\omega9c$ detected in *C. xerosis* (0.7 to 2.0%) was not present in *C. freneyi* and the fatty acid $C_{17:1}\omega8c$ was present in *C. hansenii*.

DISCUSSION

C. xerosis is rarely isolated from clinical samples, and most of the previously identified isolates were subsequently recognized as *C. amycolatum* (9). Thus, most of the studies that have analyzed the phenotypic or genetic characteristics of *C. xerosis* have included a very limited number of strains (7, 20). In the present work, most (n = 17; Table 1) of the animal clinical strains suspected to be *C. xerosis* belonged to this species. To our knowledge, this represents the largest phenotypic and genetic study of *C. xerosis* strains. Moreover, we describe the first

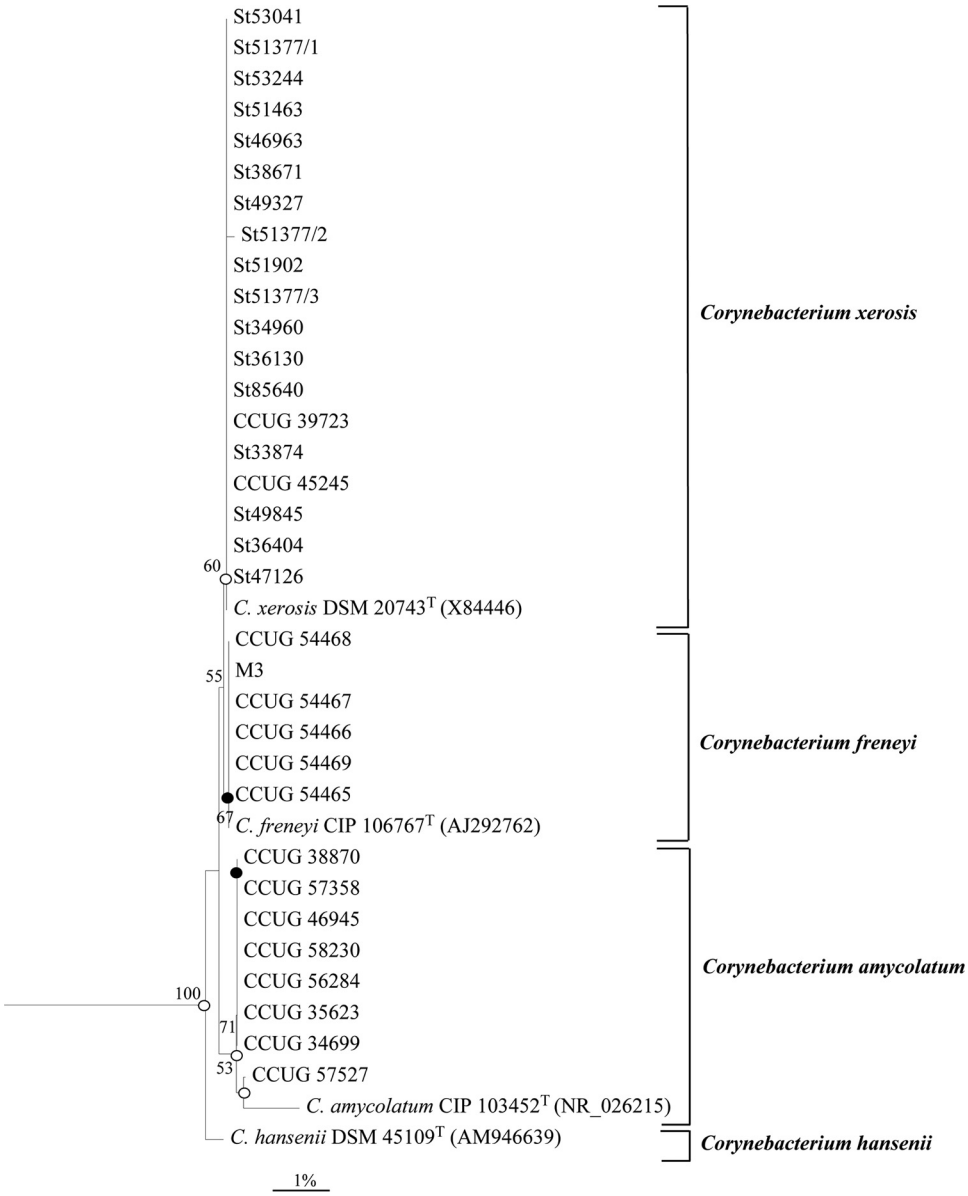

FIG. 3. Dendrogram based on 16S rRNA gene sequence comparison obtained with the neighbor-joining algorithm showing the phylogenetic relationship between animal strains of *C. xerosis* and the close relatives *C. freneyi*, *C. amycolatum*, and *C. hansenii*. Bootstrap values (expressed as percentages of 1,000 replications) higher than 50% are given at the branching points. Filled circles indicate that the corresponding nodes (groupings) are also obtained on the parsimony tree. Open circles indicate that the corresponding nodes (groupings) are also obtained on the maximum-likelihood and parsimony trees. *C. bovis* NCTC 3324^T (X82051) was used as an outgroup. Bar, 1% sequence divergence.

isolation of *C. freneyi* from animals (strain M3; Table 1). Previously, *C. freneyi* had only been isolated from human clinical samples (3, 7, 19). Macroscopically, *C. xerosis* gave dry, rough, and yellowish pigmented colonies that are considered typical for this microorganism (7); however, colonies of *C. xerosis* also had a wrinkled surface (100% of the strains; Table 1) and were whitish-grayish (50% of the strains; Table 1). These last characteristics had not been reported for this species before. The macroscopic characteristics of the colonies obtained with *C. freneyi*, *C. amycolatum*, and *C. hansenii* strains were in agreement with those previously described for these species (7, 20, 26). Therefore, the colonies formed by *C. xerosis* were very similar to those formed by *C. freneyi*, *C. amycolatum*, and *C. hansenii* and the macroscopic characteristics of colonies do not seem to be very useful for the presumptive differentiation of clinical strains of *C. xerosis*.

Most of the biochemical characteristics exhibited by *C. xerosis* were also in agreement with the current description of this species, which is based on two strains (ATCC 373^T and ATCC 7711) (6, 9, 19). The most significant discrepancy was that most of the *C. xerosis* strains reduced nitrate (90%; Table 2), and in our opinion, this trait should now be considered positive and not variable, as it has been considered so far (8). This result is relevant because reduction of nitrate is one of the

TABLE 2. Distinctive biochemical characteristics of *C. xerosis*, *C. freneyi*, *C. amycolatum*, and *C. hansenii*

Characteristic[a]	% Positive reactions			
	C. xerosis (*n* = 20)	*C. freneyi* (*n* = 7)	*C. amycolatum* (*n* = 9)	*C. hansenii* (*n* = 1)
Fermentation of glucose at 42°C	0	100	100	0
Growth at 20°C	100	100	0	100
API Coryne				
Nitrate reduction	90	71	44	0
Fermentation of sucrose	100	100	56	100
Production of:				
Alkaline phosphatase	100	100	100	0
Pyrazinamidase	100	100	89	100
α-Glucosidase (pH 7.4)	100	86	0	0
API 50CH (fermentation of:)				
Glycerol	17	0	100	100
Erythritol	5	0	0	0
D-Arabinose	5	0	0	0
L-Arabinose	5	0	11	0
Adonitol	5	0	0	0
Galactose	94	100	44	100
D-Mannose	84	83	100	100
L-Sorbose	0	16	0	0
Rhamnose	17	16	0	0
Dulcitol	0	16	0	0
Sorbitol	0	16	0	0
α-Methyl-D-mannoside	5	0	0	0
α-Methyl-D-glucoside	5	0	0	0
N-Acetyl-glucosamine	11	0	0	0
Amygdaline	27	16	11	0
Arbutin	17	33	0	0
Salicin	22	16	11	0
Cellobiose	11	33	33	0
Melibiose	5	0	22	0
Trehalose	99	100	56	100
Inulin	52	16	0	0
D-Raffinose	0	0	11	0
Amidon	22	33	11	0
β-Gentiobiose	27	16	0	0
D-Turanose	83	83	0	0
D-Lyxose	5	0	0	0
D-Tagatose	5	16	0	0
D-Fucose	11	0	0	0
L-Fucose	22	16	0	0
D-Arabitol	0	16	0	0
L-Arabitol	0	16	0	0
Gluconate	11	83	0	0
2-Ketogluconate	5	16	0	0
5-Ketogluconate	99	83	0	0
API ZYM				
Esterase (C4)	100	83	100	100
Lipase (C14)	61	66	89	0
Leucine arylamidase	99	100	89	100
Acid phosphatase	0	0	89	0
Naphthol-AS-BI-phosphohydrolase	61	16	67	0

[a] Those tests that can be useful for the differentiation of *C. xerosis* strains from those of *C. freneyi*, *C. amycolatum*, and *C. hansenii* are in bold format.

key characteristics commonly used for the differentiation of *Corynebacterium* species (6, 8). The biochemical characteristics of the *C. freneyi*, *C. amycolatum*, and *C. hansenii* strains in the present study matched those previously described for these species (9, 19, 21, 26). Using the API Coryne strips, which are widely used for the identification of coryneform microorganisms from clinical samples (1, 8, 10, 22), all of the strains of *C. xerosis* analyzed gave the numerical profile 3110325 or 2110325

(Table 1). Both of these numerical profiles were also exhibited by most of the *C. freneyi* strains (Table 1), which is in agreement with previous results (19). *C. xerosis* could be differentiated from *C. amycolatum* by the failure of this species to produce α-glucosidase and from *C. hansenii* because this species does not reduce nitrate and does not produce the enzymes alkaline phosphatase and α-glucosidase (Tables 1 and 2). However, atypical strains of *C. xerosis* cannot express α-glucosidase activity (26) and some strains of *C. amycolatum* can be α-glucosidase positive (9, 19, 26), which would limit the utility of this test. Likewise, 10% of the *C. xerosis* strains did not reduce nitrate (numerical code 2110325; Table 1). Thus, according to these data, the API Coryne system may not be sufficient for the accurate identification of *C. xerosis*. No characteristics distinguishing between *C. xerosis* and *C. freneyi* were found by using either the API 50 CH or the API ZYM system, and only glucose fermentation at 42°C would be a reliable test to differentiate these two species (Table 2). *C. xerosis* could still be distinguished from *C. amycolatum* and *C. hansenii* because these species do not produce acid from D-turanose and 5-ketogluconate when tested with API 50 CH strips (Table 2). The growth of *C. xerosis* at 20°C and its inability to ferment glucose at 42°C or produce acid phosphatase (API ZYM) could also help in its differentiation from *C. amycolatum* (Table 2). Overall, after the extensive biochemical characterization, several tests (underlined in Table 2) could be useful to improve the identification of *C. xerosis* in clinical microbiology laboratories. Phenotypic identification of those *C. xerosis* strains with atypical results for these characteristics (26) (Table 2) could be obtained by whole-cell fatty acid analysis, which is a useful tool for the identification of corynebacteria (23). *C. xerosis* can easily be differentiated from *C. freneyi* and *C. amycolatum* by the lack of the fatty acids $C_{14:0}$, $C_{15:0}$, $C_{16:1}\omega7c$, and $C_{17:1}\omega8c$. *C. xerosis* and *C. hansenii* can be differentiated by the presence of the fatty acid $C_{17:1}\omega8c$ in the latter species.

Unlike cellular fatty acid analysis, which is not available for routine diagnostics in most clinical laboratories, sequencing of the 16S rRNA gene is probably the most used molecular technique for identifying unusual bacteria in clinical microbiology (4, 15). Alternatively, partial *rpoB* gene sequencing is a successful and straightforward approach for discerning closely related *Corynebacterium* species (13, 14). Data obtained by 16S rRNA gene and partial *rpoB* gene analyses were congruent, and the phylogenetic trees constructed with both genes using the neighbor-joining method placed all of the *C. xerosis* strains in a separate branch clearly different from those formed by *C. freneyi*, *C. amycolatum*, and *C. hansenii* (Fig. 2 and 3). However, deeper branches were obtained using partial *rpoB* gene sequences as a result of the higher degree of polymorphism of the *rpoB* sequences than the 16S rRNA gene sequences (13, 14). Thus, the average similarities of partial *rpoB* gene sequences of *C. xerosis* strains compared to the partial *rpoB* gene sequences of the *C. freneyi*, *C. amycolatum*, and *C. hansenii* type strains were 94.6%, 85.7%, and 94.7%, respectively, whereas the average similarities for the almost complete 16S rRNA gene sequences were 99.1%, 97.1%, and 99.0%, respectively. A similarity of ≥95% based on the *rpoB* sequences has been proposed as a cutoff value for differentiating *Corynebacterium* species (14). These results support the utility of partial *rpoB* gene sequencing as an alternative to the 16S rRNA gene sequencing, allowing better discrimination of *C. xerosis* from closely phylogenetically related *Corynebacterium* species. Alternatively, *C. xerosis* can also be differentiated from *C. freneyi* and *C. amycolatum* by RFLP analysis of the 16S-23S rRNA gene IGS. All of the *C. xerosis* strains displayed the same pattern (Fig. 1, lanes 5 to 22), which could be distinguished from those obtained for *C. freneyi* and *C. amycolatum* (Fig. 1, lanes 1 to 3). In contrast, the digestion profiles of the *C. xerosis* strains and *C. hansenii* CCUG 53252^T were identical (Fig. 1, lanes 4 to 22), which agrees with previous results (20).

The initial description of *C. xerosis* from animals included strains from pigs and one goat isolated from different clinical specimens (25). In the present study, nine additional *C. xerosis* strains (Table 1) were recovered from different clinical samples from pigs, one cow, and one sheep, expanding the range of animals and specimens from which this microorganism has been isolated to date. These data, together with the difficulty of its correct phenotype-based identification in routine clinical microbiology laboratories, may imply that the clinical significance of *C. xerosis* in veterinary medicine could be higher than currently considered. Therefore, the results of the present study can contribute to the better recognition and identification of this microorganism in clinical samples in veterinary laboratories, thereby allowing better knowledge of its distribution and clinical relevance as an animal pathogen.

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