

## First Description of *Curtobacterium* spp. Isolated from Human Clinical Specimens

Guido Funke,<sup>1\*</sup> Max Aravena-Roman,<sup>2</sup> and Reinhard Frodl<sup>1</sup>

Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Weingarten, Germany,<sup>1</sup>  
and Department of Microbiology and Infectious Diseases, Western Australian Centre for Pathology and  
Medical Research, Nedlands, Australia<sup>2</sup>

Received 18 October 2004/Returned for modification 17 November 2004/Accepted 22 November 2004

During a 4-year period, five strains (three of which were doubtless clinically significant) of yellow- or orange-pigmented, oxidative, slowly acid-producing coryneform bacteria were recovered from human clinical specimens in two reference laboratories or referred to them. The strains were motile, catalase positive, nitrate reductase negative, and urease negative, but strongly hydrolyzed esculin. In all reference and clinical strains described in the present study, anteisopentadecanoic ( $C_{15:0ai}$ ) and anteisohexadecanoic ( $C_{16:0ai}$ ) acids represented more than 75% of all cellular fatty acids except in one clinical strain and in *Curtobacterium pusillum*, in which both the unusual  $\omega$ -cyclohexyl fatty acid (identified as  $C_{18:1\omega7cis/\omega9cis/\omega12trans}$  by the Sherlock system) represented more than 50% of all cellular fatty acids. In all clinical strains, ornithine was the diamino acid of the cell wall, the interpeptide bridge consisted of ornithine, and acetyl was the acyl type of the peptidoglycan. Therefore, the five clinical strains were unambiguously identified as *Curtobacterium* spp. Analyses of the complete 16S rRNA genes of the five clinical strains with homologies to the established *Curtobacterium* species ranging from 99.2 to 100% confirmed the identifications as *Curtobacterium* spp. Data on the antimicrobial susceptibility pattern of curtobacteria are reported, with macrolides and rifampin showing very low MICs for all strains tested. This report is the first on the isolation of *Curtobacterium* strains from human clinical specimens.

It is generally accepted that the identification of coryneform bacteria is one of the more difficult tasks for clinical microbiologists identifying microorganisms. This is mainly due to the enormous heterogeneity of these bacteria, presently comprising more than a dozen medically relevant genera with more than 100 species in total (5). Commercial identification systems do not cover the whole range of coryneform bacteria so that final identification is very often performed in a specialized laboratory. This report is on the identification of some rarely encountered oxidative, slowly acid-producing coryneform bacteria which were isolated at or received for identification by a reference laboratory in Europe and one in Australia.

Detailed phenotypic and chemotaxonomic investigations revealed that the five clinical strains included in this study belonged to the genus *Curtobacterium*. This genus had been defined by Yamada and Komagata in 1972 for some so-called motile brevibacteria. *Curtobacterium* strains had been isolated from rice and other plants and *C. flaccumfaciens*, in particular, is a well-established plant pathogen (2, 14). At present the genus *Curtobacterium* comprises six validated species (1, 14). However, curtobacteria have never been described as being isolated from humans.

This paper aims at outlining tests which may lead to the diagnosis of curtobacteria in a routine clinical laboratory. In addition, it adds further evidence that environmental coryneform bacteria might be transmitted to humans and cause dis-

ease as has been recently demonstrated for the genera *Microbacterium/Aureobacterium*, *Cellulomonas*, and *Arthrobacter* (6, 8, 10, 13).

### MATERIALS AND METHODS

**Strains, media, and growth conditions.** Table 1 lists the reference strains used in the present study. During a 4-year period, the five clinical strains were isolated at or referred to the Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Weingarten, Germany (strains 1594, 2340, and 2384), and the Department of Microbiology and Infectious Diseases, Western Australian Centre for Pathology and Medical Research, Nedlands, Australia (strains 3426 and 3430). All strains were subcultured on Columbia blood agar (BD, Heidelberg, Germany) at either 37°C or 30°C depending on the optimal growth temperature of the strains (see below).

**Biochemical tests.** Preparation of the media used for biochemical characterization of the strains studied was according to Nash and Krenz (15). Biochemical tests were performed at 30°C due to the fact that some strains had their optimal growth temperature at 30°C. Motility was observed by the hanging drop method by incubating cells in Trypticase soy broth (all media from BD, unless stated otherwise). Nitrate reduction was tested in nitrate broth, hydrolysis of urea was observed in Christensen's urea broth, and esculin hydrolysis was observed on modified esculin agar (9). Acid production from glucose, maltose, sucrose, mannitol, and xylose was observed in cystine Trypticase agar (CTA) medium containing 1% of the carbohydrates (Sigma Chemical Co., St. Louis, Mo.). The type of metabolism was observed by using CTA medium with acid production or alkalization at the surface and no change at the bottom of the tube indicating an oxidative metabolism. Enzymatic activities were determined by means of the API ZYM system (bioMérieux, Marcy l'Etoile, France) by reading the strips after 4 h of incubation. Identification of the *C. plantarum* strain was achieved by using the commercial API 32E strip (bioMérieux).

**Antimicrobial susceptibility patterns.** The MICs of 38 antimicrobial agents were determined by using the MCN microdilution system (Merlin Diagnostics, Bornheim-Hersel, Germany) combined with H-medium (Merlin) as described previously (4).

**Chemotaxonomic investigations.** For analysis of cellular fatty acid patterns, cells were processed as described previously (19), and gas-liquid chromatography

\* Corresponding author. Mailing address: Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Hoyerstrasse 51, D-88250 Weingarten, Germany. Phone: 49-751-502-630. Fax: 49-751-502-385. E-mail: ldg.funke@t-online.de.

TABLE 1. Strains included in the present study

Strain type and taxon	Strain(s) <sup>a</sup>	Source
Reference <sup>b</sup>		
<i>Curtobacterium citreum</i>	ATCC 15828, DSM 20528	Rice
<i>Curtobacterium albidum</i>	ATCC 15831, DSM 20512	Rice
<i>Curtobacterium flaccumfaciens</i> pathovar <i>flaccumfaciens</i>	CCUG 23364, NCPPB 1446	<i>Phaseolus vulgaris</i>
<i>Curtobacterium flaccumfaciens</i> pathovar <i>betae</i>	CCUG 23916, NCPPB 374	<i>Beta vulgaris</i>
<i>Curtobacterium flaccumfaciens</i> pathovar <i>oortii</i>	ATCC 25283	Diseased tulip
<i>Curtobacterium flaccumfaciens</i> pathovar <i>poinsettiae</i>	ATCC 9682	Diseased poinsettia
<i>Curtobacterium herbarum</i>	DSM 14013	Grass
<i>Curtobacterium luteum</i>	ATCC 15830, DSM 20542	Rice
<i>Curtobacterium plantarum</i>	ATCC 49174	Soybean leaves
<i>Curtobacterium pusillum</i>	ATCC 19096, CIP 81.24	Oil brine
Clinical		
<i>Curtobacterium</i> sp.	1594	Sputum, 54-year-old female with respiratory distress
<i>Curtobacterium</i> sp.	2340	Sputum, 76-year-old male with chronic bronchitis
<i>Curtobacterium</i> sp.	2384	Wound, 70-year-old male with soft tissue infection
<i>Curtobacterium</i> sp.	3426	Eye discharge, 34-year-old female with conjunctivitis
<i>Curtobacterium</i> sp.	3430	Lymph node tissue, 41-year-old male with lymphadenopathy

<sup>a</sup> ATCC, American Type Culture Collection; DSM, German Collection of Microorganisms and Cell Cultures; CCUG, Culture Collection, University of Göteborg; NCPPB, National Collection of Plant Pathogenic Bacteria; CIP, Collection Institut Pasteur.

<sup>b</sup> All reference strains are type strains.

was performed on the Sherlock system (Microbial ID, Ind., Newark, Del.). The diamino acid of the total cell walls was determined by the method described previously (9). Analysis of the partial peptidoglycan structures was performed by the methods given by Schleifer and Kandler (16), except that ascending thin-layer chromatography on cellulose sheets (Merck, Darmstadt, Germany) was used. The determination of the acyl type of the peptidoglycan was according to Uchida and Aida (18).

**16S rRNA gene sequencing and phylogenetic analysis.** A detailed description of the technique applied for 16S rRNA gene sequencing is given in a recent report (7). The purified PCR products were sequenced in both directions and the resulting sequences were deposited in the GenBank-EMBL database. The sequences were compared with all of the eubacterial 16S rRNA gene sequences available in the GenBank-EMBL database by using the BLAST software tool from the National Center for Biotechnology Information (Bethesda, Md.).

**Nucleotide sequence accession numbers.** The 16S rRNA gene sequences of isolates 1594, 2340, 2384, 3426, and 3430 have been deposited in the EMBL/GenBank database under accession numbers AY688357, AY688358, AY688359, AY688360, and AY688361, respectively.

## RESULTS

The clinical strains 2384, 3426, and 3430 grew heavily in pure culture and a moderate leukocyte reaction was observed in direct Gram stains of the clinical material suggesting a disease association in these three cases. For strains 1594 and 2340, a disease association was not so clear as these two strains were grown in mixed culture although they had been the predominant microorganisms.

All 15 strains studied grew as nonhemolytic, creamy, yellow or orange-pigmented colonies of about 1 to 1.5 mm in diameter after 24 to 48 h of incubation. Colonies of *C. pusillum* tended to exhibit glistening and mucoid colonies whereas colonies of *C. plantarum* were significantly larger (>2 mm) than the colonies of all other strains. *C. albidum*, *C. flaccumfaciens* (all four pathovars except pathovar *flaccumfaciens*), *C. herbarum*, as well as strains 2340 and 2384 showed better growth at 30°C than at 37°C whereas similar growth at 30°C and at 37°C

was observed for *C. citreum*, *C. luteum*, *C. plantarum*, *C. pusillum*, and strains 1594, 3426, and 3430. However, all strains included in the present study were able to grow at 37°C.

Gram stains of all strains showed relatively small coryneform bacteria (*curtus*, shortened) except for the *C. plantarum* strain which was a gram-negative rod. Most strains were motile and exhibited an oxidative metabolism except *C. plantarum* which was fermentative. Because of the discrepant results of the Gram stain and the oxidation/fermentation test of the *C. plantarum* type strain further biochemical identification was performed and resulted in the identification of this particular strain as *Pantoea* sp. Hence this strain was excluded from further analyses.

The remaining 14 strains were all catalase positive, did not reduce nitrate, exhibited no urease activity, but all very strongly hydrolyzed esculin. Acid was produced within 4 days from glucose by all strains except *C. citreum* and *C. pusillum*. Acid production from maltose was positive for *C. flaccumfaciens*, *C. herbarum*, *C. pusillum*, as well as strains 2340, 2384, 3426, and 3430. Sucrose was not acidified by *C. citreum*, *C. albidum*, and *C. pusillum* but all other strains. Acid production from mannitol was positive for *C. flaccumfaciens* pathovar *flaccumfaciens*, *C. flaccumfaciens* pathovar *poinsettiae*, *C. herbarum*, and strains 3426 and 3430 whereas xylose was acidified by all strains included in the present study. It is emphasized that the acid production was only very weak in comparison to other oxidative coryneform bacteria. All strains exhibited activity of the following enzymes: esterase, esterase lipase, leucine arylamidase, acid phosphatase (except *C. herbarum* and strain 3426),  $\alpha$ -galactosidase (except *C. pusillum* and strains 2340 and 3430),  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, and  $\alpha$ -mannosidase. Activities of lipase ( $C_{14}$ ), trypsin, and  $\beta$ -glucuronidase were not detected in any of the strains tested.

TABLE 2. MICs of antimicrobial agents against *Curtobacterium* strains<sup>a</sup>

Antimicrobial agent	MIC ( $\mu\text{g/ml}$ )		
	Range	50% of strains	90% of strains
Amikacin	2–32	4	16
Amoxicillin	0.5–16	4	8
Amoxicillin/clavulanic acid	0.5–32	2	16
Ampicillin/sulbactam	0.5–16	2	8
Azithromycin	$\leq 0.03$ –0.25	$\leq 0.03$	0.25
Aztreonam	>64	>64	>64
Cefaclor	0.5–16	1	4
Cefazolin	0.25–8	2	4
Cefepime	2–32	16	32
Cefotaxime	0.125–16	1	4
Cefotiam	0.25–8	1	4
Cefoxitin	4–32	8	32
Ceftazidime	8–>64	64	>64
Ceftriaxone	0.125–16	2	4
Cefuroxime	0.25–8	2	4
Chloramphenicol	0.5–4	2	4
Ciprofloxacin	0.25–16	0.5	4
Clarithromycin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$
Clindamycin	0.25–2	1	2
Cotrimoxazole	4–>256	16	64
Doxycycline	0.5–2	1	2
Erythromycin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$
Fosfomicin	>256	>256	>256
Fusidic acid	2–16	8	8
Gentamicin	0.25–4	1	4
Imipenem	0.06–64	1	32
Meropenem	0.125–16	1	4
Minocycline	0.5–2	1	2
Netilmicin	2–16	8	16
Ofloxacin	0.5–16	1	8
Oxacillin	2–64	16	32
Penicillin	0.125–4	1	4
Piperacillin	8–128	32	128
Rifampin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$
Teicoplanin	0.125–1	0.25	0.5
Tetracycline	4–8	4	8
Tobramycin	4–32	16	16
Vancomycin	0.5–1	0.5	1

<sup>a</sup> Fourteen strains (see Table 1, excluding *C. plantarum*) were included.

Table 2 outlines the antimicrobial susceptibility patterns of the nine *Curtobacterium* reference strains and the five clinical isolates. The 50% and 90% MICs of  $\beta$ -lactams were mostly greater than 1  $\mu\text{g/ml}$ . Significantly, the MICs for macrolides (except azithromycin) and rifampin were  $\leq 0.03$   $\mu\text{g/ml}$  for all strains tested. The MICs for amikacin and gentamicin were lower than for netilmicin and tobramycin as were the MICs for doxycycline and minocycline in comparison to tetracycline. The MICs of teicoplanin and vancomycin were lower than 2  $\mu\text{g/ml}$  for every strain examined.

Cellular fatty acid analysis revealed that  $C_{15:0ai}$  and  $C_{17:0ai}$  presented more than 75% of all cellular fatty acids in every strain tested except two (Table 3). Much smaller amounts of  $C_{15:0i}$ ,  $C_{16:0i}$ , and  $C_{16:0}$  were also detected. The cellular fatty acid patterns of the strains studied were very similar with the exceptions of *C. pusillum* and strain 3430. For these particular two strains the Sherlock system named the major peak feature 7, consisting of  $C_{18:1\omega 7cis/\omega 9cis/\omega 12trans}$  which could not be separated by the system.

Analysis of the peptidoglycan structure of the five clinical isolates demonstrated ornithine as the diamino acid and that the interpeptide bridge consisted of ornithine alone. The acyl type of the peptidoglycan was found to be acetyl. This combination of chemotaxonomic features is found in the genus *Curtobacterium* only (Table 4). Therefore, the five clinical isolates were unambiguously identified as *Curtobacterium* species.

The data of the 16S rRNA gene sequencing of the five clinical strains are given in Table 5. The homologies of the 16S rRNA genes between the clinical strains and their closest phylogenetic neighbors, curtobacteria, were always greater than 99.1%, unambiguously demonstrating that the strains are true members of the genus *Curtobacterium*. With no mismatches to *C. flaccumfaciens*, strains 2384 and 3426 are most likely representatives of this species.

TABLE 3. Cellular fatty acid profiles of *Curtobacterium* strains

Strain	% of total fatty acids <sup>a</sup>								
	$C_{14:0i}$	$C_{14:0}$	$C_{15:0i}$	$C_{15:0ai}$	$C_{16:0i}$	$C_{16:0}$	$C_{17:0i}$	$C_{17:0ai}$	Feature 7 <sup>b</sup>
<i>C. citreum</i> (ATCC 15828)	1		3	45	9	1	1	40	
<i>C. albidum</i> (ATCC 15831)			3	51	8			38	
<i>C. flaccumfaciens</i> pathovar <i>flaccumfaciens</i> (CCUG 23364)			3	48	7	1	1	40	
<i>C. flaccumfaciens</i> pathovar <i>betae</i> (CCUG 23916)			2	58	4	1	1	32	1
<i>C. flaccumfaciens</i> pathovar <i>oortii</i> (ATCC 25283)	1		3	59	4	2		25	3
<i>C. flaccumfaciens</i> pathovar <i>poinsettiae</i> (ATCC 9682)	1		4	47	8	1	1	37	
<i>C. herbarum</i> (DSM 14013)	1		1	46	8	2	1	40	
<i>C. luteum</i> (ATCC 15830)	1		2	58	8			30	
<i>C. pusillum</i> (ATCC 19096)			1	15	5	3	1	18	56
1594	1		2	36	7	8	1	42	
2340			3	55	8	3		25	6
2384	1		3	54	6	3	1	30	
3426		1	4	38	7	7		38	4
3430		1	1	10	3	5		20	59

<sup>a</sup> Values less than 1% are not reported.

<sup>b</sup> Includes  $C_{18:1\omega 7cis/\omega 9trans/\omega 12trans}$ , which could not be separated.

TABLE 4. Differential diagnosis of *Curtobacterium* spp. from other medically relevant, yellow- or orange-pigmented coryneform bacteria other than *Corynebacterium*<sup>a</sup>

Characteristic	<i>Curtobacterium</i>	<i>Brevibacterium</i> <sup>b</sup>	<i>Cellulomonas</i>	<i>Cellulosimicrobium</i>	<i>Exiguobacterium</i>	<i>Leifsonia</i>	<i>Microbacterium</i>
Type of metabolism <sup>c</sup>	O	O	F	F	F	O	O/F
Nitrate reduction	—	V <sup>d</sup>	+	+	V	V	V
Urease	—	—	—	—	—	—	V
Esculin hydrolysis	+	—	+	+	+	V	V
Major fatty acids	15:0 <i>ai</i> 17:0 <i>ai</i>	15:0 <i>ai</i> 17:0 <i>ai</i> 15:0 <i>i</i>	15:0 <i>ai</i> 16:0	15:0 <i>ai</i> 15:0 <i>i</i> 17:0 <i>ai</i>	17:0 <i>i</i> 15:0 <i>i</i> 16:0	17:0 <i>ai</i> 15:0 <i>ai</i> 16:0 <i>i</i>	15:0 <i>ai</i> 17:0 <i>ai</i> 16:0 <i>i</i>
Diamino acid <sup>e</sup>	ORN	<i>m</i> -DAP	ORN	LYS	LYS	DAB	LYS, ORN
Acyl type	Acetyl	Acetyl	Acetyl	Acetyl	ND <sup>f</sup>	ND	Glycolyl

<sup>a</sup> Data are from reference 5.

<sup>b</sup> Most *Brevibacterium* strains are whitish-grayish.

<sup>c</sup> O, oxidative; F, fermentative.

<sup>d</sup> V, variable.

<sup>e</sup> ORN, ornithine; *m*-DAP, *meso*-diaminopimelic acid; LYS, lysine; DAB, diaminobutyric acid.

<sup>f</sup> ND, no data.

DISCUSSION

With a polyphasic approach the five clinical strains were identified as *Curtobacterium* species. Definitive identification on the species level was not attempted since the very few *Curtobacterium* strains described in the literature did not allow the creation of a reliable database for phenotypic differentiation on the species level.

Curtobacteria had never been described before as being recovered from human clinical specimens. Since curtobacteria have so far not been isolated from blood cultures or from other normally sterile body sites (except for strain 3430 in the present study) it is suggested that they act as colonizers rather than as invasive pathogens. In contrast, strains belonging to the species *C. flaccumfaciens* are well-established plant pathogens. For epidemiological purposes this species had been divided into four different pathovars whereas *C. citreum*, *C. albidum*, and *C. luteum* are not known to cause any disease on rice from which they were primarily isolated (14), and *C. herbarum* was isolated from grass (1). Other strains belonging to genera which are primarily associated with plants and can cause disease in those but may also colonize or infect humans include *Pseudomonas*, *Stenotrophomonas*, and *Burkholderia*.

Some curtobacteria with an optimal growth temperature of 30°C may not be isolated from human clinical specimens if the plates are routinely incubated at 37°C and read after 24 h only. Curtobacteria might be underdiagnosed because they are presently not included in the databases of commercial identification systems (11, 12) and chemotaxonomic investigations are

necessary for complete identification of the organisms. However, it is the authors' experience that curtobacteria belong to the least frequently encountered yellow- or orange-pigmented coryneform bacteria (see Table 4) in clinical specimens. For example, the oxidative *Microbacterium/Aureobacterium* strains are 20 to 25 times more frequently detected in clinical specimens than curtobacteria (G. Funke, unpublished observation).

Apart from chemotaxonomic investigations simple biochemical tests like rapidity of acid production from carbohydrates (microbacteria usually within 2 days whereas in curtobacteria it may take up to 1 week or even longer) may serve in the identification of yellow- or orange-pigmented coryneform bacteria. In fact, an orange pigment in coryneform bacteria other than curtobacteria is rarely seen (e.g., *M. arborescens*, *M. imperiale*, *M. schleiferi*, and *M. testaceum* exhibit an orange pigment). It should also be noted that all curtobacteria tested until now are nitrate reductase and urease negative but strongly hydrolyze esculin (see Table 4).

*C. pusillum* is the only established *Curtobacterium* species which was not primarily isolated from plants. It is also very unusual in its characteristic that the majority of cellular fatty acids are identified as C<sub>18:1</sub>ω<sub>7</sub>cis/ω<sub>9</sub>cis/ω<sub>12</sub>trans by the Sherlock system and as ω-cyclohexyl undecanoic acid by another independent system (17). This discrepancy can be resolved by determining the precise cellular fatty acid structure by mass spectrometry. The authors are not aware of any gram-positive genus which includes both ω-cyclohexyl-containing and non-ω-cyclohexyl-containing bacteria. Some ω-cyclohexyl-containing

TABLE 5. 16S rRNA gene data on the clinical strains included in the present study compared with the closest phylogenetic neighbor

Strain no.	Sequence compared	No. of matching base pairs/total (%)	Closest phylogenetic neighbor	Detailed base pair mismatches <sup>a</sup>
1594	AJ784400	1,462/1,474 (99.2)	<i>C. pusillum</i>	64–67/CAGG/GCCC; 76–80/CCTGT/GGGTG; 444/A/G; 840/T/C; 1421/T/A
2340	AB042089	1,474/1,480 (99.6)	<i>Curtobacterium</i> sp. VKM Ac-1811	328/C/—; 598/G/—; 1105/C/G; 1112/G/C; 1253/C/—; 1328/T/—
2384	AJ312209	1,474/1,474 (100)	<i>C. flaccumfaciens</i>	
3426	AJ312209	1,471/1,471 (100)	<i>C. flaccumfaciens</i>	
3430	AJ784400	1,471/1,474 (99.8)	<i>C. pusillum</i>	444/A/G; 840/T/C; 1421/T/A

<sup>a</sup> Position of the mismatched base in the compared sequence/base detected in the clinical strain/base in the reference strain.



*Bacillus* species had recently been transferred into a separate genus, *Alicyclobacillus* (20).

*C. plantarum* (strain ATCC 49174) is certainly not a member of the genus *Curtobacterium* but a *Pantoea* strain. Since strain ATCC 49174 is the only strain that has ever been deposited (Phyllis Pienta, personal communication) the present paper raises the pursuit for a true *C. plantarum* strain as described by Dunleavy (3).

In summary, *Curtobacterium* strains are rarely isolated from clinical samples but clinical microbiologists should be aware of the possible appearance of these bacteria in material from humans although their pathogenicity is considered rather low since many people are probably exposed to curtobacteria every day. The tests outlined should facilitate the diagnosis of *Curtobacterium* spp. in the routine clinical laboratory. Finally, as with other recently described yellow-pigmented coryneform bacteria, it is expected that once a genus appears in the clinical microbiology literature, other workers will also find strains belonging to this particular genus in their specimens.

#### REFERENCES

- Behrendt, U., A. Ulrich, P. Schumann, D. Naumann, and K.-I. Suzuki. 2002. Diversity of grass-associated *Microbacteriaceae* isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of *Subtercola pratensis* sp. nov., *Curtobacterium herbarum* sp. nov. and *Planitibacter flavus* gen. nov., sp. nov. *Int. J. Syst. Evol. Microbiol.* **52**:1441–1454.
- Collins, M. D., and D. Jones. 1983. Reclassification of *Corynebacterium flaccumfaciens*, *Corynebacterium betae*, *Corynebacterium oortii* and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. nov. *J. Gen. Microbiol.* **129**:3545–3548.
- Dunleavy, J. M. 1989. *Curtobacterium plantarum* sp. nov. is ubiquitous in plant leaves and is seed transmitted in soybean and corn. *Int. J. Syst. Bacteriol.* **39**:240–249.
- Funke, G., N. Alvarez, C. Pascual, E. Falsen, E. Akervall, L. Sabbe, L. Schouls, N. Weiss, and M. D. Collins. 1997. *Actinomyces europaeus* sp. nov., isolated from human clinical specimens. *Int. J. Syst. Bacteriol.* **47**:687–692.
- Funke, G., and K. A. Bernard. 2003. Coryneform gram-positive rods, p. 472–501. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. ASM Press, Washington, D.C.
- Funke, G., E. Falsen, and C. Barreau. 1995. Primary identification of *Microbacterium* spp. encountered in clinical specimens as CDC coryneform group A-4 and A-5 bacteria. *J. Clin. Microbiol.* **33**:188–192.
- Funke, G., R. Frodl, and H. Sommer. 2004. First comprehensively documented case of *Paracoccus yeei* infection in a human. *J. Clin. Microbiol.* **42**:3366–3368.
- Funke, G., R. Hutson, K. A. Bernard, G. E. Pfyffer, G. Wauters, and M. D. Collins. 1996. Isolation of *Arthrobacter* spp. from clinical specimens and description of *Arthrobacter cumminsii* sp. nov. and *Arthrobacter woluwensis* sp. nov. *J. Clin. Microbiol.* **34**:2356–2363.
- Funke, G., G. Martinetti Lucchini, G. E. Pfyffer, M. Marchiani, and A. von Graevenitz. 1993. Characteristics of CDC group 1 and group 1-like coryneform bacteria isolated from clinical specimens. *J. Clin. Microbiol.* **31**:2907–2912.
- Funke, G., C. Pascual Ramos, and M. D. Collins. 1995. 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. *J. Clin. Microbiol.* **33**:2091–2097.
- Funke, G., K. Peters, and M. Aravena-Roman. 1998. Evaluation of the RapID CB Plus system for identification of coryneform bacteria and *Listeria* spp. *J. Clin. Microbiol.* **36**:2439–2442.
- Funke, G., F. N. R. Renaud, J. Freney, and P. Riegel. 1997. Multicenter evaluation of the updated and extended API (RAPID) Coryne data base 2.0. *J. Clin. Microbiol.* **35**:3122–3126.
- Funke, G., A. von Graevenitz, and N. Weiss. 1994. Primary identification of *Aureobacterium* spp. isolated from clinical specimens as “*Corynebacterium aquaticum*.” *J. Clin. Microbiol.* **32**:2686–2691.
- Komagata, K., and K. I. Suzuki. 1986. Genus *Curtobacterium*, p. 1313–1317. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams & Wilkins Co., Baltimore, Md.
- Nash, P., and M. M. Krenz. 1991. Culture media, p. 1226–1288. In A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407–477.
- Suzuki, K., K. Saito, A. Kawaguchi, S. Okuda, and K. Komagata. 1981. Occurrence of  $\omega$ -cyclohexyl fatty acids in *Curtobacterium pusillum* strains. *J. Gen. Appl. Microbiol.* **27**:261–266.
- Uchida, K., and K. Aida. 1984. An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J. Gen. Appl. Microbiol.* **30**:131–134.
- von Graevenitz, A., G. Osterhout, and J. Dick. 1991. Grouping of some clinically relevant gram-positive rods by automated fatty acid analysis. *APMIS* **99**:147–154.
- Wisotzkey, J. D., P. Jurtshuk, Jr., G. E. Fox, G. Deinhard, and K. Poralla. 1992. Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int. J. Syst. Bacteriol.* **42**:263–269.