Available online at www.sciencedirect.com

SCIENCE DIRECT

Diagnostic Microbiology and Infectious Disease 54 (2006) 99–104

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

www.elsevier.com/locate/diagmicrobio

Species identification of corynebacteria by cellular fatty acid analysis

Sandra Van den Velde[a], Katrien Lagrou[a,*], Koen Desmet[a], Georges Wauters[b], Jan Verhaegen[a]

[a]*Laboratory Medicine, Department of Microbiology and Immunology, University Hospitals Leuven, BE-3000 Leuven, Belgium*
[b]*Medical Microbiology Unit, University of Louvain, 1200 Brussels, Belgium*

Received 28 March 2005; accepted 30 August 2005

Abstract

We evaluated the usefulness of cellular fatty acid analysis for the identification of corynebacteria. Therefore, 219 well-characterized strains belonging to 21 *Corynebacterium* species were analyzed with the Sherlock System of MIDI (Newark, DE). Most *Corynebacterium* species have a qualitative different fatty acid profile. *Corynebacterium coyleae* (subgroup 1), *Corynebacterium riegelii*, *Corynebacterium simulans*, and *Corynebacterium imitans* differ only quantitatively. *Corynebacterium afermentans afermentans* and *C. coyleae* (subgroup 2) have both a similar qualitative and quantitative profile. The commercially available database (CLIN 40, MIDI) identified only one third of the 219 strains correctly at the species level. We created a new database with these 219 strains. This new database was tested with 34 clinical isolates and could identify 29 strains correctly. Strains that remained unidentified were 2 *Corynebacterium aurimucosum* (not included in our database), 1 *C. afermentans afermentans*, and 2 *Corynebacterium pseudodiphtheriticum*. Cellular fatty acid analysis with a self-created database can be used for the identification and differentiation of corynebacteria.
© 2006 Elsevier Inc. All rights rserved.

Keywords: Corynebacteria; Fatty acid analysis

1. Introduction

Recently, interest in corynebacteria has been increasing due to the association of multiple species with various infections, especially in immunocompromised patients. Because of the increasing number of immunocompromised patients, the incidence of opportunistic infections with corynebacteria (e.g., pneumonia, endocarditis, prosthesis-associated infections, pharyngitis, and genitourinary tract infections) is also rising (Bortolussi and Mailman, 2004; Colt et al., 1991; Coyle and Lipsky, 1990; Funke et al., 1997b; Riegel et al., 1996; Sewell et al., 1995; Soriano and Fernandez-Roblas, 1998). Moreover, some species have become multiresistant to antibiotics. *Corynebacterium jeikeium* and *Corynebacterium urealyticum* are often only susceptible to glycopeptides (Bortolussi and Mailman, 2004). Therefore, a correct species identification is important. Nevertheless, the identification of corynebacteria is still a difficult task for bacteriologists because of the diversity within the group and the fact that a number of species are biochemically inert and difficult to identify with conventional tests (Athaley et al., 1985; Funke et al., 1997a). An important drawback of commercial systems is the fact that several species are not included in these databases (Funke et al., 1997a, 1997b). The aim of this study was to evaluate the usefulness of cellular fatty acid analysis for the identification of the different corynebacteria species. Therefore, cellular fatty acid analysis was performed on 219 well-characterized strains belonging to 21 species of corynebacteria. With the data of these strains, a new database was created.

2. Materials and methods

2.1. Strains

Two hundred and nineteen strains of the genus *Corynebacterium* were used. Twenty strains were reference strains from international collections (American Type Culture Collection, Deutsche Sammlung von Mikroorganismen, Culture Collection University of Göteborg, National Collection of Type Cultures, Department of Medical Microbiology Zürich). All strains were tested biochemically with conventional tests and/or API Coryne (bioMérieux, Marcy l'Etoile,

* Corresponding author. Tel.: +32-16-347902; fax: 32-16-347931.
E-mail address: katrien.lagrou@uz.kuleuven.ac.be (K. Lagrou).

0732-8893/$ – see front matter © 2006 Elsevier Inc. All rights rserved.
doi:10.1016/j.diagmicrobio.2005.08.019

France). For 46 strains 16S rRNA gene sequence analysis was performed. The strains used were *Corynebacterium accolens* (4), *Corynebacterium afermentans afermentans* (12), *Corynebacterium amycolatum* (38), *Corynebacterium auris* (2), *Corynebacterium confusum* (2), *Corynebacterium coyleae* (10; with 2 subgroups within this species, *coyleae* 1 [6] and coyleae 2 [4]), *Corynebacterium diphtheriae* (7), *Corynebacterium falsenii* (2), *Corynebacterium glucuronolyticum* (19), *Corynebacterium imitans* (6), *C. jeikeium* (11), *Corynebacterium minutissimum* (30), *Corynebacterium mucifaciens* (4), *Corynebacterium propinquum* (2), *Corynebacterium pseudodiphtheriticum* (14), *Corynebacterium riegelii* (5), *Corynebacterium simulans* (11), *Corynebacterium singulare* (2), *Corynebacterium striatum* (28), *C. urealyticum* (6), and *Corynebacterium xerosis* (4).

To test our new database, 34 clinical isolates were analyzed with our new database as well as with the commercially available database (CLIN 40, MIDI, Newark, DE) for corynebacteria. Twelve isolates came from the collection of our laboratory from a previous study (Lagrou et al., 1998). The other 22 isolates were collected between April 2004 and July 2004 and were tested with API Coryne and/or biochemical tests (Funke and Bernard, 2003). When the results of API Coryne and the biochemical tests did not correspond and identification was not possible by use of the biochemical profile (one ore more tests against), 16S rRNA gene sequence analysis was performed (Table 1). Isolates that were used were *C. afermentans afermentans* (1), *C. amycolatum* (13), *C. aurimucosum* (2), *C. auris* (1), *C. coyleae* (1), *C. diphtheriae* (1), *C. imitans* (1), *C. jeikeium* (3), *C. minutissimum* (1), *C. pseudodiphtheriticum* (4), *C. riegelii* (1), *C. striatum* (2), and *C. urealyticum* (3).

2.2. Cellular fatty acid analysis

Bacteria were grown on Trypticase Soy Agar plates (Trypticase Soy Agar, Oxoid, Basingstoke, UK) supplemented with 5% horse blood and incubated during 24 h in a 5% CO_2 atmosphere. Approximately 2 inoculation loops of the cell mass were harvested and transferred to a tube with a Teflon-lined cap. To release the fatty acids, 1 mL of 15% NaOH in 50% aqueous methanol was added, and the tubes were heated at 100 °C during 30 minutes. The mixture was cooled and 2 mL of methanol/6N HCl (1:1.18 vol/vol) was added. The samples were then heated for 10 minutes at 80 °C and after cooling the fatty acid methyl esters were extracted by adding 1.25 mL of hexane/methyl-*tert*-butyl-ether (1:1 vol/vol). After mixing for 10 minutes, the aqueous layer was removed and the organic layer was washed with 3 mL 0.24 M NaOH. After mixing the samples for 5 minutes, the upper organic layer was transferred to a vial. Two microliters of the fatty acid methyl esters were then

Table 1
Identification of 22 clinical isolates

Final identification	API Coryne	Biochemical tests + macroscopy	16S
C. pseudodiphtheriticum	*C. pseudodiphtheriticum* 94.4% (0)		
C. amycolatum	*C. striatum/amycolatum* 81.7% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	*C. striatum/amycolatum* 97.9% (1)	Macroscopy (dry, white colonies)	
C. amycolatum	*C. striatum/amycolatum* 81.7% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	*Corynebacterium* group G 76.7% (0)	Not lipophilic	
	C. amycolatum 22.7% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	*C. striatum/amycolatum* 81.7% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	*C. striatum/amycolatum* 65.3% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	*Corynebacterium* group G 76.7% (0)	Not lipophilic	
	C. amycolatum 22.7% (0)	Macroscopy (dry, white colonies)	
C. pseudodiphtheriticum	*C. pseudodiphtheriticum* 96.7% (0)		
C. striatum	*C. striatum/amycolatum* 88.6% (0)	Macroscopy	
C. amycolatum	*C. striatum/amycolatum* 81.7% (0)	Macroscopy (dry, white colonies)	
C. pseudodiphtheriticum	*C. pseudodiphtheriticum* 99.6% (0)		
C. jeikeium	*C. jeikeium* 93.6% (0)		
C. jeikeium	*C. jeikeium* 93.6% (0)		
C. aurimucosum	*C. striatum/amycolatum* 88% (2)	N: −/U: −/AF: −/G: +/S: +/M: +	+
C. aurimucosum	*C. striatum/amycolatum* 81.6% (2)	N: −/U: −/AF: −/G: +/S: +/M: +	+
C. minutissimum	*C. striatum/amycolatum* 72.2% (1)	N: −/U: −/AF: +/G: +/S: −/M: +	
C. afermentans afermentans	*Corynebacterium* group G 96.7% (0)	Not lipophilic, N: −, U: −/AF: +/G: −/Man: −/X: −	+
C. amycolatum	*C. striatum/amycolatum* 65.3% (0)	Macroscopy (dry, white colonies)	
C. amycolatum	No corynebacteria	N: +/U: −/AF: +/G: +/S: +/M: +	+
C. amycolatum	*C. striatum/amycolatum* 65.3% (0)	Macroscopy (dry, white colonies)	
C. pseudodiphtheriticum	*C. pseudodiphtheriticum* 99.6% (0)		

Number of tests against biochemical identification database: Manual of Clinical Microbiology biochemical tests were used when API% < 90 and 1 or more tests against.

AF = alkaline phosphatase; G = fermentation of glucose; M = fermentation of maltose; Man = fermentation of mannitol; N = nitrate reduction; S = fermentation of sucrose; X = fermentation of xylose.

16S rRNA analysis was performed (+) when the results of API Coryne and the biochemical tests did not correspond and identification was not possible by use of the biochemical profile (one or more tests against).

Table 2
Average fatty acid profile of the different *Corynebacterium* species

Corynebacterium species (no. of strains)	$C_{14:0}$	$C_{15:1w8c}$	$C_{15:1w6c}$	$C_{15:0}$	Unknown 14,959	$C_{16:0}$	$C_{16:1w9c}$	SF3	$C_{17:0}$	$C_{17:1w9c}$	$C_{17:0anteiso}$	$C_{17:1w8c}$	$C_{17:02OH}$	$C_{18:1w7c}$	$C_{18:0}$	$C_{18:1w9c}$	$C_{18:3w6c(6,9,12)}$	SF5	TBSA10Me18:0	$C_{20:0}$	$C_{20:1w9c}$	$C_{20:4w6,9,12,15c}$
C. accolens (4)	0.95					34.93		1.27							9.45	14.06	2.72	34.13				0.59
C. afermentans (12)				20.08		33.61	1.66		2.19						6.97	32.37		2.57				
C. amycolatum (38)				0.71		25.41	0.61		4.98			2.17			18.15	32.56	0.95	11.80				
C. auris (2)				4.73		11.39	0.68		7.89			21.08			14.52	38.33					1.09	
C. confusum (2)				5.05		18.34	0.38		6.86			25.05	1.41		6.72	30.53		3.28	1.86			
C. coyleae 1 (6)				10.74		26.40	0.74		3.61			2.87			11.72	41.26		2.00				
C. coyleae 2 (4)	0.33			16.51		34.01	2.67		1.51						6.38	33.93		4.27				
C. diphtheriae (7)	1.40	2.50	1.77	9.50		32.13		22.29	1.25		1.47	2.04			4.14	11.05	0.51	9.22				
C. falsenii (2)				1.82		20.01			12.18	1.64	0.75	0.93			23.35	34.41		4.62				
C. glucuronolyticum (19)	1.06			5.54	2.28	32.01	1.27		3.36	1.09	2.25	1.91			2.81	39.73		5.04				
C. imitans (6)				14.28		34.15	0.79		1.31			3.52			4.22	40.30		1.30				
C. jeikeium (11)	0.48			0.81		26.80			2.48	1.08	9.36				12.50	16.39		28.65				
C. minutissimum subgroup A (13)		2.06		3.22		28.23	1.21		1.96	15.56			0.69		6.51	37.01	0.90	1.09	0.72			
C. minutissimum subgroup B (17)				7.49		29.32	0.93		2.30			13.47			6.09	36.56		1.01	1.32			
C. mucifaciens (4)				1.7		38.34	2.3								7.62	43.23		2.6	2.27			
C. propinquum (2)	1.39		1.29	6.23		33.89	2.12		2.37	8.36					4.96	35.41	0.67	3.31				
pseudodiphthericum (14)	1.09	2.34		3.83		30.69	1.84		3.73	11.14	1.14				4.84	36.34		2.58				
C. riegelii (5)				9.03		33.43	1.37		1.31			4.04			6.10	39.54		4.29				
C. simulans (11)				4.75		23.83	1.78		0.85			11.66			5.94	48.39		1.68				
C. singulare (2)				4.84		24.26	2.50		1.12			16.12	1.28		5.78	37.19		1.55	4.92			
C. striatum subgroup A (14)	0.60			13.34		37.38			1.00			5.40			2.61	37.03		1.43				
C. striatum subgroup B (14)	0.91			11.65		35.67			1.39	6.57					2.81	37.22		1.73				
C. urealyticum (6)						24.43		0.66							12.36	8.75	1.09	42.00	8.11			0.51
C. xerosis (4)				1.33		11.89	0.79		6.24	3.53	1.26	1.76		0.99	18.61	44.59		5.50		0.96		

Data are given in average percentages of the fatty acids for the different species. Only fatty acids recorded in 100% of the strains or in ≥50 % with a percentage ≥0.5% are shown.
SF = summed feature; 2 or more fatty acids that cannot be resolved from each other; SF3 = $C_{16:1w7c}/C_{15:0iso2OH}$; SF5 = $C_{18:2w6,9c}/C_{18:0anteiso}$.

analyzed using a 5890 A gas chromatograph fitted with a 5% phenyl methyl silicone capillary column, a flame ionization detector, an automatic sampler, and an integrator (Agilent Technologies, Diegem, Belgium) with the Microbial Identification System (MIS, Microbial ID, Newark, DE). The parameter settings of the gas chromatograph were as follows: injection volume 2 μL, column split ratio 1:100, injection port temperature 250 °C, detector temperature 300 °C, column temperature 170–270 °C at 5 °C/min. For samples with a low response, the organic layer was dried and concentrated in 100 μL hexane/methyl-*tert*-butyl-ether (1:1 vol/vol).

2.3. Evaluation of data

Evaluation of data occurred with the MIS of MIDI. The equivalent chain length (ECL), which is derived from the retention time of the peaks, was used to identify the fatty acids. The peak area value of every fatty acid was calculated as a percentage of the total peak area. The similarity index (SI value between 0 and 1; 1 is a perfect match) expresses the similarity between the fatty acid profile of a strain and that of a species in the database. Using the Library Generation System (LGS, MIDI), we created a new database (Coryne). The presence of wide peaks, which are the result

Table 3
Cellular fatty acid analysis of 34 clinical isolates with the new database and CLIN 40 database

Strain	New database		CLIN 40 database	
	Identification	SI	Identification	SI
C. striatum	*C. striatum* (A)	0.681	*C. glucuronolyticum*	0.613
C. coyleae	*C. coyleae*	0.541	*C. auris*	0.586
C. riegelii	*C. riegelii*	0.670	*C. pseudodiphtheriticum*	0.370
	C. simulans	0.657		
C. imitans	*C. imitans*	0.392	*C. pseudodiphtheriticum*	0.352
	C. simulans	0.385		
C. amycolatum	*C. amycolatum*	0.758	*C. amycolatum*	0.496
C. amycolatum	*C. amycolatum*	0.593	*C. amycolatum*	0.741
C. diphtheriae	*C. diphtheriae*	0.174	*No identification*	–
C. urealyticum	*C. urealyticum*	0.760	*No identification*	–
C. jeikeium	*C. jeikeium*	0.269	*C. bovis*	0.108
C. urealyticum	*C. urealyticum*	0.793	*C. urealyticum*	0.103
C. urealyticum	*C. urealyticum*	0.134	*No identification*	–
C. pseudodiphtheriticum	*C. pseudodiphtheriticum*	0.014	*C. auris*	0.315
C. pseudodiphtheriticum	*C. pseudodiphtheriticum*	0.616	*C. pseudodiphtheriticum*	0.674
C. amycolatum	*C. amycolatum*	0.279	*C. amycolatum*	0.121
C. amycolatum	*C. amycolatum*	0.217	*C. amycolatum*	0.138
			C. xerosis	0.083
C. amycolatum	*C. amycolatum*	0.519	*C. amycolatum*	0.784
C. amycolatum	*C. amycolatum*	0.341	*C. amycolatum*	0.224
C. amycolatum	*C. amycolatum*	0.636	*C. amycolatum*	0.721
C. amycolatum	*C. amycolatum*	0.530	*C. amycolatum*	0.320
			C. xerosis	0.294
C. amycolatum	*C. amycolatum*	0.604	*C. amycolatum*	0.711
C. pseudodiphtheriticum	*C. minutissimum* (A)	0.728	*C. pseudodiphtheriticum*	0.898
C. striatum	*C. striatum* (A)	0.603	*C. striatum* (B)	0.242
			C. pseudodiphtheriticum	0.163
C. amycolatum	*C. amycolatum*	0.716	*C. amycolatum*	0.632
C. pseudodiphtheriticum	*C. pseudodiphtheriticum*	0.759	*C. auris*	0.312
C. jeikeium	*C. jeikeium*	0.107	*No identification*	
C. jeikeium	*C. jeikeium*	0.013	*No identification*	
C. aurimucosum	*No identification*		*No identification*	
C. aurimucosum	*No identification*		*No identification*	
C. minutissimum	*C. minutissimum*	0.865	*C. minutissimum*	0.251
			C. pseudodiphtheriticum	0.157
C. afermentans afermentans	*No identification*		*No identification*	
C. amycolatum	*C. amycolatum*	0.369	*C. afermentans afermentans*	0.594
			C. amycolatum	0.578
C. amycolatum	*C. amycolatum*	0.158	*C. amycolatum*	0.163
			C. xerosis	0.123
C. amycolatum	*C. amycolatum*	0.596	*C. auris*	0.605
			C. amycolatum	0.517
C. pseudodiphtheriticum	*C. minutissimum* (B)	0.440	*C. pseudodiphtheriticum*	0.703
	C. pseudodiphtheriticum	0.275		

(A) = subgroup A, 0.300 < SI < 0.500, atypical strain; SI > 0.500: good match; (B) = subgroup B, SI < 0.300, poorly discriminating.

of cleavages of mycolic acids in the cell envelope, in some strains made it necessary to adapt certain parameters in the peak recognition algorithm: for each peak minimum area/height = 0.017, maximum area/height = 0.165, maximum peak response = 400 000; minimum total response = 45 000, minimum solvent response = 15 000 000, minimum percentage named = 70%, maximum ECL shift = 0.013 (6.7). The 2-dimensional plot based on principal component analysis (LGS, MIDI) was used to detect subspecies within a species.

3. Results

3.1. Fatty acid profile of corynebacteria

All *Corynebacterium* species had a quantitatively and usually also qualitatively different profile (Table 2). All species were characterized by a general pattern with mainly straight-chain and monounsaturated fatty acids. Iso- and anteiso-methyl-branched fatty acids were mostly not present or at a very small level. $C_{17:0anteiso}$ is an exception to this rule and was found in multiple species (*C. diphtheriae*, *C. falsenii*, *C. glucuronolyticum*, *C. jeikeium*, *C. pseudodiphtheriticum*, and *C. xerosis*). In *C. jeikeium*, $C_{17:0anteiso}$ accounted for 9.4% of all fatty acids. Oleic acid ($C_{18:1w9c}$), palmitic acid ($C_{16:0}$), and stearic acid ($C_{18:0}$) were present in all corynebacteria. In most species, oleic acid and palmitic acid were found at relatively high amounts (mean 33.6% and 28.4%). However, a number of species show smaller quantities (e.g. *C. auris* and *C. xerosis* had a smaller percentage of $C_{16:0}$, 11.4% and 11.9%, respectively, whereas *C. diphtheriae* and *C. urealyticum* had a smaller quantity of $C_{18:1w9c}$, 11.1% and 8.6%, respectively). Stearic acid ($C_{18:0}$) appeared also in all species but at a lower concentration (mean 8.55%). Other fatty acids are rather specific for certain groups. *C. diphtheriae* can easily be recognized by the large amount of $C_{16:1w7c}$/$C_{15:0iso2OH}$ (so-called summed feature 3) (mean 22.3%). Tuberculostearic acid (TBSA10Me18:0) can only be found in a limited number of species (*C. urealyticum*, *C. mucifaciens*, *C. minutissimum*, *C. singulare*, and *C. confusum*). The largest quantity was found in *C. urealyticum* (8%).

In most species with the exception of *C. urealyticum* and *C. accolens*, a large specific peak was found (ECL ± 15,000), which is identified by the MIS as $C_{15:0}$. In *C. afermentans afermentans*, this peak accounts for 20% of the total fatty acids. In fact this peak is the result of cleavages of the mycolic acids in the cell envelope of corynebacteria. Mycolic acids cleave at a temperature of 300 °C in the injection port liner (Funke and Bernard, 2003; Funke et al., 1995).

3.2. Analysis of the strains with CLIN 40 database

Only 70 of 219 strains (32%) were identified correctly with the commercially available database (Table 3). For 20 other strains (9%), the correct identification had not the largest SI. For 140 strains, the MIS made a remark mainly for 2 reasons: the presence of very large peaks derived from mycolic acids and the low response rate for lipophilic corynebacteria due to insufficient growth of these species (*C. urealyticum*, *C. jeikeium*, *C. accolens*, and *C. macginleyi* [2]). Concentrating the samples of lipophilic corynebacteria could solve the problem except for *C. macginleyi*. *C. macginleyi* was not included in our new database.

3.3. Making and testing of our own database

Using the 219 fatty acid profiles of the analyzed strains, a new database was created, ending up with 21 species. In 2 species, *C. minutissimum* and *C. striatum*, 2 clusters could be identified with the 2-dimensional plot. The fatty acid profiles of the different clusters showed clear differences. *C. striatum* subgroup A contained $C_{17:1w8c}$, whereas subgroup B contained $C_{17:1w9c}$. *C. minutissimum* subgroup A showed some additional fatty acids ($C_{15:1w8c}$, $C_{17:0\ 2OH}$, and $C_{18:3w6c(6,9,12)}$) different from subgroup B and contained $C_{17:1w9c}$ instead of $C_{17:1w8c}$ in subgroup B.

To test both databases, 34 clinical isolates were analyzed with our new database as well as with the existing database (Table 3). Twenty-nine isolates were identified correctly

Table 4
Cellular fatty acid analysis of 219 strains of corynebacteria with CLIN 40 database

	1	2		1	2
C. accolens	0/4		*C. minutissimum* subgroup A	8/13	2/13
C. afermentans afermentans	9/12	3/12	*C. minutissimum* subgroup B	6/17	0/17
C. amycolatum	20/38	9/38	*C. mucifaciens*	0/4	0/4
C. auris	2/2	0/2	*C. propinquum*	0/2	0/2
C. confusum	0/2	0/2	*C. pseudodiphtheriticum*	6/14	1/14
C. coyleae (1)	0/6	0/6	*C. riegelii*	0/5	0/5
C. coyleae (2)	0/4	0/4	*C. simulans*	0/11	0/11
C. diphtheriae	0/7	0/7	*C. singulare*	0/2	0/2
C. falsenii	0/2	0/2	*C. striatum subgroup A*	8/14	1/14
C. glucuronolyticum	6/19	2/19	*C. striatum subgroup B*	3/14	2/14
C. imitans	0/6	0/6	*C. urealyticum*	0/6	0/6
C. jeikeium	0/11	0/11	*C. xerosis*	2/4	0/4
			Total	70/219	20/219

1 = Number of strains correctly identified with CLIN 40 database; 2 = number of strains for which the correct identification was given but not with the highest SI.

with our new database, whereas only 17 isolates could be classified with the CLIN 40 database. For a number of isolates, the SI was very low (Table 4). For 2 of the 29 isolates correctly identified with our database and for 5 of the 17 isolates correctly identified with the CLIN 40 database, 2 possible identifications were given with similar similarity indices (Table 3).

4. Discussion

In this study, we evaluated the usefulness of cellular fatty acid analysis for the identification of corynebacteria. Therefore, 219 strains were tested and evaluated with the commercial available database. With these strains, we created a new database containing 21 species. The database was then tested by analyzing 34 clinical isolates.

The fatty acid profiles we obtained for the different species of corynebacteria were similar to those reported in other studies (Athaley et al., 1985; Bernard et al., 1991; Von Graevenitz et al., 1991). All strains were identified correctly at the genus level with the commercially available database. However, only one third of these strains could be identified correctly at the species level. These results clearly indicate that the commercially available library is not well suited for the identification and differentiation of corynebacteria.

The new database was tested with 34 clinical isolates, and the results obtained were compared with those obtained with the CLIN 40 database. With our new database, 29 of the 34 testing were identified correctly. With the commercial available database, only 17 testing isolates were identified correctly. Isolates that could not be identified with our database were 2 *C. aurimucosum*, 2 *C. pseudodiphtheriticum*, and 1 *C. afermentans afermentans*. As *C. aurimucosum* was not included in our database, it was obvious that no identification was possible. The 2 *C. pseudodiphtheriticum* isolates were both identified as *C. minutissimum*. It is known that *C. pseudodiphtheriticum* shows some heterogeneity in his fatty acid profile (Von Graevenitz et al., 1991), which might be an explanation for the incorrect identification. The incorrect identification of the 1 *C. afermentans afermentans* isolate is because of an exceptional fatty acid profile. The isolate contained some iso- and anteiso-methyl-branched fatty acids ($C_{15:0\ iso}$, $C_{15:0\ anteiso}$, $C_{16:0\ iso}$, $C_{17:0\ anteiso}$, and $C_{17:0\ iso}$), which are very rare in corynebacteria (Athaley et al., 1985). It is also important to mention that, for some isolates, the SI was very low (*C. pseudodiphtheriticum* and *C. jeikeium*). In such cases, it is recommended to do some additional tests to confirm the identification obtained by cellular fatty acid analysis. Because of the similarity of the fatty acid profiles of some species (e.g. between *C. afermentans afermentans* and *C. coyleae* [subgroup 2] and among *C. riegelii, C. coyleae* [subgroup 1], *C. imitans*, and *C. simulans*), multiple identifications with similar similarity indices may be given. Also in this case, additional tests may be needed for identification.

For lipophilic corynebacteria, a concentrating step is needed to obtain adequate response values. Because a concentrating step did not result in adequate response values for *C. macginleyi* analysis of cellular fatty acids by gas chromatography is not suited for the identification of this species. *C. macginleyi* can be recognized by its lack of pyrazinamidase activity and by the fermentation of mannitol, which is exceptional for corynebacteria. *C. macginleyi* is also a species that is not frequently seen in the clinical laboratory.

We conclude that cellular fatty analysis is a valuable tool for the identification of corynebacteria. For identification at the species level, the commercial database was not adequate, and it was necessary to make a new database. For identifications with a low SI or when multiple possible identifications are given, additional tests are needed.

References

Athaley M, Noble WC, Minnikin DE (1985) Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. *J Appl Bacteriol* 58:507–512.

Bernard KA, Bellefeuille M, Ewan EP (1991) Cellular fatty acid composition as an adjunct to the identification of asporogenous, aerobic, gram-positive rods. *J Clin Microbiol* 29:83–89.

Bortolussi R, Mailman T (2004) Aerobic Gram-positive bacilli. In *Infectious diseases.* Eds, J Cohen and WG Powderly. 2nd ed. Edinburgh: Mosby, pp 2153–2172.

Colt HG, Morris JF, Marston BJ, Sewell DL (1991) Necrotizing tracheitis caused by *Corynebacterium pseudodiphtheriticum*: unique case and review. *Rev Infect Dis* 13:73–76.

Coyle MB, Lipsky BA (1990) Coryneform bacteria in infectious diseases: Clinical and laboratory aspects. *Clin Microbiol Rev* 3:227–246.

Funke G, Bernard KA (2003) Coryneform Gram-positive rods. In *Manual of clinical microbiology.* Eds, PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, and RH Yolken. 8th ed. Washington (DC): American Society for Microbiology, pp 472–501.

Funke G, Lawson PA, Collins MD (1995) Heterogeneity within Centers for Disease Control and Prevention coryneforme group ANF1-like bacteria and description of *Corynebacterium auris* sp. nov. *Int J Syst Bacteriol* 45:735–739.

Funke G, Renaud FNR, Freney J, Riegel P (1997a) Multicenter evaluation of the updated and extended Api (RAPID) Coryne Database 2.0. *J Clin Microbiol* 35:3122–3126.

Funke G, Von Graevenitz A, Clarridge III JE, Bernard KA (1997b) Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* 10:125–159.

Lagrou K, Verhaegen J, Janssens M, Wauters G, Verbist L (1998) Prospective study of catalase-positive coryneform organisms in clinical specimens, identification, clinical relevance and antibiotic susceptibility. *Diagn Microbiol Infect Dis* 30:7–15.

Riegel P, Ruimy R, Christen R, Monteil H (1996) Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. *Eur J Clin Microbiol Infect Dis* 15:657–662.

Sewell DL, Coyle MB, Funke G (1995) Prosthetic valve endocarditis caused by *Corynebacterium afermentans* subsp. *lipophilum* (CDC coryneforme group ANF-1). *J Clin Microbiol* 33:759–761.

Soriano F, Fernandez-Roblas R (1998) Infections caused by antibiotic-resistant *Corynebacterium* group D-2. *Eur J Clin Microbiol Infect Dis* 7:337–341.

Von Graevenitz A, Osterhout G, Dick J (1991) Grouping of some clinically relevant gram-positive rods by automated fatty acid analysis. *APMIS* 99:147–154.