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Identities of *Microbacterium* spp. Encountered in Human Clinical Specimens▽

Kathrina Gneiding, Reinhard Frodl, and Guido Funke*

Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Ravensburg, Germany

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In the present study, 50 strains of yellow-pigmented gram-positive rods that had been isolated from human clinical specimens and collected over a 5-year period were further characterized by phenotypic and molecular genetic methods. All 50 strains belonged to the genus *Microbacterium*, and together they represented 18 different species. *Microbacterium oxydans* (n = 11), *M. paraoxydans* (n = 9), and *M. foliorum* (n = 7) represented more than half of the strains included in the present study. The isolation of strains belonging to *M. hydrocarbonoxydans* (n = 2), *M. esteraromaticum* (n = 1), *M. oleivorans* (n = 1), *M. phyllosphaerae* (n = 1), and *M. thalassium* (n = 1) from humans is reported for the first time. *Microbacterium* sp. strain VKM Ac-1389 (n = 1) and the previously uncultured *Microbacterium* sp. clone YJQ-29 (n = 1) probably represent new species. Comprehensive antimicrobial susceptibility data are given for the 50 *Microbacterium* isolates. This study is, so far, the largest on *Microbacterium* spp. encountered in human clinical specimens and outlines the heterogeneity of clinical *Microbacterium* strains.

Among the coryneform bacteria, the phenotypically and phylogenetically closely related genera *Microbacterium* and *Aureobacterium* have been united in the redefined genus *Microbacterium* (20). At present, the genus *Microbacterium* comprises 55 species (www.bacterio.cict.fr/m/microbacterium.html), all of which exhibit more or less yellow-pigmented gram-positive rods. Despite this large number of species, only in the mid-1990s was the presence of microbacteria in human clinical specimens recognized (7, 8, 11). Since then, only eight other reports on microbacteria have appeared in the relevant clinical microbiology literature (1, 2, 9, 12–16). The aim of the present study was to reveal the distribution of individual *Microbacterium* species in human clinical specimens by applying phenotypic and molecular genetic methods. Because no comprehensive data on the antimicrobial susceptibility patterns of *Microbacterium* spp. were available, we also determined the MICs of 10 antimicrobial agents against all 50 strains included in the present study. We observed that three species, namely, *Microbacterium oxydans*, *M. paraoxydans*, and *M. foliorum*, accounted for more than 50% of all strains included in the present study, but overall, 18 different taxa were encountered, indicating the heterogeneity of microbacteria isolated from clinical specimens.

(This paper is part of the medical doctoral thesis of K. Gneiding at the medical faculty of the University of Ulm, Ulm, Germany.)

MATERIALS AND METHODS

Strains. During a 5-year period, the 50 strains investigated in the present study were isolated in the routine clinical microbiology laboratories of Gärtner & Colleagues Laboratories, Ravensburg, Germany, or referred to the reference laboratory for coryneform bacteria at this institution by collaborating laboratories. None of the isolates had been included in any of our previous studies (7–9, 11). None of the patients were epidemiologically linked. The strains had been stored at −20°C in skim milk. For the investigations, strains were grown on Columbia sheep blood agar plates (BD, Heidelberg, Germany) and passaged twice on Columbia sheep blood agar at 35°C in ambient air before use.

Biochemical identification. The techniques used have been described in detail previously (10). The commercial API Coryne and API ZYM kits (both from bioMérieux, Marcy l'Etoile, France) were used according to the manufacturer's instructions, and reading was done after 48 h of incubation at 35°C for the API Coryne and after 4 h for the API ZYM system.

Molecular genetic investigations. The 16S rRNA gene sequences were analyzed according to a published protocol (3). Almost complete (>1,400-bp) 16S rRNA gene sequences were determined for each clinical strain by aligning multiple overlapping sequences by use of the Lasergene 5 package (DNAStar Inc., Madison, WI). The 16S rRNA genes of the different *Microbacterium* species were aligned and compared by using the Web-based BLAST 2 Sequences software tool (www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).

Identification. A strain was identified to the species level if its 16S rRNA gene sequence shared >98.70% base pair homology with the type strain or with other representative strains of a valid species (19) and if phenotypic testing did not indicate any aberrant reactions relative to the published data for this particular species.

Antimicrobial susceptibility testing. The CLSI standard for the determination and interpretation of antimicrobial MICs for *Corynebacterium* spp. (5) was applied. Briefly, by use of a broth microdilution method, bacterial cells with an inoculum equivalent to a 0.5 McFarland standard were grown in cation-adjusted Mueller-Hinton broth with lysed horse blood and were incubated for as long as 48 h. MICs were read by two independent researchers.

Nucleotide sequence accession numbers. The GenBank accession numbers of the almost complete 16S rRNA gene sequences of all 50 clinical isolates included in the present study are given in Table 1.

RESULTS

Table 1 outlines the patients' data as well as the identities of the 50 *Microbacterium* strains included in the present study. Twenty-nine patients were male and 21 female. The ages of the patients ranged from 1 to 79 years, with an average of 43.1 years. Sixteen strains came from blood cultures; 13 strains were isolated from wounds; 11 strains came from normally sterile anatomical sites or sterile materials; 6 strains came from urines; and 4 strains were isolated from miscellaneous materials.

The 50 strains were found to belong to 18 different taxa: *M. oxydans* (n = 11), *M. paraoxydans* (n = 9), *M. foliorum* (n = 7), *M. aurum* (n = 3), *M. lacticum* (n = 3), "*M. binotii*" (n = 2), *M.*

* Corresponding author. Mailing address: Department of Medical Microbiology and Hygiene, Gärtner & Colleagues Laboratories, Elisabethenstrasse 11, D-88212 Ravensburg, Germany. Phone: 49-751-502-230. Fax: 49-751-502-385. E-mail: ldg.funke@t-online.de.
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TABLE 1. Strains included in the present study

Running no.	Strain collection no.	Patient's age (yr), sex[a]	Clinical source	Identification	GenBank accession no.
1	1	31, f	Knee puncture fluid	*M. paraoxydans*	EU714331
2	9	34, m	Wound swab	*M. foliorum*	EU714380
3	58	45, f	Urine	*M. paraoxydans*	EU714372
4	76	56, m	Wound swab	*M. paraoxydans*	EU714377
5	118	74, f	Gall bladder	*M. schleiferi*	EU714332
6	150	70, m	Wound swab	*M. foliorum*	EU714333
7	297	8, m	Throat swab	*M. oxydans*	EU714348
8	314	1, m	Blood culture	*M. aurum*	EU714355
9	327	36, m	Wound swab	*M. foliorum*	EU714358
10	331	65, f	Prosthetic hip infection	*M. phyllosphaerae*	EU714359
11	332	55, f	Blood culture	*M. trichothecenolyticum*	EU714360
12	343	5, f	Urine	*M. testaceum*	EU714365
13	346	66, m	Blood culture	*M. laevaniformans*	EU714366
14	407	25, f	Superficial wound	*M. oxydans*	EU714369
15	428	79, m	Pleural fluid	*M. paraoxydans*	EU714370
16	556	49, m	Wound swab	*M. foliorum*	EU714371
17	591	39, m	Blood culture	*M. paraoxydans*	EU714373
18	698	47, m	Endophthalmitis	*M. oxydans*	EU714374
19	699	25, f	Urine	*M. oxydans*	EU714375
20	720	74, f	Pleural fluid	*M. foliorum*	EU714376
21	768	68, m	Wound swab	*Microbacterium* sp. strain VKM Ac-1389	EU714378
22	798	24, f	Blood culture	*M. foliorum*	EU714379
23	985	18, m	Blood culture	*M. oleivorans*	EU714381
24	2083	40, f	Sinus aspirate	*M. oxydans*	EU714335
25	2121	5, m	Superficial wound	"*M. binotii*"	EU714336
26	2122	59, m	Blood culture	*M. esteraromaticum*	EU714337
27	2229	23, m	Bone infection	"*M. binotii*"	EU714338
28	2345	54, m	Dialysis fluid	*M. oxydans*	EU714339
29	2350	28, f	Blood culture	*M. oxydans*	EU714340
30	2400	67, m	Wound swab	*M. foliorum*	EU714341
31	2470	54, f	Urine	*M. paraoxydans*	EU714342
32	2588	65, m	Blood culture	*M. aurum*	EU714343
33	2704	41, m	Lymph node	*M. oxydans*	EU714344
34	2761	45, f	Wound swab	*Microbacterium* sp. strain YJQ-29	EU714345
35	2833	54, f	Blood culture	*M. lacticum*	EU714346
36	2841	8, m	Wound swab	*M. oxydans*	EU714347
37	3043	29, f	Blood culture	*M. oxydans*	EU714349
38	3047	NK, f	Urine	*M. lacticum*	EU714350
39	3075	66, m	Wound swab	*M. lacticum*	EU714351
40	3084	7, m	Blood culture	*M. hydrocarbonoxydans*	EU714352
41	3109	35, m	Tracheal secretion	*M. paraoxydans*	EU714353
42	3131	75, f	Urine	*M. paraoxydans*	EU714354
43	3200	11, m	Blood culture	*M. paraoxydans*	EU714356
44	3227	74, m	Blood culture	*M. oxydans*	EU714357
45	3352	31, m	Wound swab	*M. resistens*	EU714361
46	3370	60, m	Blood culture	*M. trichothecenolyticum*	EU714362
47	3373	49, m	Urethral swab	*M. thalassium*	EU714363
48	3388	51, f	Conjunctival swab	*M. aurum*	EU714364
49	3502	42, f	Blood culture	*M. testaceum*	EU714367
50	3517	45, f	Catheter tip	*M. hydrocarbonoxydans*	EU714368

[a] m, male; f, female; NK, not known.

hydrocarbonoxydans ($n = 2$), *M. testaceum* ($n = 2$), *M. trichothecenolyticum* ($n = 2$), *M. esteraromaticum* ($n = 1$), *M. laevaniformans* ($n = 1$), *M. oleivorans* ($n = 1$), *M. phyllosphaerae* ($n = 1$), *M. resistens* ($n = 1$), *M. schleiferi* ($n = 1$), *M. thalassium* ($n = 1$), *Microbacterium* sp. strain VKM Ac-1389 ($n = 1$), and the uncultured *Microbacterium* sp. clone YJQ-29 ($n = 1$). For all 50 strains, the 16S rRNA gene homology of the individual clinical strain with the type strain or another representative strain of the corresponding species ranged from 98.84% to 100%, with a mean homology of 99.60%.

The 16S rRNA gene homologies between all 55 *Microbacterium* species defined to date are given in Table 2. A total of 1,485 16S rRNA gene homologies were calculated. Two different clinically relevant *Microbacterium* species always shared less than 98.70% homology except for the species *M. arborescens* and *M. imperiale* (99.73% homology), *M. oxydans* and M. *paraoxydans* (99.25%), *M. foliorum* and *M. phyllosphaerae* (99.19%), *M. lacticum* and *M. schleiferi* (98.91%), *M. foliorum* and *M. hydrocarbonoxydans* (98.85%), *M. hydrocarbonoxydans* and *M. oxydans* (98.77%), *M. oleivorans* and *M. phyllosphaerae* (98.73%), *M. hydrocarbonoxydans* and *M. phyllosphaerae* (98.72%), and *M. foliorum* and *M. oxydans* (98.70%).

Table 3 shows the antimicrobial susceptibility patterns of *Microbacterium* spp. All 50 isolates were susceptible to lin-

TABLE 2. Percentages of 16S rRNA gene homologies of *Microbacterium* spp.

Species (GenBank accession no.)	% 16S rRNA gene homology with:											
	M. aerolatum	*M. aoyamense*	*M. aquimaris*	*M. arabinogalactanolyticum*	*M. arborescens*	*M. aurantiacum*	*M. aurum*	*M. barkeri*	*M. chocolatum*	*M. deminutum*	*M. dextranolyticum*	*M. esteraromaticum*
Microbacterium aerolatum (AJ309929)												
Microbacterium aoyamense (AB234028)	96.43											
Microbacterium aquimaris (AM778449)	96.58	96.22										
Microbacterium arabinogalactanolyticum (Y17228)	97.43	96.53	96.99									
Microbacterium arborescens (X77443)	95.94	97.16	95.88	95.83								
Microbacterium aurantiacum (AB004726)	96.37	96.54	96.72	96.65	95.44							
Microbacterium aurum (Y17229)	96.09	98.89	95.81	96.17	96.67	96.39						
Microbacterium barkeri (X77446)	95.51	94.19	95.05	96.16	96.19	95.03	93.81					
Microbacterium chocolatum (AB004725)	95.93	95.75	96.06	96.21	95.12	98.42	95.92	94.5				
Microbacterium deminutum (AB234026)	96.08	99.09	95.99	96.23	97	96.93	98.39	94.23	96.3			
Microbacterium dextranolyticum (Y17230)	96.72	96.94	96.28	97.33	96.13	97.09	96.54	95.24	96.77	97.49		
Microbacterium esteraromaticum (Y17231)	97.65	96.54	96.86	99.18	95.83	96.52	96.18	96.37	96.08	96.24	97.19	
Microbacterium flavescens (Y17232)	96.58	96.68	96.5	97.13	96.39	97.34	96.33	95.44	96.84	97	97.82	97.13
Microbacterium flavum (AB286029)	97.43	98.75	96.35	96.56	97.04	96.88	98.34	94.58	96.21	98.4	97.38	96.56
Microbacterium foliorum (AJ249780)	98.53	97.37	97.14	97.4	96.67	97	97.15	95.38	96.24	97.08	96.68	97.27
Microbacterium ginsengisoli (AB271048)	94.73	97.23	94.77	94.67	98.55	95.01	96.49	95.46	94.36	97.21	95.48	94.67
Microbacterium gubbeenense (AF263563)	94.42	94.53	94.77	93.6	93.9	93.82	94.9	95.42	92.95	94.3	92.62	94.61
Microbacterium halophilum (AB004714)	95.22	94.95	97.14	95.97	95.38	95.58	94.64	94.77	95.08	95.33	95.66	95.84
Microbacterium halotolerans (AY376165)	95.02	94.27	95.12	95.4	93.87	95.3	93.93	94.55	94.14	94.44	94.97	95.34
Microbacterium hatanonis (AB274908)	95.59	97.23	95.96	96.01	96.51	95.66	96.93	94.95	94.61	97.01	96.39	96.02
Microbacterium hominis (AB004727)	96.93	96.81	96.65	96.84	97.06	97.15	96.38	95.57	96.61	97.07	97.63	96.7
Microbacterium hydrocarbonoxydans (AJ698726)	97.95	97.58	97	97.47	96.6	97.37	97.42	95.45	96.65	97.22	97.23	97.2
Microbacterium imperiale (X77442)	96.08	97.09	95.95	95.83	99.73	95.71	96.6	96.05	95.2	97	96.27	95.83
Microbacterium indicum (AM158907)	94.6	94.29	95.05	95.33	95.08	94.63	94.46	96.4	93.55	93.76	94.54	95.13
Microbacterium keratanolyticum (Y17233)	97.43	97.02	96.79	96.24	96.3	96.58	96.99	94.25	95.53	96.59	96.16	96.38
Microbacterium ketosireducens (AB004724)	97.08	96.39	96.74	98.44	96.1	97.73	96.1	96.04	97.02	96.95	97.73	97.59
Microbacterium kitamiense (AB013907)	96.51	96.68	96.86	96.92	95.92	99.39	96.88	95.31	98.17	97.07	97.43	96.79
Microbacterium koreense (AY962574)	96.39	98.32	96.86	96.1	96.66	96.31	98.05	93.96	95.52	97.83	96.16	95.9
Microbacterium lacticum (X77441)	96.58	98.62	96.44	96.51	96.94	96.53	98.44	94.15	96.09	98.26	96.95	96.52
Microbacterium lacus (AB286030)	96.22	99.45	96.09	96.4	97.04	96.79	98.99	94.09	95.62	98.95	96.71	96.41
Microbacterium laevaniformans (Y17234)	96.71	97.49	96.01	96.79	96.44	97.64	97.3	95.02	97.29	97.99	98.72	96.65
Microbacterium liquefaciens (X77444)	97.58	97.23	97	97.2	96.53	96.66	97.08	95.17	95.88	96.86	96.61	97.34
Microbacterium luteolum (Y17235)	96.73	96.75	96.79	97.06	96.24	96.51	96.72	95.01	95.74	96.38	96.64	97.2
Microbacterium luticocti (AM747814)	94.38	94.32	95.26	94.59	94.62	94.8	94.07	95.32	94.68	94.22	93.96	94.32
Microbacterium marinilacus (AB286020)	95.52	95.16	95.05	95.65	97.11	95.84	94.71	96.43	95.21	95.06	95.55	95.58
Microbacterium maritypicum (AB004728)	96.81	97.16	95.88	96.84	96.24	97.55	96.72	95.23	97.13	97.63	98.31	96.84
Microbacterium natoriense (AY566291)	97.87	97.19	97.18	97.54	96.83	97.19	96.84	95.5	96.68	96.9	97.11	97.26
Microbacterium oleivorans (AJ698725)	97.56	97.65	97.97	97.4	97.14	97.17	97.15	95.3	96.79	97.42	97.22	97.27
Microbacterium oxydans (Y17227)	97.8	97.44	97.2	97.4	96.72	96.86	96.72	95.35	96.09	97.07	96.92	97.54
Microbacterium paludicola (AJ853909)	94.94	95.25	94.84	95.25	97.53	95.03	97.53	96.37	94.51	94.94	94.8	95.06
Microbacterium paraoxydans (AJ491806)	98.4	97.23	97.56	97.74	96.66	96.96	96.66	95.92	96.35	97.01	97.08	98.02
Microbacterium phyllosphaerae (AJ277840)	98.24	97.3	97.14	97.81	97.01	97.28	97.01	95.78	96.65	96.93	97.35	97.68
Microbacterium pumilum (AB234027)	96.22	99.29	96.02	96.37	97.15	97.01	97.15	94.25	96.5	99.79	97.58	96.3
Microbacterium resistens (Y14699)	97.22	96.75	97.48	97.67	96.3	96.92	96.3	95.82	96.49	96.45	97.33	97.68
Microbacterium saperdae (Y17236)	97.36	96.89	96.79	96.86	96.33	96.46	96.33	94.77	95.67	96.59	96.34	97
Microbacterium schleiferi (Y17237)	96.86	98.89	96.92	96.65	97.74	96.92	97.74	94.73	96.42	98.53	97.46	96.79
Microbacterium sediminicola (AB286021)	96.01	96.4	95.53	95.71	98.28	96.86	98.28	95.88	95.76	96.17	96.1	95.72
Microbacterium terrae (AB004720)	96.23	95.71	96.46	97.06	95.42	96.72	95.42	95.22	96.35	96.37	97.45	96.79
Microbacterium terregens (AB004721)	96.3	98.95	96.03	96.45	96.87	96.11	96.87	94.24	95.51	98.6	96.39	96.24
Microbacterium terricola (AB234025)	96.58	97.23	96.69	96.87	96.93	96.73	96.93	94.85	96.03	98.33	97.08	96.87
Microbacterium testaceum (X77445)	97.65	97.02	97.76	97.74	96.67	97.75	96.67	95.98	97.18	96.72	97.48	97.74
Microbacterium thalassium (AB004713)	96.66	96.55	97.35	96.84	96.25	97.15	96.25	95.38	96.28	97.08	97.76	97.12
Microbacterium trichothecenolyticum (AB004722)	96.29	96.12	96.16	97.47	96.3	97.47	96.3	95.75	96.9	96.3	97.19	97.33
Microbacterium ulmi (AY062021)	95.31	95.93	95.81	95.83	97.76	95.22	97.76	95.78	94.86	95.76	95.61	95.57
Microbacterium xylanilyticum (AJ853908)	96.73	96.13	96.16	97.04	95.96	96.74	95.96	95.63	96.05	96.45	96.66	97.04

ezolid and meropenem. Only strain 3352 was resistant to vancomycin, and only strain 985 was resistant to doxycycline. Ciprofloxacin had the weakest activity against microbacteria; 22% of the isolates were intermediately susceptible, and 22% were resistant.

DISCUSSION

From the work of Stackebrandt and Ebers, it has been clear that a cutoff of 98.7% 16S rRNA gene homology is appropriate for species differentiation within a genus (19). As is evident from Table 2, the genus *Microbacterium* is a very tight genus regarding the 16S rRNA gene homology between two valid species. However, applying the recommendations of Stackebrandt and Ebers, we were able to easily identify every *Microbacterium* strain included in the present study.

Of note is the molecular genetic differentiation between *M. oxydans* and *M. paraoxydans*, the two most frequently encountered species in the present study. Compared to the *M. oxydans*

TABLE 2—*Continued*

% 16S rRNA gene homology with:																	
M. flavescens	*M. flavum*	*M. foliorum*	*M. ginsengisoli*	*M. gubbeenense*	*M. halophilum*	*M. halotolerans*	*M. hatanonis*	*M. hominis*	*M. hydrocarbonoxydans*	*M. imperiale*	*M. indicum*	*M. keratanolyticum*	*M. ketosireducens*	*M. kitamiense*	*M. koreense*	*M. lacticum*	*M. lacus*
96.83																	
96.74	97.24																
95.66	97.08	95.53															
93.07	94.09	94.37	93.39														
96.4	95.05	95.55	94.17	92.76													
95.29	94.73	95.02	92.97	94.29	93.93												
96.31	97.23	96.59	96.65	93.18	95.16	94.03											
98.02	97.22	96.82	95.83	93.42	96.36	94.93	96.35										
96.67	98	98.85	95.97	93.37	95.56	95.06	96.75	96.85									
96.32	96.97	96.68	98.41	93.75	95.26	93.8	96.44	96.95	96.54								
94.53	94.68	94.59	94.21	96.27	94.47	95.24	94.15	94.56	95.52	94.94							
96.51	97.18	98.02	95.22	94.41	95.08	94.51	96.84	96.15	97.67	96.17	94.56						
98.09	96.67	97.03	94.97	93.38	96.32	95.76	95.83	97.45	97.17	96.11	94.77	96.18					
97.62	96.83	97.29	95.21	94.06	95.62	95.41	96.02	97.21	97.59	96	94.97	97.2	97.73				
96.24	98.18	97.08	96.1	94.69	94.96	94.3	95.97	96.31	97.43	96.59	94.71	96.45	96.25	96.38			
96.67	98.55	97.36	96.84	93.97	95.19	94.07	96.86	96.81	97.76	96.95	94.25	96.99	96.39	96.88	98.05		
96.49	98.61	97.53	97.12	94.69	94.86	94.41	97.54	96.72	97.75	96.97	94.62	97.58	96.24	97.27	98.05	98.35	
97.29	98.22	97.22	95.98	93.59	95.37	95.27	96.51	97.58	98.15	96.37	94.71	96.65	97.14	97.72	96.81	97.44	97.35
96.67	97.38	98.51	96.84	94.33	95.26	94.95	97.27	96.47	98.58	96.4	94.66	97.74	96.89	96.95	96.8	97.56	97.39
96.51	96.97	98.29	95.09	94.09	95.09	94.79	96.84	96.36	98.36	96.1	94.42	98.02	96.6	96.79	96.38	96.99	96.89
94.07	94.5	94.69	94.78	94.91	94.16	94.04	94.13	94.26	95.21	94.55	95.45	93.83	94.22	95.01	95	94	94.25
95.94	95.77	95.6	96.44	94.36	94.45	94.89	94.49	95.57	96.32	97.04	96.14	94.89	95.97	96.05	94.92	95.26	95.09
97.13	97.85	96.69	95.76	93.57	95.35	95.08	96.49	98.12	97.59	96.2	94.62	96.15	97.24	97.6	96.38	97.15	97.06
97.11	97.47	98.66	95.57	93.01	93.01	95.02	96.49	97.11	98.45	96.83	94.74	97.61	97.59	97.33	96.91	97.33	97.04
97.62	97.94	98.11	96.17	93.58	94.49	94.99	97.01	97.65	98.06	97.22	94.66	97.67	97.52	97.38	97.15	97.9	97.54
96.93	97.59	98.7	95.78	94.49	93.58	95.13	97.46	96.66	98.77	96.59	94.77	97.95	97.1	97.14	97	97.61	97.58
95.27	95.59	95.63	96.35	94.78	94.78	94.22	94.86	95.05	98.36	97.25	95.94	95.11	95.37	95.41	95.05	95.07	95.03
97.13	97.59	98.5	95.76	95.28	94.53	95.08	97.31	96.96	98.65	96.6	95.18	97.6	97.38	97.18	96.66	97.41	97.14
97.41	97.45	99.19	95.66	93.3	93.3	95.57	96.86	97.63	98.72	96.88	95.36	98.15	97.73	97.69	97.01	97.49	97.46
97.08	98.58	97.16	97.22	94.35	94.35	94.6	96.87	97.22	97.37	97.08	94.58	96.65	97.08	97.15	98.01	98.29	99.14
97.26	97.18	97.74	95.15	94.21	94.21	95.06	96.77	96.91	97.74	96.24	95.46	97.47	97.17	97.33	96.38	97.06	97.06
96.46	97.17	98.03	95.19	94.25	94.25	94.54	97	96.32	98.1	96.19	94.26	98.15	96.46	96.68	96.59	97.08	97.08
97.33	98.62	97.61	97.23	93.85	93.85	94.24	97.32	97.25	98.02	97.74	94.42	97.33	96.88	97.19	98.4	98.91	96.61
96.56	96.53	96.36	97.74	95.32	95.32	94.43	95.87	96.53	96.86	98.21	95.66	96.27	95.96	97.22	95.83	96.5	96.98
97.81	95.94	96.31	94.26	93.17	93.17	94.79	95.13	96.7	96.38	95.42	94.01	95.83	98.58	96.99	95.55	95.76	95.76
96.39	98.32	97.16	97.01	94.45	94.45	93.84	96.95	96.6	97.36	96.8	93.96	96.59	98.46	96.11	97.71	98.13	98.13
97.08	97.98	97.43	96.72	94.61	94.61	93.98	97.64	96.59	97.71	96.93	94.67	97.08	96.45	96.87	97.83	97.98	97.98
97.62	97.32	97.76	95.46	94.29	94.29	95.16	97.13	97.47	97.89	96.8	95.22	97.4	97.45	98.03	96.73	97.21	97.21
97.47	96.75	96.9	95.23	93.83	93.83	94.8	96.56	96.95	97.06	96.39	94.63	96.78	97.67	97.09	96.25	96.66	96.66
98.43	96.14	96.58	95.43	93.89	93.89	95.13	95.87	97.66	96.79	96.17	94.42	96.03	97.8	97.74	95.82	96.31	96.31
95.72	95.95	96.22	96.78	94.59	94.59	94.07	95.62	96.36	95.92	97.76	95.17	95.22	95.54	95.52	95.83	95.6	95.6
97.46	96.18	97.6	94.95	93.45	94.78	95.36	95.59	97.83	96.8	95.9	94.56	96.07	97.03	96.88	95.62	96.03	96.03

Continued on next page

type strain sequence (GenBank accession no. Y17227 [18]), all nine *M. paraoxydans* strains from the present study showed the following nucleotide differences: at position 168, T instead of C; at position 177, T instead of A; at position 181, T instead of a deletion; at position 374, T instead of C; at position 555, C instead of G; at position 569, G instead of C; at position 588, G instead of N; and at position 1211, T instead of C. In general, we can confirm the data of Laffineur et al. (15) for the biochemical differentiation of *M. oxydans* and *M. paraoxydans*: in the present study, 9 of 11 *M. oxydans* strains expressed β-glucosidase activity (10 of 10 in reference 15), whereas all *M. paraoxydans* strains were negative in both studies. Another distinguishing reaction might be the strong pyrrolidonyl arylamidase activity detected in the present study for 8 of 11 *M.*

TABLE 2—*Continued*

% 16S rRNA gene homology with:																							
M. laevaniformans	*M. liquefaciens*	*M. luteolum*	*M. luticocti*	*M. marinilacus*	*M. maritypicum*	*M. natoriense*	*M. oleivorans*	*M. oxydans*	*M. paludicola*	*M. paraoxydans*	*M. phyllosphaerae*	*M. pumilum*	*M. resistens*	*M. saperdae*	*M. schleiferi*	*M. sediminicola*	*M. terrae*	*M. terregens*	*M. terricola*	*M. testaceum*	*M. thalassium*	*M. trichothecenolyticum*	*M. ulmi*
97																							
97	98.97																						
94.66	94.42	94.24																					
95.98	95.12	94.96	94.38																				
99	96.61	96.5	94.33	95.91																			
97.93	97.75	97.61	94.39	95.99	97.11																		
97.58	97.83	97.68	94.46	95.63	97.45	98.58																	
97.22	99.66	99.18	94.59	95.3	96.8	97.96	98.02																
95.65	95.21	95.04	94.48	98.6	95.47	96.07	95.73	95.39															
97.57	99.05	98.84	95.08	95.77	97.24	98.24	98.38	99.25	95.82														
97.43	98.37	98.22	94.35	96.22	97.36	98.73	98.73	98.56	95.84	98.3													
98.17	97.01	96.51	94.19	95.09	97.79	97.01	97.01	97.22	95.14	97.01	97.08												
97.58	97.74	97.61	95.35	95.71	97.25	97.82	97.82	97.95	95.81	98.36	97.88	96.51											
97.15	98.78	99.11	94.15	94.79	96.46	97.47	97.47	99.04	94.86	98.5	97.96	96.73	97.61										
97.79	97.67	97.33	94.24	95.65	97.46	97.82	97.82	97.88	95.82	97.67	97.88	98.57	97.26	97.47									
96.48	95.88	95.72	94.93	97.82	96.47	96.41	96.41	96.06	97.14	96.27	96.91	96.23	96.26	95.75	96.75								
96.94	96.1	96.31	94.04	95.17	96.5	96.77	96.77	96.31	97.47	96.58	96.85	96.44	96.37	95.97	96.3	95.23							
97.04	96.95	96.45	94.05	95.21	96.74	96.91	96.91	97.14	95.27	96.95	97.08	98.86	96.45	96.6	98.26	96.04	95.34						
97.34	97.63	97.22	94.63	95.76	97.21	97.26	97.26	97.84	95.79	97.77	97.42	98.36	97.71	97.43	98.4	96.66	95.89	98.32					
97.51	98.03	97.88	94.97	96.43	97.54	97.96	97.96	98.22	95.84	98.64	98.3	96.79	98.5	97.83	97.54	96.91	96.72	96.6	97.84				
97.51	97.21	96.92	94.4	95.38	97.26	97.26	97.26	97.28	95.55	97.84	97.1	97.16	98.01	96.87	97.19	96	96.78	96.33	97.5	97.82			
96.81	96.58	96.45	93.79	95.85	96.91	96.9	96.9	96.79	95.46	97.12	97.13	96.37	97.47	96.51	96.65	96.54	97.26	96.03	96.8	98.01	97.19		
96.3	95.47	95.29	95.28	96.66	96.07	96.28	96.28	95.65	96.52	96.12	96.08	95.81	95.76	95.26	95.9	97.15	94.95	95.91	95.76	96.27	95.38	95.7	
96.92	96.65	96.49	94.46	95.5	96.54	96.91	96.91	96.83	95.52	97.15	97.26	96.51	97.38	96.31	96.28	95.98	96.28	96.05	96.52	96.78	96.95	97.31	95.86

oxydans strains, whereas weak activity was detected for 1 of 9 *M. paraoxydans* strains. The reason why *M. oxydans* and *M. paraoxydans* were the most frequently encountered species in our series is unclear but might be related to the distribution of these species in the environment.

In their important study of microbacteria, Laffineur et al. (15) observed that *M. oxydans* (9 of 30 strains) and *M. paraoxydans* (5 of 30 strains) were the microbacteria most frequently found in clinical specimens. These authors also detected *M. aurum* (4 of 30 strains), *M. lacticum* (4 of 30 strains), *M. schleiferi* (1 of 30 strains), and *M. testaceum* (1 of 30 strains), but only 1 of 30 strains was identified as *M. foliorum*, whereas in our study, 7 of 50 strains belonged to this species.

In the present study, we describe the second and third *M. trichothecenolyticum* strains from humans, whereas to date, only one strain had been isolated from clinical specimens and

TABLE 3. Antimicrobial susceptibility patterns of *Microbacterium* strains ($n = 50$)

Antimicrobial agent	MIC (μg/ml)			No. (%) of isolates in the following category:		
	Range	50%	90%	Susceptible	Intermediate	Resistant
Cefotaxime	0.06–4	0.5	4	36 (72)	9 (18)	5 (10)
Ciprofloxacin	0.25–32	1	8	28 (56)	11 (22)	11 (22)
Doxycycline	≤0.06–>128	0.12	1	49 (98)	0 (0)	1 (2)
Erythromycin	0.03–>32	0.12	1	42 (84)	5 (10)	3 (6)
Gentamicin	0.25–32	1	4	45 (90)	3 (6)	2 (4)
Linezolid	0.25–2	1	2	50 (100)	0 (0)	0 (0)
Meropenem	0.06–4	1	4	50 (100)	0 (0)	0 (0)
Penicillin	0.12–2	1	2	39 (78)	11 (22)	0 (0)
Rifampin	≤0.015–32	0.12	4	44 (88)	1 (2)	5 (10)
Vancomycin	0.25–16	0.5	2	49 (98)	0 (0)	1 (2)

another from soil (16, 22). We report on the first two *M. hydrocarbonoxydans* strains and the first *M. oleivorans* strain from humans, whereas only one strain of *M. hydrocarbonoxydans* had been isolated from oil-contaminated soil and only one strain of *M. oleivorans* had been isolated from an oil storage cavern (17). *M. esteraromaticum* also has not been reported for humans but had been used as an aroma-producing bacterium (22), and *M. thalassium* had been isolated from soil (21). One *M. laevaniformans* strain (previously CDC group A-5 coryneform bacteria) isolated from blood had been described previously (7).

M. foliorum and *M. phyllosphaerae* cannot be distinguished phenotypically but were reported to share 12 differences in 1,480 bp (10 substitutions and 2 additional bases) of their 16S rRNA genes (4). All seven *M. foliorum* strains from the present study shared the following mismatches with the type strain of *M. phyllosphaerae* (AJ277840): at positions 45 to 47, CAG instead of GCC; at positions 49 and 50, GG instead of C and a deletion; at position 60, T instead of G; and at position 65, G instead of a deletion. The latter two *Microbacterium* species were isolated from phyllospheres and grasses and from decaying grasses of a litter layer (4). It is not unlikely that our patients acquired their *M. foliorum* and *M. phyllosphaerae* strains from grasses.

Strains 2121 and 2229 were identified as "*Microbacterium binotii*," a taxon that has been proposed as a new species by D. Clermont, S. Diard, L. Motreff, C. Vivier, F. Bimet, C. Bouchier, M. Welker, W. Kallow, and C. Bizet (unpublished data) (GenBank accession no. EF567306) but has not been validated so far. Strain 768 is a member of a presently undescribed *Microbacterium* species of which strain VKM Ac-1389, isolated from an interacting plant and nematode (GenBank accession no. AB0402070), is a representative. Finally, strain 2761 is a representative of the uncultured *Microbacterium* sp. clone YJQ-29, which had been isolated from a hot spring (GenBank accession no. AY569297).

It should be noted that, except for *M. resistens*, *M. hominis*, *M. paraoxydans*, and "*M. binotii*," all microbacteria were initially defined by strains that originated from the environment. It is not known at present whether microbacteria have a habitat in humans or are solely acquired from the environment.

The 50 *Microbacterium* isolates of our current series exhibited a level of susceptibility to penicillin (78%) similar to that of isolates reported in previous publications, about 80 to 90% of which showed susceptibility (7, 11). Interestingly, higher MICs for gentamicin (range, 1 to 64 μg/ml) were reported in previous studies of microbacteria (7, 11) than in the present study. The reason for this is unclear, but the different results might result from different MIC determination methods (microdilution in the present study versus agar dilution in the previous studies). The results of the present study correlated well with the antimicrobial MIC data obtained for six *M. paraoxydans* strains by use of Etest strips (15). In contrast to other coryneform bacteria and, in particular, other yellow-pigmented strains (6), for which rifampin usually has very low MICs, the MICs were slightly higher than usual (i.e., ≥0.12 μg/ml), although 88% of the *Microbacterium* strains were still fully susceptible. The present study reports only the third isolate of *M. resistens*, which shows the vancomycin resistance inherent in this species (9).

It is acknowledged that microbacteria are not frequently found as pathogens in human clinical specimens, as evidenced by the fact that just 50 isolates were collected in a reference center over a 5-year period.

Because of the heterogeneity of clinical isolates belonging to the genus *Microbacterium*, we strongly recommend to clinical microbiology laboratories that for yellow-pigmented gram-positive rods, of which *Microbacterium* is the most frequently encountered genus (6), almost-complete (i.e., >1,400-bp) 16S rRNA gene sequences should be determined in order to identify the strains, if indicated, to the species level, although the present study, together with the study of Laffineur et al. (15), indicates that *M. oxydans* and *M. paraoxydans* are the most frequently isolated microbacteria in human clinical specimens.

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