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Bruker Biotyper Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of *Nocardia*, *Rhodococcus*, *Kocuria*, *Gordonia*, *Tsukamurella*, and *Listeria* Species

Po-Ren Hsueh,[a,b] Tai-Fen Lee,[a,c] Shin-Hei Du,[a] Shih-Hua Teng,[d,e] Chun-Hsing Liao,[f] Wang-Hui Sheng,[b] Lee-Jene Teng[a,c]

Departments of Laboratory Medicine[a] and Internal Medicine,[b] National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan; Department and Graduate Institute of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan[c]; Department of Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, Taiwan[d]; Bruker Taiwan Co., Ltd., Taipei, Taiwan[e]; Department of Internal Medicine, Far Eastern Memorial Hospital, Taipei, Taiwan[f]

We evaluated whether the Bruker Biotyper matrix-associated laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) system provides accurate species-level identifications of 147 isolates of aerobically growing Gram-positive rods (GPRs). The bacterial isolates included *Nocardia* ($n = 74$), *Listeria* ($n = 39$), *Kocuria* ($n = 15$), *Rhodococcus* ($n = 10$), *Gordonia* ($n = 7$), and *Tsukamurella* ($n = 2$) species, which had all been identified by conventional methods, molecular methods, or both. In total, 89.7% of *Listeria monocytogenes*, 80% of *Rhodococcus* species, 26.7% of *Kocuria* species, and 14.9% of *Nocardia* species ($n = 11$, all *N. nova* and *N. otitidiscaviarum*) were correctly identified to the species level (score values, ≥2.0). A clustering analysis of spectra generated by the Bruker Biotyper identified six clusters of *Nocardia* species, i.e., cluster 1 (*N. cyriacigeorgica*), cluster 2 (*N. brasiliensis*), cluster 3 (*N. farcinica*), cluster 4 (*N. puris*), cluster 5 (*N. asiatica*), and cluster 6 (*N. beijingensis*), based on the six peaks generated by ClinProTools with the genetic algorithm, i.e., *m/z* 2,774.477 (cluster 1), *m/z* 5,389.792 (cluster 2), *m/z* 6,505.720 (cluster 3), *m/z* 5,428.795 (cluster 4), *m/z* 6,525.326 (cluster 5), and *m/z* 16,085.216 (cluster 6). Two clusters of *L. monocytogenes* spectra were also found according to the five peaks, i.e., *m/z* 5,594.85, *m/z* 6,184.39, and *m/z* 11,187.31, for cluster 1 (serotype 1/2a) and *m/z* 5,601.21 and *m/z* 11,199.33 for cluster 2 (serotypes 1/2b and 4b). The Bruker Biotyper system was unable to accurately identify *Nocardia* (except for *N. nova* and *N. otitidiscaviarum*), *Tsukamurella*, or *Gordonia* species. Continuous expansion of the MALDI-TOF MS databases to include more GPRs is necessary.

Aerobically growing Gram-positive rods (GPRs) constitute a very heterogeneous and extensive group of bacterial species (1, 2). Some of them, such as *Listeria monocytogenes* and *Nocardia*, *Kocuria*, *Rhodococcus*, *Gordonia*, and *Tsukamurella* species, are associated with severe community-acquired infections, including meningitis, bacteremia, pneumonia, brain abscesses, and skin and soft tissue infections (3–16). These organisms also cause various health care-associated infections, including catheter-related bacteremia (5, 9, 12–14). Some species, especially *L. monocytogenes*, can cause outbreaks (17, 18). Since these GPRs differ in the clinical spectrum of diseases they cause and in their susceptibilities to antimicrobial agents, it is important to precisely identify these isolates beyond the genus level for both therapeutic and infection control measures (9, 19–23).

In clinical microbiology laboratories, bacteria are traditionally identified using manual or automated phenotypic and biochemical methods. These methods are generally reliable for species-level identification but are often cumbersome and time-consuming to perform (1, 2). However, for some clinically important Gram-positive bacilli, performing these manual or automated methods is not always sufficient for identifying necessary organisms. Studies have clearly demonstrated that molecular methods, such as 16S rRNA gene sequencing analysis, are more accurate than conventional phenotypic methods for identifying unusual or rarely encountered pathogens, such as *Nocardia*, *Rhodococcus*, *Kocuria*, *Gordonia*, and *Tsukamurella* species (5, 9, 11–14).

Several commercially available matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems are now widely used in clinical microbiology laboratories for the rapid identification of commonly encountered bacteria and fungi (24–26). Numerous studies have compared the performances of MALDI-TOF MS systems with those of commonly used phenotypic testing and DNA sequence analysis techniques for identifying infrequently encountered bacterial pathogens; however, few studies have investigated the performance of MALDI-TOF MS for GPRs (1, 2, 27).

In this study, we assessed the performance of the Bruker Biotyper MALDI-TOF MS system for identifying a large collection of aerobically growing GPRs, including *Nocardia*, *Listeria*, *Kocuria* (which are coccoid but are always described as being rod-like), *Rhodococcus*, *Gordonia*, and *Tsukamurella* species, that were isolated from various clinical sources.

MATERIALS AND METHODS

Bacterial isolates. A total of 147 nonduplicate isolates of GPRs that were recovered from various sources from patients treated at the National Taiwan University Hospital (NTUH) were evaluated. These included 74 isolates of *Nocardia* species, 39 isolates of *L. monocytogenes*, 15 isolates of

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Address correspondence to Po-Ren Hsueh, hsporen@ntu.edu.tw.

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TABLE 1 Identification results and scores of 108 clinical isolates of *Nocardia*, *Kocuria*, *Rhodococcus*, *Gordonia*, and *Tsukamurella* species with the MALDI Bruker Biotyper database (DB 5627)[a]

	Identification results by 16S rRNA sequencing					
Isolate no. by genus (*n*)	Bacterial species	GenBank accession no.	Organism (best match)	Score	Organism (second best match)	Score
Nocardia species (74)						
1	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.957	*N. brasiliensis*	1.856
2	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.985	*N. brasiliensis*	1.748
3	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.904	*Lactobacillus paracasei* (NRI)	1.570
4	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.041	*N. brasiliensis*	1.852
5	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.967	*N. brasiliensis*	1.773
6	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.093	*N. brasiliensis*	1.938
7	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.97	*N. brasiliensis*	1.715
8	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.912	*N. brasiliensis*	1.754
9	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.048	*N. brasiliensis*	1.897
10	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.019	*N. brasiliensis* (NRI)	1.68
11	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.009	*N. brasiliensis*	1.807
12	*N. brasiliensis*	FJ172109.1	*Clostridium bifermentans* (NRI)[b]	1.455	*Lactobacillus paracasei* (NRI)	1.406
13	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.867	*N. brasiliensis*	1.691
14	*N. brasiliensis*	FJ172109.1	*N. brasiliensis*	1.872	*N. brasiliensis*	1.845
15	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.041	*N. brasiliensis*	1.845
16	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.995	*N. brasiliensis*	1.973
17	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.074	*N. brasiliensis*	1.923
18	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.068	*N. brasiliensis*	1.857
19	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	1.971	*N. brasiliensis*	1.867
20	*N. brasiliensis*	FJ172109.1	*Nocardia* sp.	2.033	*N. brasiliensis*	1.858
21	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.918	*N. cyriacigeorgica*	1.908
22	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.842	*N. cyriacigeorgica*	1.841
23	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.747	*N. cyriacigeorgica*	1.722
24	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.745	*N. cyriacigeorgica*	1.719
25	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.844	*N. cyriacigeorgica*	1.839
26	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica* (NRI)	1.601	*N. cyriacigeorgica*	1.551
27	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.816	*N. cyriacigeorgica*	1.73
28	*N. cyriacigeorgica*	GQ376180.1	*Pseudomonas jinjuensis* (NRI)	1.305	*Aromatoleum tolulyticus* (NRI)	1.256
29	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.712	NRI	1.611
30	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.924	*N. cyriacigeorgica*	1.862
31	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.806	NRI	1.577
32	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.787	NRI	1.607
33	*N. cyriacigeorgica*	GQ376180.1	*Staphylococcus lutrae* (NRI)	1.414	*Lactobacillus fructivorans* (NRI)	1.412
34	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.788	NRI	1.694
35	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica* (NRI)	1.661	*N. cyriacigeorgica* (NRI)	1.59
36	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	2.177	*N. cyriacigeorgica*	2.165
37	*N. cyriacigeorgica*	GQ376180.1	*Staphylococcus epidermidis* (NRI)	1.404	*Staphylococcus aureus* (NRI)	1.387
38	*N. cyriacigeorgica*	GQ376180.1	*N. cyriacigeorgica*	1.784	NRI	1.562
39	*N. farcinica*	GQ853065.1	*N. farcinica*	2.036	*N. farcinica*	1.956
40	*N. farcinica*	GQ853065.1	*Lactobacillus paracasei*	1.447	*Lactobacillus vini* (NRI)	1.391
41	*N. farcinica*	GQ853065.1	*N. farcinica* (NRI)	1.632	*N. farcinica* (NRI)	1.608
42	*N. farcinica*	GQ853065.1	*N. farcinica*	1.944	NRI	1.647
43	*N. farcinica*	GQ853065.1	*N. farcinica*	1.943	*N. farcinica*	1.929
44	*N. farcinica*	GQ853065.1	*N. farcinica*	1.773	NRI	1.615
45	*N. farcinica*	GQ853065.1	*N. farcinica*	1.749	NRI	1.668
46	*N. farcinica*	GQ853065.1	*N. farcinica* (NRI)	1.677	*N. farcinica* (NRI)	1.558
47	*N. asiatica*	GQ217495.1	*N. cyriacigeorgica*	2.256	*N. cyriacigeorgica*	2.245
48	*N. asiatica*	GQ217495.1	*N. cyriacigeorgica*	1.905	*N. cyriacigeorgica*	1.869
49	*N. asiatica*	GQ217495.1	*N. asiatica*	1.843	*Aeromonas veronii* (NRI)	1.44
50	*N. asiatica*	GQ217495.1	*N. asiatica*	1.859	*Lactobacillus paracasei* (NRI)	1.513
51	*N. nova*	GQ376190.1	*N. nova*	2.221	NRI	1.571
52	*N. nova*	AF430031.1	*N. nova*	2.245	NRI	1.513
53	*N. nova*	GQ376190.1	*N. nova*	2.327	NRI	1.378
54	*N. nova*	GQ376190.1	*N. nova*	2.342	NRI	1.479
55	*N. nova*	GQ376190.1	*N. nova*	2.076	NRI	1.3
56	*N. nova*	FJ172123.1	*N. nova*	2.437	NRI	1.638
57	*N. beijingensis*	GQ217493.1	*N. asiatica* (NRI)	1.576	*Lactobacillus paracasei* (NRI)	1.552
58	*N. beijingensis*	GQ217493.1	*N. asiatica* (NRI)	1.444	*Nocardia* sp. (NRI)	1.381
59	*N. beijingensis*	GQ217493.1	*Nocardia* sp. (NRI)	1.328	*N. asiatica* (NRI)	1.305
60	*N. puris*	GQ217500.1	*N. cyriacigeorgica* (NRI)	1.521	NRI	1.363
61	*N. puris*	GQ217500.1	*Aromatoleum alkani* (NRI)	1.393	*Streptomyces lavendulae* (NRI)	1.362
62	*N. puris*	GQ217500.1	*Lactobacillus aviarius* (NRI)	1.32	*Streptomyces hirsutus* (NRI)	1.306
63	*N. puris*	GQ217500.1	*Rhodococcus equi* (NRI)	1.42	*Lactobacillus paracasei* (NRI)	1.404
64	*N. puris*	AB097453.1	*Rhizobium radiobacter* (NRI)	1.418	*Xenorhabdus ehlersii* (NRI)	1.281
65	*N. otitidiscaviarum*	GQ376191.1	*N. otitidiscaviarum*	2.138	*N. otitidiscaviarum*	2.044

(Continued on following page)

TABLE 1 (Continued)

Isolate no. by genus (*n*)	Identification results by 16S rRNA sequencing					
	Bacterial species	GenBank accession no.	Organism (best match)	Score	Organism (second best match)	Score
66	*N. otitidiscaviarum*	GQ376191.1	*N. otitidiscaviarum*	2.132	*N. otitidiscaviarum*	1.778
67	*N. otitidiscaviarum*	GQ376191.1	*N. otitidiscaviarum*	2.114	*N. otitidiscaviarum* (NRI)	1.397
68	*N. abscessus*	GU471235.1	*Lactobacillus fuchuensis* (NRI)	1.323	*Nocardia* sp. (NRI)	1.294
69	*N. abscessus*	GU471235.1	*Clostridium tetani* (NRI)	1.277	*Lactobacillus amylovorus* (NRI)	1.274
70	*N. rhamnosiphila*	EF418604.1	*Agromyces rhizospherae* (NRI)	1.325	*Lactobacillus fructivorans* (NRI)	1.266
71	*N. asteroides*	AF430025.1	*N. asteroides*	1.777	NRI	1.580
72	*N. elegans*	GQ376166.1	*N. nova*	2.071	*Lactobacillus paracasei* (NRI)	1.499
73	*N. carnea*	GU433886.1	*Staphylococcus equorum* (NRI)	1.419	*Lactobacillus amylotrophicus* (NRI)	1.41
74	*N. transvalensis*	AB084446.1	*L. paracasei* (NRI)	1.494	*Aeromonas salmonicida* (NRI)	1.416
Kocuria species (15)						
75	*K. kristinae*	EU379300.1	*K. kristinae*	1.992	*K. kristinae*	1.911
76	*K. kristinae*	EU379300.1	*K. kristinae*	2.115	*K. kristinae*	2.085
77	*K. kristinae*	EU379300.1	*K. kristinae*	1.973	*K. kristinae*	1.931
78	*K. kristinae*	EU379300.1	*K. kristinae*	2.003	*K. kristinae*	1.944
79	*K. kristinae*	EU379300.1	*K. kristinae*	2.005	*K. kristinae*	1.808
80	*K. kristinae*	EU379300.1	*K. kristinae*	1.91	*K. kristinae*	1.823
81	*K. kristinae*	EU379300.1	*K. kristinae*	1.984	*K. kristinae*	1.93
82	*K. kristinae*	EU379300.1	*K. kristinae*	1.756	*K. kristinae*	1.747
83	*K. kristinae*	EU379300.1	*K. kristinae*	2.113	*K. kristinae*	1.93
84	*K. kristinae*	EU379300.1	*K. kristinae*	1.861	NRI	1.507
85	*K. kristinae*	EU379300.1	*K. kristinae*	1.95	*K. kristinae*	1.81
86	*K. kristinae*	EU379300.1	*K. kristinae*	1.717	NRI	1.667
87	*K. kristinae*	EU379300.1	*K. kristinae*	1.831	NRI	1.639
88	*K. marina*	KF306369.1	*K. marina*	1.955	NRI	1.507
89	*K. marina*	KF306369.1	*K. marina*	1.824	NRI	1.477
Rhodococcus species (10)						
90	*R. equi*	JQ965789.1	*R. equi*	2.134	*R. equi*	2.010
91	*R. equi*	JQ965789.1	*R. equi*	2.240	*R. equi*	2.202
92	*R. equi*	JQ965789.1	*R. equi*	2.087	*R. equi*	2.060
93	*R. equi*	JQ965789.1	*R. equi*	2.188	*R. equi*	2.073
94	*R. equi*	JQ965789.1	*R. equi*	2.255	*R. equi*	2.201
95	*R. equi*	JQ965789.1	*R. equi*	2.258	*R. equi*	2.204
96	*R. equi*	JQ965789.1	*R. equi*	2.278	*R. equi*	2.175
97	*R. equi*	JQ965789.1	*R. equi*	2.192	*R. equi*	2.141
98	*R. kroppenstedtii*	KC346296.1	*R. kroppenstedtii*	1.912	NRI	1.529
99	*R. kroppenstedtii*	KC346296.1	*R. kroppenstedtii* (NRI)	1.595	NRI	1.519
Gordonia species (7)						
100	*G. bronchialis*	HQ316182.1	*G. bronchialis* (NRI)	1.662	*Morganella morganii* (NRI)	1.543
101	*G. bronchialis*	HQ316182.1	*Morganella morganii* (NRI)	1.568	*Nocardia* sp. (NRI)	1.302
102	*G. bronchialis*	HQ316182.1	*G. bronchialis* (NRI)	1.588	*Morganella morganii*	1.482
103	*G. amicalis*	HQ842811.1	*G. rubripertincta* (NRI)	1.629	*G. rubripertincta* (NRI)	1.593
104	*G. amicalis*	HQ842811.1	*G. rubripertincta* (NRI)	1.465	*G. terrae* (NRI)	1.404
105	*G. amicalis*	HQ842811.1	*G. rubripertincta* (NRI)	1.436	*Staphylococcus chromogenes* (NRI)	1.406
106	*G. sputi*	FJ536318.1	*G. sputi*	1.984	*G. sputi* (NRI)	1.617
Tsukamurella species (2)						
107	*T. tyrosinosolvens*	JX154557.1	*Tsukamurella paurometabola* (NRI)	1.598	*Tsukamurella* sp. (NRI)	1.511
108	*T. tyrosinosolvens*	JX154557.1	*Tsukamurella* sp.	1.786	*T. inchonensis* (NRI)	1.622

[a] The Bruker Biotyper database (DB 5627) does not include *N. beijingensis*, *N. puris*, *N. rhamnosiphila*, *T. tyrosinosolvens*, or *G. amicalis*.
[b] NRI, not a reliable identification.

Kocuria species, 10 isolates of *Rhodococcus* species, seven isolates of *Gordonia* species, and two isolates of *Tsukamurella* species. All of these isolates were associated various clinical infections from patients who were treated at NTUH, and some of these infections were previously reported (5–9, 11–16, 20–22).

Bacterial identification. The 108 isolates of *Nocardia*, *Kocuria*, *Rhodococcus*, *Gordonia*, and *Tsukamurella* species were obtained from a clinical microbiology laboratory and were initially identified to the genus and/or species level by conventional methods. Due to the previously reported high misidentification rates by routine identification systems for these GPRs (5–9, 11–16, 20–22), these isolates were further confirmed to the species level using several molecular methods. For isolates of *Nocardia*, *Kocuria*, *Rhodococcus*, and *Tsukamurella* species, partial sequencing analysis of the 16S rRNA gene was performed using the primers *Noc*1 (5′-GCTTAACACATGCAAGTCG-3′) (positions 46 to 64, *Escherichia coli* numbering system) and *Noc*2 (5′-GAATTCCAGTCTCCCCTG-3′). The sequences were compared with published sequences in the 16S rRNA database. For *Nocardia* species, a second molecular method by sequencing the subunit A of SecA preprotein translocase gene (*sec*A1) was performed using a pair of primers, NsecA1 (5′-GTAAAACGACGGCCAGGACAGYGAGTGGATGGGYCGSGTGCACCG-3′) and NsecA1 R (5′-CAGGAAACAGCTATGACGCGGACGATGTAGTCCTTGTC-3′) (28).

Two molecular methods were used to identify *Gordonia* and *Tsukamurella* organisms to the species level (11, 12). The first molecular method was a previously described PCR restriction fragment length polymorphism (RFLP) identification scheme that used an amplified 440-bp segment of the 65-kDa heat shock protein gene (*hsp*65) performed using two primers, TB11 (5′-ACCAACGATGGTGTGTCCAT-3′) and TB12 (5′-CTTGTCGAACCGCATACCCT-3′) (12–14), and digestion by MspI and HinfI. The second molecular method was 16S rRNA gene sequencing using a pair of universal primers, 8FLP (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492RPL (5′-GGTTACCTTGTTACGACTT-3′) (12–14). The sequences (1,080 bp) obtained were compared with published sequences in the GenBank database using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov/blast). The accession number and closest match observed for each sequence were obtained. The criteria used to determine the closest match for identification using 16S rRNA sequencing were >99.5% sequence similarity to the unknown strain and the difference between the closest and next closest match having >0.5% divergence (29). The identification of *Gordonia* and *Tsukamurella* isolates to species level was based on >99.5% similarity by 16S rRNA gene sequencing and compatible RFLP results.

L. monocytogenes ($n = 39$) was identified using conventional identification methods, including hemolysis on sheep blood agar plates and the Christie-Atkins-Munch-Petersen (CAMP) reaction, as well as the API Coryne system (bioMérieux, Marcy l'Etoile, France). The serotypes of the isolates were determined by PCR, as previously described (15, 30).

Performance of the MALDI Bruker Biotyper. For analysis of the 147 GPRs by the Bruker MALDI-TOF Biotyper system, the samples were prepared as previously described (27). All isolates were incubated on Trypticase soy agar with 5% sheep blood (BAP) (Becton, Dickinson Microbiology Systems, Sparks, MD, USA) and incubated for 48 h at 37°C. Two to three colonies were transferred to a 1.5-ml screw-cap Eppendorf tube containing 300 μl of distilled water and then mixed with 900 μl of ethanol by pipetting. The suspension was pelleted by centrifugation at 13,000 rpm for 2 min, evaporated to dryness, and then reconstituted in 50 μl of 70% formic acid. After incubation for 30 s, 50 μl of acetonitrile (Sigma-Aldrich) was added. The suspension was then centrifuged at 13,000 rpm for 2 min. Next, 1.0 μl of the supernatant was applied to a 96-spot polished steel target plate (Bruker Daltonik GmbH, Bremen, Germany) and dried. A saturated solution of 1.0 μl of MALDI matrix (α-cyano-4-hydroxycinnamic acid [HCCA]; Bruker Daltonik GmbH) was applied to each sample and dried. Measurements were performed with the Bruker microflex LT MALDI-TOF MS (Bruker Daltonik GmbH) using FlexControl software with Compass Flex Series version 1.3 software and a 60-Hz nitrogen laser (337 nm wavelength). The spectra were collected in the linear positive mode in a mass range covering *m/z* 1,960 to 20,132. Spectra ranging from the mass-to-charge ratio (*m/z*) 2,000 to 20,000 were analyzed using Bruker Biotyper automation control and the Bruker Biotyper 3.1 software and library (database [DB] 5627 with 5,627 entries). Identification scores of ≥2.000 indicated species-level identification, scores of 1.700 to 1.999 indicated genus-level identification, and scores of <1.700 indicated no reliable identification. All isolates with discrepant identification results between the molecular and Bruker Biotyper methods were retested twice.

Cluster analysis by the Bruker Biotyper for six *Nocardia* species and *L. monocytogenes* isolates. A clustering analysis of the isolates was performed using ClinProTools 3.0 (Bruker Daltonics GmbH) (31). Fifty-five isolates belonging to six *Nocardia* species (*N. cyriacigeorgica* [$n = 18$], *N. brasiliensis* [$n = 20$], *N. farcinica* [$n = 8$], *N. puris* [$n = 5$], *N. asiatica* [$n = 4$], and *N. beijingensis* [$n = 3$] were identified by molecular methods, and 39 isolates of *L. monocytogenes*, including 10 isolates of serotype 1/2a, 17 of serotype 1/2b, and 12 of serotype 4b, were analyzed for specific signals by clustering analysis (31, 32). A model based on a genetic algorithm was created with the parameter of 15 as the maximum number of best peaks, 15 as the maximum number of generation, a mutation rate of 0.2%, and a crossover rate of 0.5%.

TABLE 2 Serotypes, cluster analysis, and score values of MALDI-TOF MS spectra of 39 clinical isolates of *L. monocytogenes*

Serotype	No. of isolates	Cluster[a]	No. of isolates with indicated score values by MALDI-TOF Bruker Biotyper		
			<1.7	1.7–1.999	≥2.0
1/2a	10	A	0	2 (1.889–1.902)	8 (2.058–2.321)
1/2b	17	B	0	0	17 (2.045–2.375)
4b	12	B	0	2 (1.82–1.98)	10 (2.069–2.437)

[a] See Fig. 2 for definition of clusters A and B based on cluster analysis by spectra generated by MALDI-TOF Bruker Biotyper.

RESULTS

Bacterial identification. Table 1 shows the identification results using molecular methods for the 108 isolates of *Nocardia*, *Kocuria*, *Rhodococcus*, *Gordonia*, and *Tsukamurella* species. A total of 14 different *Nocardia* species, two *Kocuria* species, two *Rhodococcus* species, three *Gordonia* species, and one *Tsukamurella* species were identified by various molecular methods. All isolates were identified to the species level, with >99.5% sequence similarity at the level of the 16S rRNA sequence. All isolates of *N. cyriacigeorgica*, *N. farcinica*, *N. beijingensis*, *N. otitidiscaviarum*, *N. puris*, *Kocuria marina*, *Gordonia bronchialis*, *Gordonia amicalis*, *Gordonia sputi*, *Rhodococcus equi*, and *Rhodococcus kroppenstedtii* exhibited 100% sequence similarity by 16S rRNA sequence analysis. All but three *Nocardia* species identified by 16S rRNA sequence analysis possessed compatible identification results by *sec*A1 gene sequencing (>95.0% [range, 97.0% to 100%] similarity). The three *Nocardia* species with discordant results by 16S rRNA and *sec*A1 gene sequencing included *N. transvalensis* (99.6% similarity by 16S rRNA) identified as *N. wallacei* (99.5% similarity by *sec*A1; GenBank accession no. JN562389.1), *N. beijingensis* (100% similarity by 16S rRNA) identified as *N. arthritidis* (97.6% similarity by *sec*A1; GenBank accession no. DQ360262.1), and *N. abscessus* (99.5% similarity by 16S rRNA) identified as *N. asiatica* (100% similarity by *sec*A1; GenBank accession no. JQ773453.1). As for the 39 *L. monocytogenes* isolates, 17 (43.6%) belonged to serotype 1/2b, 12 (30.8%) were serotype 4b, and 10 (25.6%) were serotype 1/2a (Table 2).

Identification by the Bruker Biotyper. The MALDI-TOF MS spectra of six selected species of GPRs are shown in Fig. 1A and 2A. Of the 22 species of GPRs evaluated in this study, *N. beijingensis*, *N. puris*, *Nocardia rhamnosiphila*, *Tsukamurella tyrosinosolvens*, and *G. amicalis* are not included in the Bruker Biotyper database (DB 5627) (Table 1). Of the 20 isolates of *N. brasiliensis*, only one isolate was identified as *N. brasiliensis* and had an identification score value of 1.872. The other 19 isolates were reported as *Nocardia* species. However, 18 of the second-matched organisms were correctly identified (score values, 1.680 to 1.938). Of the 18 isolates of *N. cyriacigeorgica*, 15 were identified as *N. cyriacigeorgica* (one isolate with a score value of 2.177 and 14 with score values ranging from 1.601 to 1.924). Three *N. cyriacigeorgica* isolates were misidentified as *Pseudomonas* and *Staphylococcus* species. The rates of consistency between species using molecular identification and best-matched species by the Bruker Biotyper among other *Nocardia* isolates were 100% (6/6) for *N. nova* (score values, all >2.0) and *N. otitidiscaviarum* (3/3; score values, all >2.0), 87.5% (7/8) for *N. farcinica* (one isolate with a score value of 2.036 and 6 with score values ranging from 1.632 to 1.944), and 50%

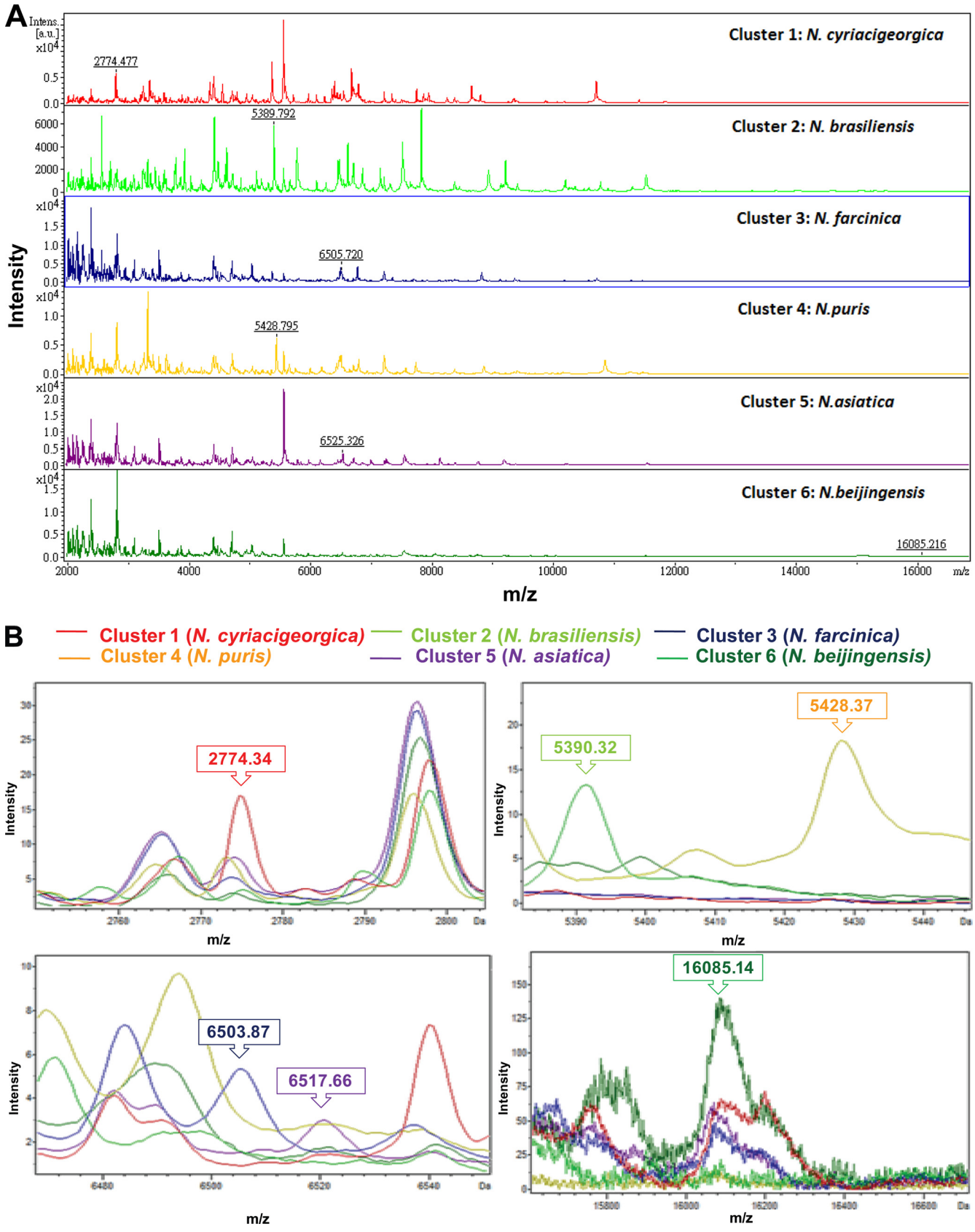
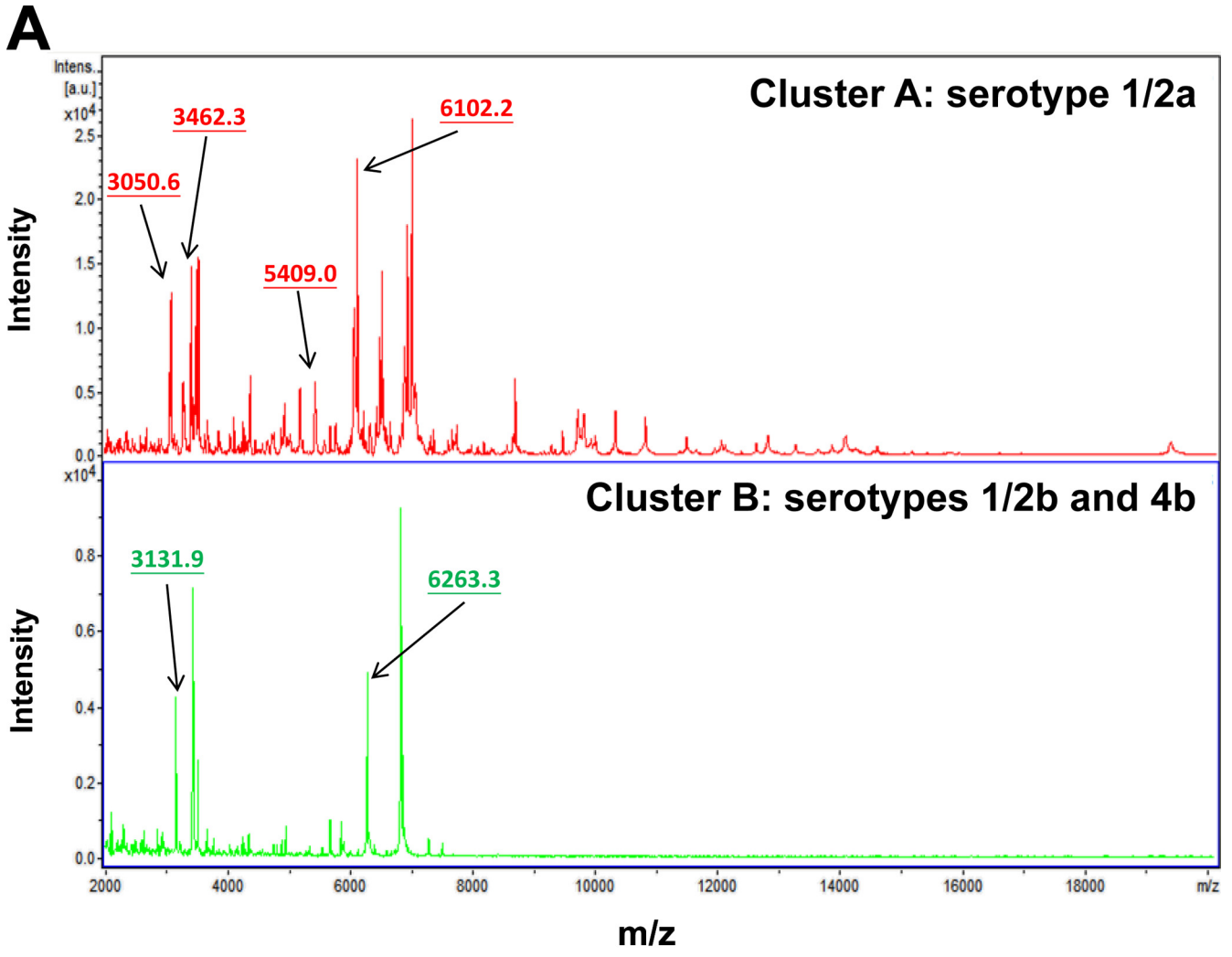


FIG 1 Clustering analysis of spectra generated by the Bruker Biotyper MALDI-TOF MS system for six common *Nocardia* species with poor identification performance or absence in the Bruker Biotyper database. (A) Six clusters of *Nocardia* species spectra, i.e., cluster 1, *N. cyriacigeorgica*; cluster 2, *N. brasiliensis*; cluster 3, *N. farcinica*; cluster 4, *N. puris*; cluster 5, *N. asiatica*; and cluster 6, *N. beijingensis*. (B) The six peaks (indicated by numbers and arrows) used to define the six clusters which were generated by ClinProTools with the genetic algorithm are *m/z* 2,774.477 (cluster 1), *m/z* 5,389.792 (cluster 2), *m/z* 6,505.720 (cluster 3), *m/z* 5,428.795 (cluster 4), *m/z* 6,525.326 (cluster 5), and *m/z* 16,085.216 (cluster 6). The absolute intensities of the ions are shown on the *y* axis, and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* values represent the mass-to-charge ratio.


A
Intens. [a.u.]
x10^4
Cluster A: serotype 1/2a
3462.3
6102.2
3050.6
5409.0
Intensity
Cluster B: serotypes 1/2b and 4b
3131.9
6263.3
Intensity
m/z
B
Cluster A: serotype 1/2a
Cluster B: serotypes 1/2b and 4b
5601.21
5594.85
Intensity
m/z
6184.39
Intensity
m/z
11199.33
11187.31
Intensity
m/z

(2/4) for *N. asiatica* (score values, 1.843 and 1.859). Of the 13 isolates of *Kocuria kristinae*, all were correctly identified, including four with score values of 2.003 to 2.115 and nine with score values of 1.831 to 1.992. Two *K. marina* isolates were identified to the species level but had score values of 1.824 and 1.955. For the 10 *Rhodococcus* isolates, eight *R. equi* isolates were all correctly identified to the species level (score values, >2.0), and the remaining two *R. kroppenstedtii* isolates were also identified correctly but with low score values. The Bruker Biotyper was unable to correctly identify the other *Nocardia* and *Tsukamurella* species. Although two *G. bronchialis* isolates were correctly identified by the Bruker Biotyper, the score values were low (<1.7). All *G. amicalis* isolates were identified as *Gordonia rubripertincta* (score values, <1.7).

All isolates of *L. monocytogenes* were correctly identified by the Bruker Biotyper, and 89.7% had high identification score values (≥2.0) (Table 2).

Clustering analysis of the spectra of six *Nocardia* species and *L. monocytogenes* by the Bruker Biotyper. A clustering analysis was performed for the spectra generated by the Bruker Biotyper results for 63 isolates of six common *Nocardia* species with poor identification performance or that were absent in the Bruker database. Six clusters of *Nocardia* species spectra, i.e., cluster 1 (*N. cyriacigeorgica*), cluster 2 (*N. brasiliensis*), cluster 3 (*N. farcinica*), cluster 4 (*N. puris*), cluster 5 (*N. asiatica*), and cluster 6 (*N. beijingensis*), were identified based on the six peaks generated by ClinProTools with the genetic algorithm, i.e., *m/z* 2,774.477 (cluster 1), *m/z* 5,389.792 (cluster 2), *m/z* 6,505.720 (cluster 3), *m/z* 5,428.795 (cluster 4), *m/z* 6,525.326 (cluster 5), and *m/z* 16,085.216 (cluster 6) (Fig. 1A and B). A model was used that included the six specific peaks that were analyzed by the 63 spectra of six clusters. The recognition capacity for the 63 spectra was 100% correct in each cluster. Two clusters of *L. monocytogenes* spectra, i.e., cluster 1 (serotype 1/2a) and cluster 2 (serotypes 1/2b and 4b), were also found according to the five peaks, i.e., *m/z* 5,594.85, *m/z* 6,184.39, and *m/z* 11,187.31 for cluster 1, and *m/z* 5,601.21 and *m/z* 11,199.33 for cluster 2 (Fig. 2A and B).

DISCUSSION

Our analysis of the performance of the Bruker Biotyper MALDI-TOF system for identifying aerobically growing Gram-positive rod-shaped bacilli that had been confirmed by molecular identification methods disclosed several important findings. First, 89.7% of *L. monocytogenes*, 14.9% of *Nocardia* species, 80% of *Rhodococcus* species, and 26.7% of *Kocuria* species were correctly identified to the species level (score values, ≥2.0) by the Bruker Biotyper system. However, none of the *Gordonia* and *Tsukamurella* isolates were correctly identified. Second, although *N. brasiliensis*, *N. cyriacigeorgica*, and *N. farcinica*, the most commonly encountered *Nocardia* species in clinical settings, were listed in the updated Bruker Biotyper database (DB 5627), the system was not able to accurately identify them to the species level (score values, ≥2.0; identification rate, 10.9% [5/46]). Of the 47 isolates of the three species, only 35 (74.5%) were identified as the same species, and all had identification score values of ≥1.7 (both best-matched and second-matched organisms). Finally, six peaks generated by the cluster analysis of MALDI-TOF MS spectra were found to be markers that could differentiate among the six commonly encountered *Nocardia* species. Moreover, five peaks were also demonstrated to be characteristic of two clusters of *L. monocytogenes* based on the two serotype groups (serotype 1/2a in cluster A and serotypes 1/2b and 4b in cluster B).

The analysis of the 468-bp region of the *secA1* gene has been demonstrated to be sufficient for the identification of all pathogenic species of *Nocardia* and may provide a more discriminative and precise method than 16S rRNA gene sequencing for identifying *Nocardia* isolates (28). In the present study, all but three of the *Nocardia* species identified by 16S rRNA gene sequencing were compatible with those identified by *secA1* gene sequencing. Further molecular methods are needed to solve the discrepant results by 16S rRNA and *secA1* gene sequencing for these three *Nocardia* species (28).

The Bruker Biotyper database used in this study (DS 5627) contains 32 different *Nocardia* species, 10 different *Kocuria* species, 27 different *Rhodococcus* species, seven different *Gordonia* species, two different *Tsukamurella* species, and six different *Listeria* species. Several species evaluated in the present study, including three *Nocardia* species (*N. beijingensis*, *N. puris*, and *N. rhamnosiphila*), *T. tyrosinosolvens*, and *G. amicalis* species were not listed in the database. Although the analyses of all isolates with discrepant identification results between the molecular and Bruker Biotyper methods were repeated twice, repeated analysis did not lead to better identification scores than analysis of single deposits. Our findings are consistent with those reported by Verroken et al. (27).

Farfour et al. (1) compared the accuracy of the Andromas MALDI-TOF MS system with that of a direct colony method to identify a set of 659 GPR isolates representing 16 bacterial genera and 72 species. Their bacterial collection included 32 isolates of *L. monocytogenes* ($n = 32$), 24 isolates of *Listeria* species ($n = 24$), and 46 isolates of *Nocardia* and other GPR bacterial species. In total, 98.5% of the non-*Listeria* GPR isolates were identified to the species level, and 1.2% were identified to the genus level. However, no isolates of *Kocuria*, *Gordonia*, or *Tsukamurella* species were evaluated in their study (1).

Of the 46 isolates of 12 different *Nocardia* species evaluated by Farfour et al. (1), 42 (91.3%) were identified to the species level by the Bruker Biotyper system, including six of seven isolates of *N. brasiliensis*, 10 of 11 isolates of *N. farcinica*, and all isolates of *N. nova* ($n = 5$), *N. abscessus* ($n = 4$), *N. beijingensis* ($n = 3$), *N. asteroides* ($n = 2$), and *N. otitidiscaviarum* ($n = 2$). In addition, Verroken et al. (27) reported that of 43 blind-coded *Nocardia* spectra after pretreatment by 30 min of boiling and ethanol-formic acid extraction of the organisms and alignment with the Bruker Biotyper database, 19 isolates (44%) were correctly identified, of which 10 (23%) were identified to the species level (log scores, ≥2) and 9 (21%) were identified to the genus level (log

FIG 2 Clustering analysis of spectra generated by the Bruker Biotyper MALDI-TOF system for three different serotypes of 39 *L. monocytogenes* isolates. (A) Two clusters of *L. monocytogenes* spectra, i.e., cluster A (serotype 1/2a) and cluster B (serotypes 1/2b and 4b). Peaks are labeled with arrows. (B) The five peaks (indicated by numbers and arrows) used to define the two clusters that were generated by ClinProTools with the genetic algorithm are *m/z* 5,594.85, *m/z* 6,184.39, and *m/z* 11,187.31 for cluster 1, and *m/z* 5,601.21 and *m/z* 11,199.33 for cluster 2. The absolute intensities of the ions are shown on the *y* axis and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* values represent the mass-to-charge ratio.

scores, ≥1.7 to 2) (27). The remaining 24 isolates (56%) yielded log scores of <1.7 and were thereby considered not identifiable (27). Of the 10 *Nocardia* species that were identified to the species level, nine were *N. nova* ($n = 15$), and one *N. brasiliensis* isolate was identified as *Nocardia* species, with a log score of 2.084 (27). The Bruker Biotyper failed to accurately identify all *N. brasiliensis*, *N. cyriacigeorgica*, and *N. farcinica* isolates. Of the remaining six *N. nova* isolates, one was identified as *Nocardia aobensis* (log score, 1.720) and five were identified as *N. nova*, with score values ranging from 1.87 to 1.988 (27). Their findings were partly in accordance with our results.

Correct identification to the genus level for *L. monocytogenes* isolates was reported by Farfour et al. (1) to be 100% (32/32) using the Bruker Daltonics system and by Rychert et al. (2) to be 76% (34/55) using the Vitek MS version 2.0 system (27). Farfour et al. (1) further demonstrated that with the exception of *Listeria grayi* isolates that were identified to the species level, all other *Listeria* isolates were identified to the genus level because of highly similar spectra. Barbuddhe et al. (33) demonstrated that the Bruker Daltonics MALDI-TOF system was an accurate system for the rapid identification of six *Listeria* species isolated from humans and various environmental sources. Of 86 *L. monocytogenes* isolates belonging to 15 different serotypes in their study, three lineages (I, II, and III) were identified and separated by two different peak pairs (5,590/11,179 Da and 5,597/11,193 Da) generated by MALDI-TOF. Furthermore, the MALDI-TOF lineages were in complete agreement with the pulsed-field gel electrophoresis lineage (33). They also found that the lineage of serotype 1/2a *L. monocytogenes* isolates (lineage II) differed from the lineage of serotypes 1/2b and 4b (lineage I). Their findings partially support our results, mainly because only isolates from human infection (bacteremia and/or meningitis) were evaluated in the present study.

Few studies have evaluated the accuracy of MALDI-TOF MS for the identification of *Kocuria*, *Gordonia*, *Rhodococcus*, and *Tsukamurella* species (1, 2). The consistent result of one bacteremic isolate of *K. kristinae* identified by MALDI-TOF MS and 16S rRNA gene sequencing analysis has been reported (10).

There are several limitations in this study. First, the number of isolates of several rare species tested in this study was small. For example, there are currently 86 recognized *Nocardia* species, >50% of which have been described during the last 10 years, and most have been implicated in various clinical infections in humans (19). Second, although several peaks were found to be characteristic of the six species of *Nocardia* and two clusters of serotypes of *L. monocytogenes*, further study is needed to establish in-house databases and to validate the accuracy of the performance of the Bruker Biotyper MALDI-TOF system.

In conclusion, our data suggest that the Bruker Biotyper MALDI-TOF system is ineffective for identifying *Nocardia* and other unusual GPRs (*Gordonia* and *Tsukamurella* species) because of the current database limitations. Taxonomic changes and characterization of novel species of GPRs are common. Therefore, it is necessary to continuously update the MALDI-TOF MS databases. Further expansion of the database with a larger number of recently described isolates of *Nocardia* species and other unusual GPRs is warranted.

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