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Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Identification of *Burkholderia pseudomallei*: Importance of Expanding Databases with Pathogens Endemic to Different Localities

Burkholderia pseudomallei is the causative agent of melioidosis, a serious disease endemic in southeast Asia. Accurate identification of *B. pseudomallei* is important, since treatment of melioidosis requires prolonged antibiotics to prevent relapse (9). Although *B. pseudomallei* differs greatly from other *Burkholderia* species in pathogenicity and epidemiology, identification of *B. pseudomallei* is often difficult, as phenotypic tests and even 16S rRNA gene sequencing may not offer adequate discrimination from related species, such as *B. thailandensis* and *B. cepacia* complex (BCC) (3–6, 8).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently emerged as a revolutionary technique for rapid bacterial identification at a low cost. Although MALDI-TOF MS has been shown to be useful for the identification of various nonfermenting Gram-negative bacilli, including some *Burkholderia* species (1), its application in identifying *B. pseudomallei* has not been explored.

Using 76 *Burkholderia* strains, we evaluated the performance of MALDI-TOF MS for the identification of *B. pseudomallei* (Table 1). All isolates were phenotypically identified by using the API 20NE system (bioMérieux) supplemented with biochemical methods. The identities of *B. pseudomallei* and *B. thailandensis* isolates were confirmed by *groEL* gene sequencing, and that of BCC by *recA* gene sequencing (6). All isolates were grown on horse blood agar at 37°C for 18 to 24 h and analyzed by the direct transfer method (2) (except that two BCC strains were analyzed by ethanol-formic acid extraction due to suboptimal results) in biosafety level II cabinets. Samples were processed in a MALDI-TOF MS spectrometer (Bruker Daltonik) with 1 μl of matrix solution (Bruker α-cyano). Spectra were obtained with an accelerating voltage of 20 kV in linear mode and analyzed within an *m/z* range of 2,000 to 20,000 Da. Spectra were analyzed with MALDI Biotyper 3.0 and Reference Library version 3.1.2.0 (Bruker Daltonik), which contained 41 *Burkholderia* main spectra (MSPs) comprising 26 species. Since *B. pseudomallei* is not represented in the Bruker database, 21 *B. pseudomallei* strains and, later, one *B. thailandensis* strain were added as reference strains.

The spectra obtained with *B. pseudomallei* compared to those obtained with other species are shown in Fig. 1. Using the Bruker database extended with *B. pseudomallei* reference strains, all isolates except for *B. thailandensis* were correctly identified (the score of the top match was ≥2.0, and the score of the second match was lower by ≥10%) (Table 1). Notably, the three *B. thailandensis* isolates were misidentified as *B. pseudomallei*. Further extension of the database with one additional *B. thailandensis* reference strain enabled the correct identification of two other *B. thailandensis* isolates. The misidentification of *B. thailandensis* by using the Bruker database is probably due to the inclusion of only one MSP from the species, which fails to cover intraspecies variability.

MALDI-TOF MS is potentially useful for accurate routine identification of *B. pseudomallei* and *B. thailandensis*. However, this requires optimization of the database by adding reference MSPs for *B. pseudomallei* and expanding the number of MSPs for *B. thailandensis*. While the present BCC isolates were correctly identified as BCC, species identification may require additional reference strains (1). Expansion of commercial databases with pathogens endemic in different localities is important to improve the usefulness of MALDI-TOF MS.

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TABLE 1 Identification of *Burkholderia* species by MALDI-TOF MS

Species	No. of strains of species	Origin or reference; strain(s) (no. of strains from origin)	No. of isolates identified using[a]: *B. pseudomallei* reference strains	No. of isolates identified using[a]: *B. pseudomallei* and *B. thailandensis* reference strains
B. cepacia complex	20	Clinical isolates ($n = 17$) BCCM/LMG collection; LMG1222, LMG14191, LMG20980 ($n = 3$)	20	20
B. gladioli	1	BCCM/LMG collection; LMG2216	1	1
B. pseudomallei	52	Clinical isolates ($n = 15$) Veterinary isolates ($n = 25$) Environmental isolates ($n = 12$)	52	52
B. thailandensis	3	7	0 (misidentified as *B. pseudomallei*)	3

[a] Isolates were identified by MALDI-TOF MS using extended database with *B. pseudomallei* reference strains or with *B. pseudomallei* and *B. thailandensis* reference strains in this study.

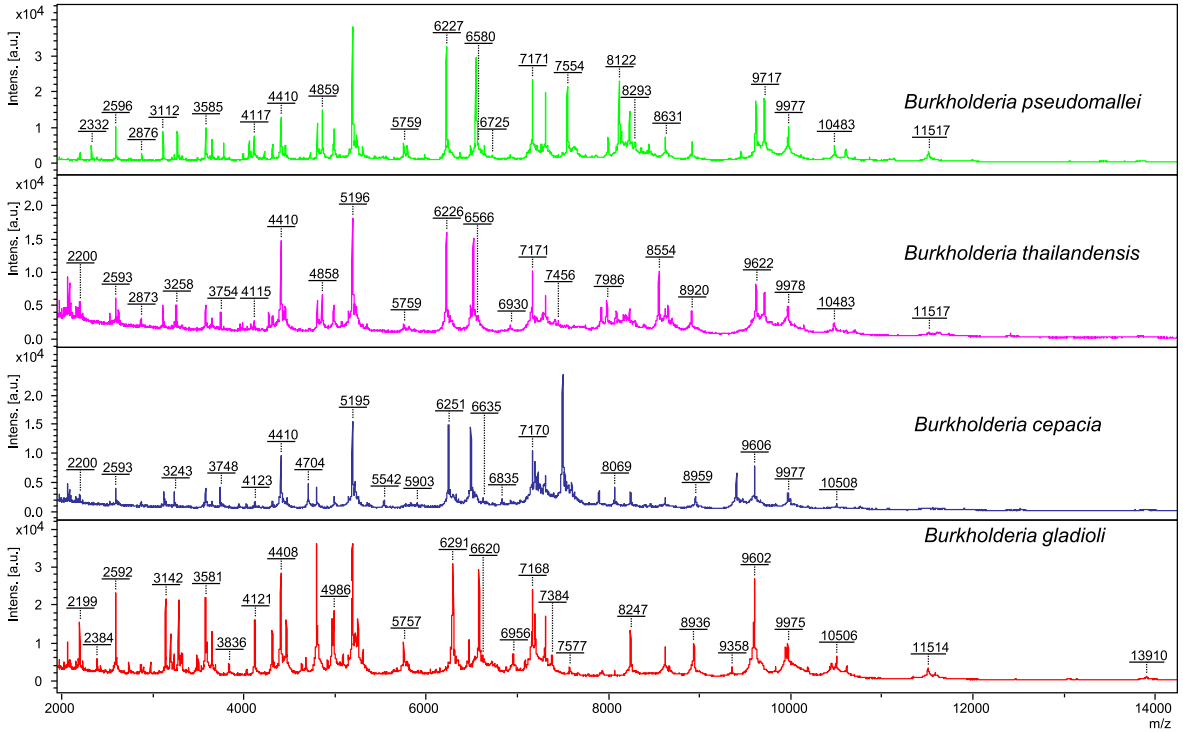

FIG 1 MALDI-TOF MS spectra of *B. pseudomallei* compared to those of other species. Intens. [a.u.], intensity in arbitrary units.

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