IDENTIFICATION OF BIOMARKERS IN *BURKHOLDERIA PSEUDOMALLEI* USING WHOLE-CELL MALDI-TOF MS

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ABSTRACT

Burkholderia pseudomallei is a pathogenic bacterium causing melioidosis, which is a serious human infectious disease with high mortality rates. Rapid identification and classification of *B. pseudomallei* from various sources could be advantageous for epidemiological tracking, medical prevention, and treatment of melioidosis. The whole-cell matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (whole-cell MALDI-TOF MS), a recently developed proteomics-based bacterial identification approach, could be employed for a rapid, robust, and accurate identification of *B. pseudomallei*. In this study, eleven isolates of *B. pseudomallei* collected from environmental and clinical sources were initially used for the whole-cell MALDI-TOF MS analyses; these included five environmental and six clinical isolates. The morphological appearance of most of these bacterial samples was purple, dry, and rough and was classified as morphotype I *B. pseudomallei*. By whole-cell MALDI-TOF MS approach, these bacteria were reliably identified as *B. pseudomallei*, based on five taxon-specific biomarkers and the identification scores obtained from BioTyper analysis. Cluster analysis of the environmental and clinical isolates revealed that six out of eleven isolates were correctly clustered according to their isolation sources. Upon further analysis using ClinProTools software, ten source-specific biomarkers were obtained according to their respective sources. Among these, six biomarkers (m/z 4056, 4214, 5814, 7545, 7895, and 8112 Da) were detected specific for the isolates of environmental source, and four (m/z 3658, 6322, 7035, and 7984 Da) were specific for clinical source. The clinical isolate-specific biomarkers were also observed in three laboratory-constructed *rpoS*, *ppk*, and *bpsI* mutants of clinical isolates that possessed single gene mutations in their respective gene locations, confirming their source of origin. Cluster analysis indicated that the wild-type PP844 reference strain, *rpoS*, *ppk*, and *bpsI* strains were separately dispersed. Thus the whole-cell MALDI-TOF MS method could distinguish *B. pseudomallei* isolates originated from either environmental or clinical sources as well as mutants bearing genetic changes. Moreover, subsequent ClinProTools analysis could identify the potential biomarkers specific to each of the three mutants. In this respect, a total of twelve candidate biomarkers were discovered, specific for each of the three mutant strains. These biomarkers included m/z 2721 and 2748 Da which were specific for the *rpoS* mutant, m/z 3150, 3378, and 7994 Da for *ppk*, and seven mass peaks at m/z 3420, 3520, 3587, 3688, 4623, 4708, and 5450 Da for *bpsI*. The present study is thus the first to establish and identify the source-specific and mutant-specific biomarkers for the identification and classification of *B. pseudomallei* based on whole-cell MALDI-TOF MS approach. These findings have also broadened the applicability of the whole-cell MALDI-TOF as a laboratory-based tool to rapidly, accurately, and reproducibly identify extensive libraries of the genetically modified bacteria.

KEY WORDS: *BURKHOLDERIA PSEUDOMALLEI* / BIOMARKERS / WHOLE-CELL MALDI-TOF MS

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