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Supplemental Materials

for

Teaching Microbial Identification with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and Bioinformatics Tools

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Theoretical overview

Central to the laboratory exercise is MALDI-TOF MS and interpretation of the generated mass spectra with bioinformatics tools for phylogenetic analysis. MALDI-TOF MS, originally developed for analysis of peptides and proteins in proteomics studies, is a soft ionization technique that, with the help of a laser and matrix, aid the ionization of various biomolecules from a cell sample without inducing molecular fragmentation (2). The sample is usually deposited as a thin film on a target plate and overlaid with a matrix - whose function is to facilitate the transfer of pulsed laser energy to the biomolecules. After ionization, the singly charged intact biomolecules – predominantly proteins as they constitute about 50% of a cell's dry mass – are separated by their mass-dependent velocities, in a field-free time-of-flight mass analyzer; at the end of which, a detector - scanning through a range of mass/charge (*m/z*) ratios - would count the absolute number of molecular ions at each *m/z* ratio to construct a mass spectrum (2). MALDI-TOF MS-based microbial identification operates on the premise that each species has a unique mass spectrum – both in terms of *m/z* ratios as well as their relative intensities. The postulation has been verified by the discovery of conserved biomarker ion peaks – across a variety of sample preparation conditions - within the mass range from 2000 to 20000 Daltons (Da), and the confirmation that many biomarker ions are ribosomal proteins – the most abundant group of basic proteins with moderate hydrophobicity in a microbial cell (2, 3). Additionally, since ribosomal proteins have been known to be highly conserved; with small changes in the genetic sequence encoding them representative of the divergence in evolutionary trajectories within and between species (5, 6), the MALDI-TOF MS approach has a firm theoretical basis that affords its data to be used in deciphering phylogenetic relationships amongst microorganisms.

Currently, two approaches are available for analyzing the generated mass spectra: (i) pattern recognition – that is, comparison of mass spectra against a reference database and species identification made based on a statistical similarity score; an approach utilized in commercial MALDI-TOF MS microbial typing software packages (1), and (ii) biomarker ion-based proteome database search approach (3, 7). Given the lack of an open-source MALDI-TOF mass spectra reference database for microorganisms and the desire to integrate practical aspects of bioinformatics search tools into the laboratory exercise, the proteome database search approach (using the Microbial Genome Database for Comparative Analysis), http://mbgd.genome.ad.jp/) was adopted in this exercise.

Practical tips for exercise implementation

Though conceptually simple, difficulties may arise during the exercise's implementation. Specifically, working on native water samples with unknown microbial population, it is anticipated that mass spectra with few characteristic mass peaks might be obtained due to: (i) lack of sufficient cells from slow-growing microorganisms; (ii) difficulty in ionizing biomolecules from spores (5); and (iii) thick cell wall structure of Gram-positive bacteria preventing the ionization of cytoplasmic proteins (5). Additionally, since not all microorganisms present on the planet have had their genomes sequenced, there may be instances where isolated microorganisms might not be identifiable via the approach.

Exercise implementation necessitates securing MALDI-TOF mass spectrometer instrument time – most cost-effectively on a pay-per-use basis, thus saving significant capital investment. Specifically, MALDI-TOF mass spectrometers should be available in chemistry, biological sciences, or engineering departments. As the exercise's objective is to demonstrate the concepts and methods underlying mass spectrometry-based microbial identification - and not the generation of research data - a MALDI-TOF mass spectrometer with mass resolution of about 500 parts-per-million (ppm) would suffice (4). Due to the instrument's complexity, a graduate student should be available to explain and guide the students in its use. This activity can be part of a laboratory sequence complementing a regular course on bioinformatics or analytical instrumentation, but the significant investment of time – on the part of students due to the cultivation of slow-growing environmental microorganisms on agar – renders it more suitable as a week-long laboratory exercise - at the end of which, a group report is deliverable together with individual *viva* to assess the students' understanding of key concepts pertinent to the experiment. Finally, modular in design, the exercise's content and activities can be flexibly tuned to cater to the specific learning needs of students.

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