MICROBIAL FORENSICS USING MALDI-TOF MS SPECTRAL FINGERPRINTS

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Homeland security, forensics, and medical diagnostics need rapid and efficient methods to detect and identify biological samples ranging from bacteria and viruses to toxic contaminants in air, water and food. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can analyze femtomole (10^{-15} M) concentrations of peptides in 10 minutes and is the ideal instrument to analyze limited environmental samples. Rapid detection and identification of biological samples without extensive manipulation (e.g., PCR, sequencing, protein digestion, and 2D gel electrophoresis) will help to protect human and environmental health.

E. coli ATCC 15597*, B. subtilis* var. *globigii*, ATCC 9372, *B. subtilis* ATCC 6051A, *B. subtilis* ATCC 82, *B. cereus* ATCC 2 and ATCC 11778, and *B. thuringiensis* ATCC 10792 were examined by growing colonies on agar, transferring to liquid media, and growing until they reached a pre-specified growth stage (logarithmic, stationary, decay). Cell pellets, generated by centrifugation, or cell colonies, grown on agar, were washed and resuspended in NH_4Cl, and mixed with a ferulic acid matrix. One µl of this solution was analyzed by MALDI-TOF MS and each spectrum was the average of 100 laser shots. The results showed that MALDI-TOF MS produces distinguishable spectral fingerprints of the proteomes of different bacterial genera, species, and strains using whole cells and eliminating the protein digest and 2D gel electrophoresis steps. Individual colonies grown on agar or in broth produced very similar results. Freezing at –20°C for 15 weeks had no effect. The growth stage did change the spectral fingerprint, but characteristic identifying mass peaks remained in all stages. Reproducibility of spectra from the same bacteria grown under similar conditions was good.

Our conclusions are that MALDI-TOF MS can distinguish between different bacterial genus, species, and strains quickly (10 minutes) and efficiently at femtomole levels and can be used by first responders to enable them to rapidly react to these dangers and hopefully, prevent any damage. Detection of low levels of microorganisms has become extremely important with mounting concerns regarding biological warfare and contaminated food, soil, air, and water.