Molecular fingerprinting of 47 Clostridium botulinum isolates by Focal-Plane-Array Fourier Transform Infrared (FPA-FTIR) spectroscopy

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Recent advances in focal plane array Fourier transform infrared (FPA-FTIR) instrumentation and data analysis techniques have provided new opportunities for bacteria identification down to the strain level, with unprecedented throughput and sensitivity. In the present study, 47 isolates of *C. botulinum* have been differentiated based on their infrared (IR) spectra recorded with an FPA-FTIR imaging system equipped with an infrared microscope and a 16 × 16 mercury-cadmium-telluride FPA detector. Over 150,000 infrared spectra were acquired from the 47 isolates after growth on three different media. Group I (proteolytic) and Group II (non-proteolytic) strains each exhibited unique spectral features that allowed their differentiation from each other by FPA-FTIR spectroscopy. Spectral regions corresponding to the variations between serotypes have been identified, allowing for the complete taxonomic separation of all *C. botulinum* isolates. Comparison of the infrared spectra of isolates grown on the three different media established that the use of a consistent growth medium is a prerequisite for successful differentiation by IR spectroscopy. The results demonstrate that FPA-FTIR spectroscopy has the potential for the rapid identification of *C. botulinum* strains from a few hundred intact cells in less than two minutes with minimal sample preparation. Comparison between the accuracy of the IR-based method and that of the pulse-field gel electrophoresis (PFGE) analysis will be discussed.