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Main topic : Other Topics

DISCRIMINATION OF VACCINE BACILLUS ANTHRACIS STRAINS FROM VIRULENT ONES BY IR BIOTYPER: PRELIMINARY DATA

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Introduction
Fourier-transform infrared spectroscopy (FTIR) is a recently technique used to characterize microorganisms according to strain-specific absorbance patterns in the infrared spectrum (1). This method is quick, inexpensive, relatively easy and provides relevant information about the biomolecular contents of microorganisms, including lipids, carbohydrates, proteins, and nucleic acids, derived from IR spectra. In particular, IR Biotyper (IRBT) based on FTIR technology was launched in 2017 as a very promising system in the field of microbial strain typing with multiple reported successful applications (1,2).
In this study, we evaluated the discriminatory power of IRBT to differentiate *Bacillus anthracis* vaccine strains from virulent strains isolated in field.

Methods
A total of 14 *B.anthracis* strains, including 2 vaccine strains, Carbosap and Sterne 34F2, and 12 *B.anthracis* virulent strains isolated during anthrax outbreaks in Italy, were tested in this study. For IRBT analysis, isolates were grown on TSA at 37°C for 24 hours. 15 ?L of bacterial suspension was spotted onto the IRBT silicon sample plate and dried at room temperature. Three replicates were prepared for each sample. For each run, quality control was performed with the Infrared Test Standards (IRTS 1 and 2) from the IR Biotyper kit.
Spectra were recorded in transmission mode in the spectral range of 4,000–500 cm^{-1} (mid-IR) using an IR Biotyper spectrometer (Bruker Daltonics GmbH). The spectra were then acquired by OPUS v 8.2.28 software (Bruker Daltonics GmbH). Dendrograms were constructed by IR Biotyper v 3.1.2 sofware (Bruker Daltonics GmbH) using Euclidian distances and the average linkage clustering method.

Results
The data obtained by the dendrogram (Fig.1) show that the Sterne strain, which lacks the pXO2 plasmid, is located on a different branch compared to the other virulent *B.anthracis* strains. This is presumably related to the fact that the bacterial cell surface structures of this acapsulated strain are clearly different from the other strains.
Furthermore, in the group of strains owning both plasmids, the Carbosap, that is a vaccine strain naturally attenuated, stands separately, suggesting that probably it has a different biomolecular structure compared to the pathogenic strains.
The Carbosap strain, even if is generated from the same node than the pathogenic strains, is located in a separate cluster, while the Sterne strain appears to be located on a completely different separate cluster, not common with the other strains.

Conclusions
IR Biotyper was developed as an easy, fast and cost-effective method to typing different microorganisms belonging to the same species, with the main objective of get epidemiological data in a shorter time. The present study, for our knowledge, is the first one that evaluates the ability of IRBT to discriminate different *B.anthracis* strains, even if, at the moment, only a few samples have been analyzed to provide relevant feedback on the usefulness of this new diagnostic tool. On the basis of these preliminary data, IRBT successfully distinguished *B.anthracis* vaccine strains from pathogenic ones. Our next goal is to test a larger number of *B.anthracis*, different each other by the genotypic point of view.