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Application of Fourier transform infrared spectroscopy to discriminate *B. anthracis* from *B. cereus* sensu stricto

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BACKGROUND

Due to the long phases of metabolic dormancy in the spore form which led to a slow evolution, species belonging to the *Bacillus cereus* group are genetically and phenotypically highly homogeneous. In order to distinguish *B. anthracis* from *B. cereus* sensu stricto (s.s.), the two main species of the group, that have an important impact on human and animal health, it's necessary to resort to sophisticated biomolecular methods. Although extremely effective, these techniques require long times, high costs and highly qualified personnel.

Fourier transform infrared spectroscopy (FTIRS) is a diagnostic technique (Fig.1) historically used in the chemical field, and only recently applied in the microbiological field for the characterization of bacterial strains in relation to the specific composition of their lipid, protein, and polysaccharide components (1,2). For each bacterial strain it is possible to obtain a unique absorption spectrum that represents the fingerprint obtained based on the components of the outer cell membrane (3). In this study, Fourier-transform infrared spectroscopy (FTIRS) has been applied to discriminate the two pathogenic species of the *B. cereus* group, *B. anthracis* and *B. cereus* s.s.

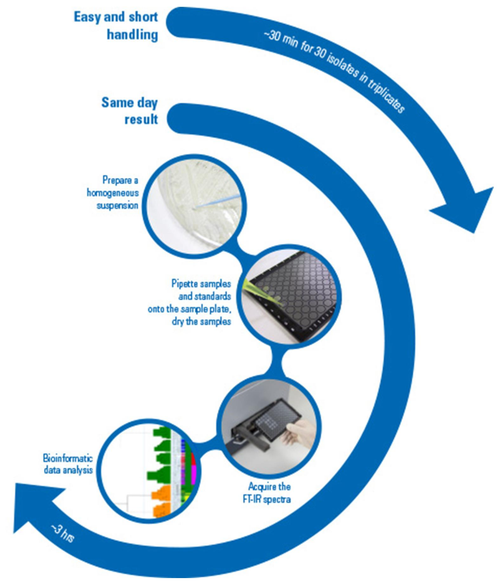

Figure 1. Workflow IR Biotyper analysis

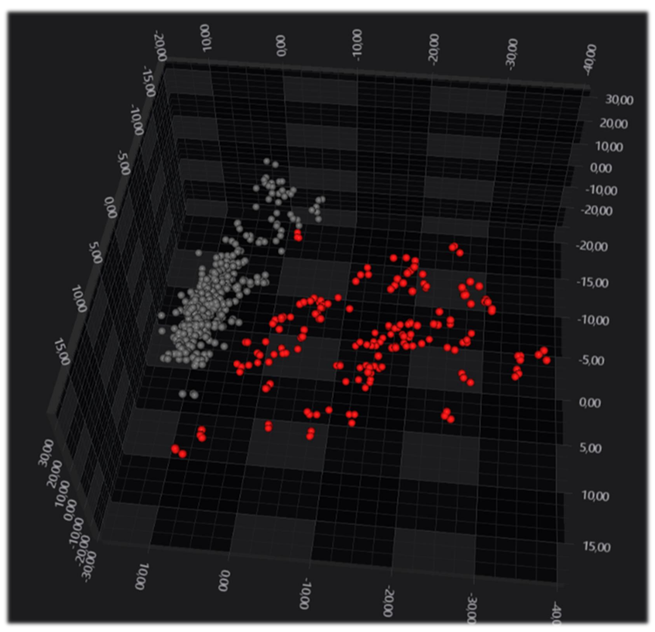

Figure. 2. 2D Scatterplot showing the IRBT clustering of *B. anthracis* (grey) from *B. cereus* s.s. (red)

MATERIALS

A total of 132 previously characterized *B. anthracis* (n=77) and *B. cereus*.s.s. (n=52) isolates were included in this study. The strains were isolated from clinical or environmental samples in Italy, collected and tested at the Anthrax Reference Institute of Italy at the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata. Furthermore, n=3 *B. anthracis* vaccine strains were also included in the study. For the IRBT analysis, a loopful of pure bacterial culture was collected and resuspended in 100 µL of sterile H_2O dd and incubated at 98°C for 30 minutes (to inactivate the vegetative and spore-forming forms). Subsequently, 50 µL was taken and added to 50 µL of 70% (vol/vol) ethanol in the appropriate tubes provided by the IR Biotyper kit (Bruker Daltonics, Germany), spitting to obtain a homogeneous suspension. Then, 15 µL was placed on the IRBT silicon 96-well plate and allowed to dry at room temperature. For each sample, three replicates were set up and, for each run, quality control was performed with the standards, IRTS 1 and 2, provided by the IR Biotyper kit (Bruker Daltonics, Germany). Spectra were acquired in transmission mode in the spectral range of 4,000-500 cm^{-1} (mid-IR) using a Biotyper IR spectrometer (Bruker Daltonik, Germany) and OPUS software Bruker Daltonik, Germany). The IR Biotyper (V3.1) software (Bruker Daltonik, Germany) was used to process and analyze the acquired spectra. Exploratory data analysis was performed by Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA).

RESULTS

PCA/LDA shows that *B. anthracis* and *B. cereus* s.s. form two well separated clusters (Fig.2). Additionally, the three *B. anthracis* vaccine strains are distinguishable from *B. anthracis* field strains (Fig.3).

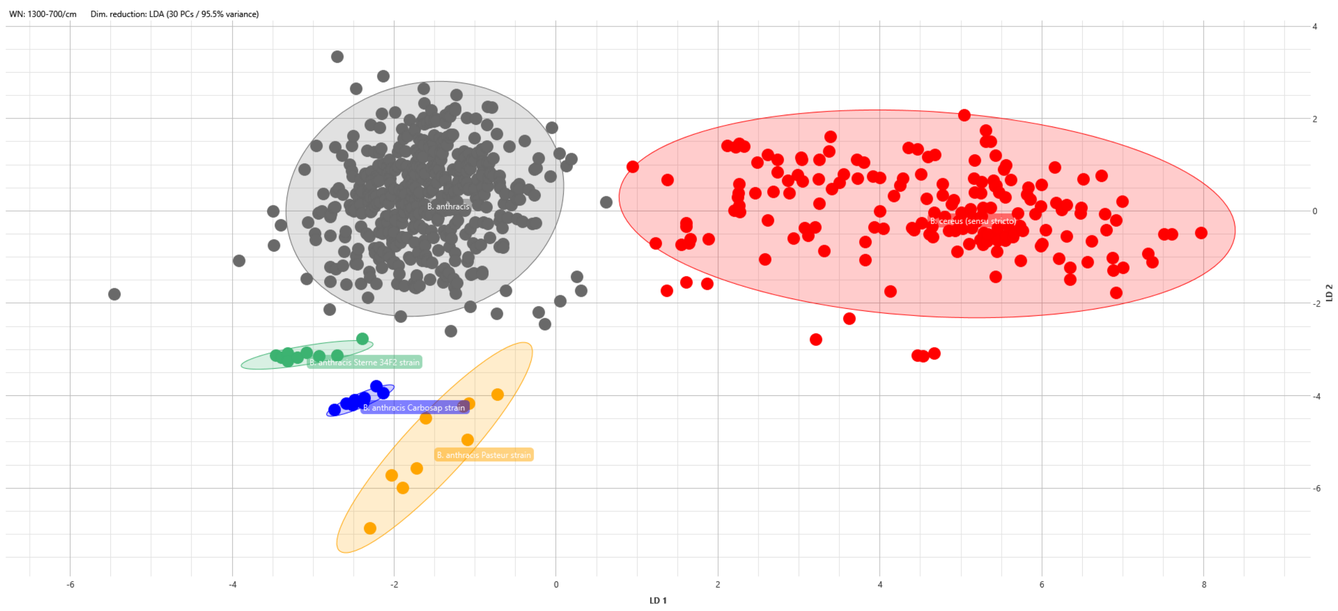

Figure 3. 2D Scatterplot showing the differentiation between *B. anthracis* vaccine and field strains

CONCLUSIONS

In this study, the IR Biotyper® system was successfully applied for the first time as experimental analytical tool for the discrimination of *B. anthracis* and *B. cereus* s.s. representing an innovative technology in the field of microbiology. Future studies expanding the investigation to the other species belonging to the *B. cereus* group are necessary, to assess the possibility to propose FTIRS as a novel routine method for a prompt identification of bacterial strains.

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REFERENCES

1. Li X, et al. 2022. Evaluation of IR Biotyper for Lactiplantibacillus plantarum Typing and Its Application Potential in Probiotic Preliminary Screening. Front Microbiol.;13:823120.
2. Baker MJ, Trevisan J, Bassan P, et al. 2014. Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc.;9(8):1771-1791.
3. Cordovana M, Mauder N, Kostrzewa M, et al. 2021. Classification of Salmonella enterica of the (Para)Typhoid Fever Group by Fourier-Transform Infrared (FTIR) Spectroscopy. Microorganisms;9(4).

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