Biomarkers for rapid differentiation of *Bacillus anthracis from B. cereus* group non-anthracis strains using matrix-assisted laser desorption/ionization - time of flight mass-spectrometry (MALDI-TOF).

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Purpose

- The current MALDI-TOF databases (Biotyper Bruker) including standard and security-relevant (SR) peak spectra cannot reliably differentiate between species within the *Bacillus cereus* group.
- A reliable and rapid identification of *B. anthracis* with MALDI-TOF could offer a critical time advantage in the clinical management of human anthrax including bioterror attacks.

Clinical case

- A 61-year old woman, living in Switzerland, visited her family in Turkey.
- When cows of neighbour unexpectedly died, she helped butchering the cows.
- After returning to Switzerland, she presented with a skin lesion (**Figure 1.**).
- Culture of a wound swab showed typical *B. anthracis* morphology.
- *B. anthracis* was confirmed by PCR in the Swiss reference center for Anthrax.

→ First confirmed human anthrax case since 1991 in Switzerland!

- Treatment with ciprofloxacin (500mg BID) was initiated with rapid success.

Figure 1. Skin lesion during treatment

MALDI-TOF technology

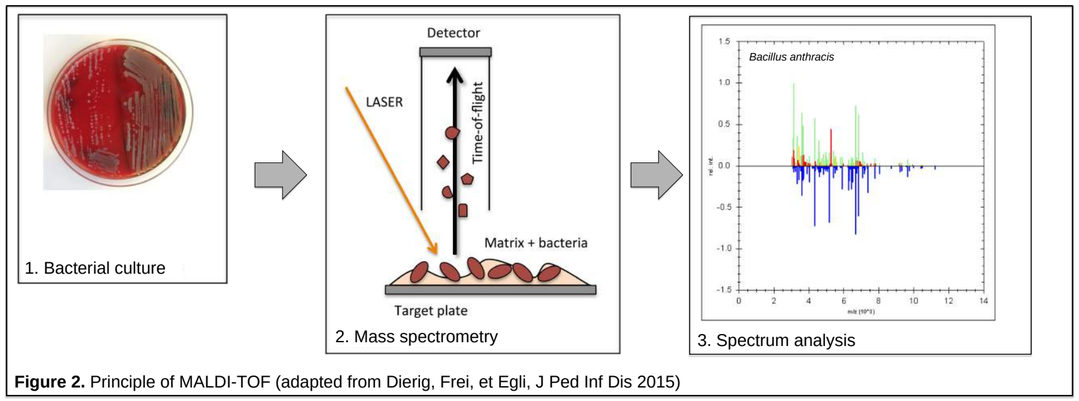

Figure 2. Principle of MALDI-TOF (adapted from Dierig, Frei, et Egli, J Ped Inf Dis 2015)

Methods

- MALDI-TOF full protein extraction protocol was performed in BS3 lab (**Figure 2.**) using ethanol (70%) washing, followed by formic acid and acetonitril treatment. (Egli et al., Plos one 2015, in press)
- Mass peaks of *B. anthracis* (n=1) were compared to *B. cereus* group non-anthracis isolates (n=6).
- Peaks with an arbitrary value of >1000 intensity units were considered for evaluation.
- List of species specific peaks was generated (see results).

Results

- A total of 155 peak positions were initally identified. Signal to noise ratio of >10 reduced the number of unspecific peaks.
- Using a principal component analysis the *B. anthracis* isolate could be easily determined (**Figure 3 A/B**).

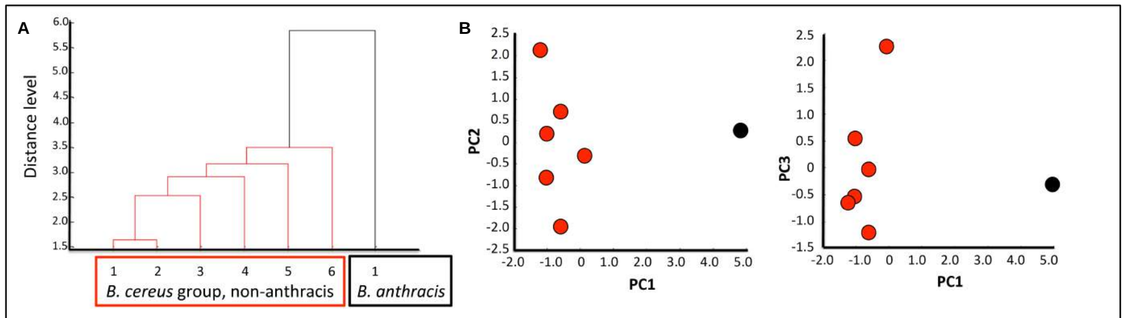

Figure 3. A. Dendrogram using a standard principal component analysis (PCA) algorithm **B.** Principal component 1 vs. 2 vs. 3.

- Detailed spectrum analysis reveiled specific peaks within the *B. cereus* group (**Figure 4A/B**).
- *B. cereus* group non-anthracis peaks: 3885, 5217, 6265, 6795, 7368, 9646, and 9990 dalton (m/z).
- *B. anthracis* specific peaks: 3652, 4551, 6679 dalton (m/z).

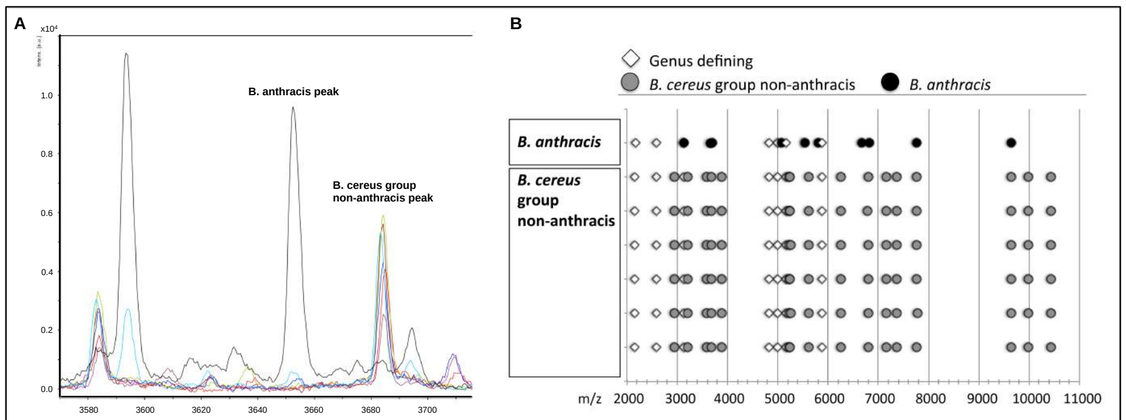

Figure 4. A. Peak spectrum of *B. anthracis* (black line) and *B. cereus* group non-anthracis (colored lines) **B.** Peak list of isolates

Summary and Conclusions

- The identified MALDI-TOF peaks could be used as specific biomarkers to differentiate strains within the *B. cereus* group.
- Previously, Lasch et al. identified a specific peak for *B. anthracis* at 6679 Da (Lasch et al., App En Micro 2009).
- Rapid and reliable identification with an improved database and/or algorithm may be very important for identification of critical bioterror-associated pathogens.

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