Abstract

- Microbial attribution generally requires fundamental knowledge of the natural variation or mutation rate across organisms in varied environments.
- Genomic data not providing all answers to single spore attribution.
- PlusALL-72 hours compared with case where Ca excluded from sporulation media below:

Mass Spectrometry

- Large Geometry Secondary Ion Mass Spectrometry (LG-SIMS) was used to obtain single Bacillus thuringiensis spore profiles:
  - For common elemental signatures Mg, Ca, and Fe at a dynamic signal range of \( \times 10^4 \) orders of magnitude.
  - Removal of Ca from the sporulation conditions resulted in a significant reduction in Ca signal with respect to Mg.
  - Efforts underway to establish measurement/spore variability to provide context for measurement values of unknown elemental concentrations within spores.

Spore Preparation

- B. thuringiensis sporulation procedure:
  - PGSM solution prepared by combining Bacto-peptone, glucose, MgSO_4, MnSO_4, MgSO_4, FeSO_4 and CaCl_2 prepared separately to supplement PGSM (Table 1).
  - PGSM solution autoclaved for 20 min at 121 °C.
  - Individual positive ion isotopes of 24Mg, 44Ca, and 56Fe at a dynamic signal range of \( \times 10^4 \) orders of magnitude.
  - Enough individual spore analyses to place the natural variability of a single spore within spore profiles.

Single Spore Profiles

- Single 1-µm bacterial spores consistently analyzed with LG-SIMS:
  - Elemental signatures detected over 4 orders of signal magnitude.
  - Key developments towards establishing natural spore variability.

Elemental Ratios

- Elemental ratios measured using total counts throughout full profiles:
  - Ca/Mg ratios consistent; a bit less so for Ca/Fe and Mg/Fe.
  - Dramatic difference in Ca ratios when Ca not in sporulation media.

Conclusions

- LG-SIMS method being established for single bacterial spore analysis.
  - Natural variability of elemental ratios being measured to provide context.
  - Preliminary data gives confidence for quantification.
  - Work towards single spore analysis in dirtier samples; other elements lacking.