

# Rapid Detection and Discrimination of *Bacillus* Species Using IMS-SERS

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## ABSTRACT

*Bacillus* species are gram positive, spore-forming bacteria which include *B. anthracis*, a class A bioterrorism agent according to the Centers for Disease Control and Prevention. Because of anthrax's high toxicity, it is important to be able to quickly and accurately detect *B. anthracis* spores. Immunomagnetic separation (IMS) has previously been shown to capture toxins from complex media, such as food. Subsequent detection with Surface-Enhanced Raman Spectroscopy (SERS) has been shown to be highly sensitive for detection and discrimination of other toxins. This research aimed to discriminate three different *Bacillus* species and establish a procedure based on IMS-SERS that can detect *B. anthracis* in under 20 minutes.

We found that the *Bacillus* species could be differentiated when SERS spectra were analyzed using principal component analysis as the discrimination between cell states could be achieved with hierarchical cluster analysis. The limit of detection was  $\sim 2 \times 10^7$  spores/mL due to the difficulty to optically locate the spores under a Raman microscope. In order to achieve a lower limit of detection, the spores were treated with dodecylamine, which rapidly digests the spore coat, which follows IMS, and adds little time to sample preparation. Upon digestion, the spore biomarker dipicolinic acid (DPA) is released. DPA comprises  $\sim 8$  to 15 % of the dry weight of bacterial spores, and is a highly Raman-active compound. A lower limit of detection at  $2 \times 10^3$  spores/mL was achieved due to the correlation of DPA and *B. anthracis*' viable spore count. Based on published toxicological data, detection at these limits is sufficient for protecting the public in the case of deliberate contamination of food.

## INTRODUCTION

*Bacillus anthracis*

- ❖ Class A potential bioterrorism agent
- ❖ Gram positive endospore forming bacteria
- ❖ Spores were sent via the Postal Service in 2001
- ❖ Spores are very resistant to heat, freezing, light, & some chemicals

IMS-SERS

- ❖ Immunomagnetic separation (IMS) is used to capture the spores out of complex media, such as food, eg. milk
- ❖ Surface enhanced Raman spectroscopy (SERS) is used as a detection method to visualize complex organisms
- ❖ SERS uses gold or silver nanostructures to increase the Raman signals
- ❖ IMS-SERS has been previously used to detect ricin and ovalbumin from food (1)

## OBJECTIVES

- ❖ Employ SERS to see if method can discriminate between *Bacillus* species and cell states
- ❖ Develop an IMS-SERS method to detect *B. anthracis* under 20 minutes

## METHODS

### Discrimination

This study used cultures of *B. anthracis*, *B. mycoides*, and *B. thuringiensis* to evaluate whether SERS could discriminate between the cultures in the vegetative cell, visible spore, and inactivated spore (boiled for 30 min) stages. These cultures were deposited onto silver dendrites and read using an Almega Raman microscope (Thermo Fisher Scientific) at 785nm.

### IMS-SERS

When the discrimination studies were completed, it was realized that the limit of detection for the spores was high, at  $2 \times 10^7$  CFU/mL. Because of this high limit of detection, it was decided that the spore coat would be digested to release dipicolinic acid (DPA), which is a spore biomarker.

Immunomagnetic beads (Millipore, Germany) were bound to an anti-*B. anthracis* spore monoclonal antibody (Thermo Fisher Scientific). These beads were used to capture the spores from PBS. The spores were released from the beads and digested with dodecylamine (DDA), which releases the DPA. The supernatant is mixed with the dendrites and deposited on a gold slide. These are allowed to dry and read at 785nm.

## RESULTS

### Discrimination

Figure 1 shows how the species and cell states are separated by hierarchical cluster analysis (HCA). The difference between the viable and spore cells is larger than the difference between the live and inactivated spores.

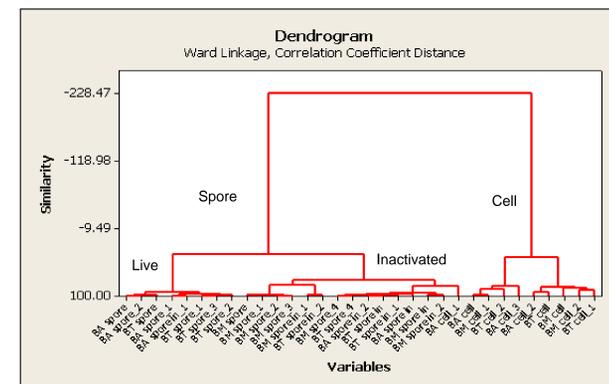


Fig. 1 A HCA diagram of three *Bacillus* species and their cell states

Figure 2 shows the difference between the three cell states of *B. anthracis*. One can see that it is easy to tell the difference between vegetative cell and spore states. Also, the inactivated spore shows patterns related to protein denaturation.

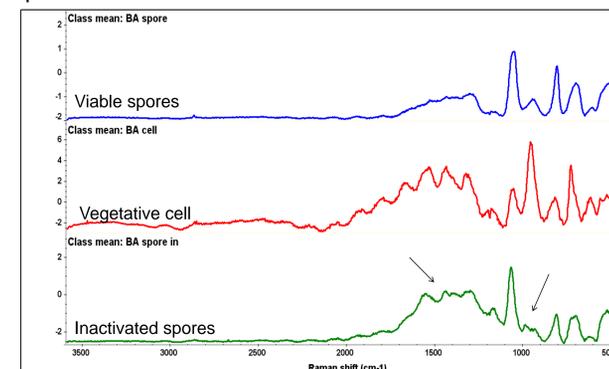


Fig. 2 SERS spectra of the three different cell states

Figure 3 shows that the spores of different *Bacillus* species can be differentiated using principle component analysis (PCA).

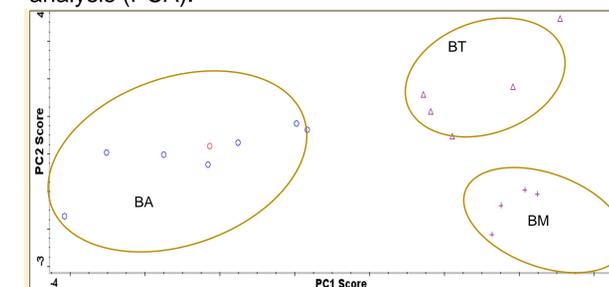


Fig 3. A PCA plot of three *Bacillus* spores

Different concentrations of *B. anthracis* spores were digested with DDA to release DPA. This showed that the limit of detection could be lowered to  $2 \times 10^3$ .

## IMS-SERS

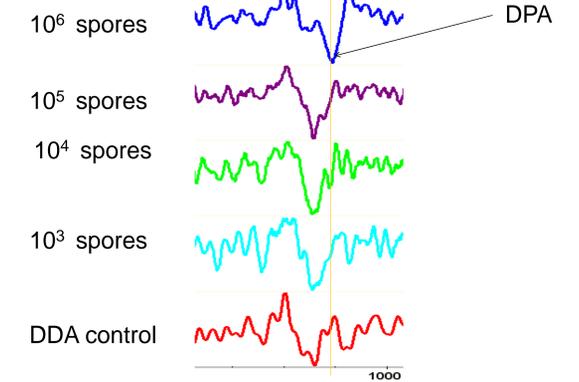


Fig. 4 Second derivative of the SERS spectra of a DDA digestion of *B. anthracis* spores

## CONCLUSIONS

- ❖ We successfully discriminated between *Bacillus* species as well as cell states (viable spores, inactive spores, vegetative).
- ❖ Preliminary trials of IMS-SERS method can detect  $2 \times 10^3$  spores/mL in PBS within 20 min. Further optimization and validation needed to be done in food matrices.

## REFERENCES

- (1) He L.; Deen B.; Rodda T.; Ronningen I.; Blasius T.; Haynes C.L.; Diez-Gonzalez F.; Labuza T.P.\* 2011. Rapid detection of ricin in milk using immunogenic separation coupled with surface enhanced Raman spectroscopy. *J. Food Sci.* 2011, 76, N49-N53

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