Bio-Threats Detection in International Mail using Raman Spectroscopy

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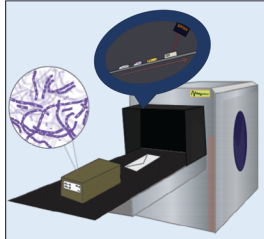
Homeland Security Challenge

Bacteria, ***viruses***, and ***toxins*** are the potential groups considered by DHS as biological threats (BTs). Unlike chemical hazards, detection of BTs tends to be more complicated due to such organisms being less than 5 µm in diameter, leading to delays in identification. The evidence produced by these BTs can delay identifying targets for hours, days, and even weeks until a pattern can be recognized. This slow process in acquiring the required evidence for identification/detection makes BTs a high risk to national security. They are relatively invisible and from various settings where they can directly be used for terrorism. Since powdered anthrax spores were deliberately put into letters and mailed through the US postal system in 2001, investigations on BTs have taken great importance to prevent events that could result in sicknesses and, in the worst scenarios, even deaths. In 2001, at least five envelopes containing 1 g of *Ba* endospores infected 22 victims where 5 died from infections, 31 people tested positive for exposure to spores, and 10,000 were deemed "at-risk" from possible exposure. From this incident, 35 postal facilities and commercial mailrooms were contaminated while USPS closed two processing and distribution centers (P&DC). The Amerithrax Task Force, composed of FBI special agents and USPS inspectors, spent 7 years investigating potential suspects.[4] This incident shows the potential reach, anonymity, and danger that BTs can have. There is a need for rapid on-line detection of BTs in international mail facilities (IMF) (e.g., ports, airports, etc.) while reducing employee's cognitive load. Our research is based on detecting the biological simulant *Bacillus thuringiensis* (*Bt*) as a simulant for *Bacillus anthracis* (anthrax) due to their genetic resemblance. The project's success should lead to non-destructive analysis and reduce the risks of anthrax attacks.

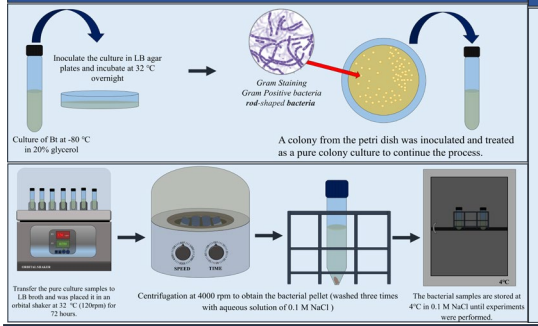
Future Application



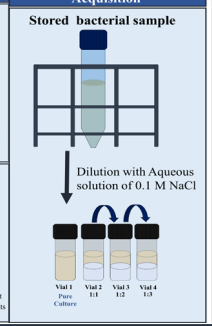
- ✓**Personnel Safety:** Reduce Cognitive Load and increase protection of possible BTs in packages
- ✓**Speed:** Increases speed of package inspection
- ✓**Fast Analysis:** Match/No Match system for nonintrusive inspection
- ✓**Detection/Discrimination:** BWMD spectral libraries and MVA models

Approach / Methodology

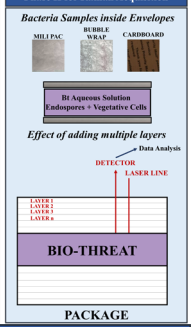
Bacillus *thuringiensis* (Bt) culture protocol



Dilution for initial Raman Acquisition



Sample Preparation: Phase II for Raman Acquisition



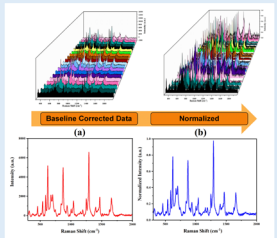
Raman spectral acquisition and Data Processing

Raman Spectral Acquisition

Thermo Scientific AhuraFD
AHURA First Defender
Raman System 785 nm

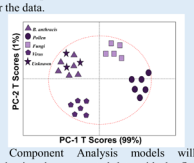
Data Processing

After acquiring the RS spectrum, it can be processed to have a better visualization of the data. For the samples, a baseline correction and normalization will be applied before continuing with other data treatments. The Origin Pro™2020 software will be used to apply a normalization algorithm to spectral data.



Multivariate Analysis (MVA)

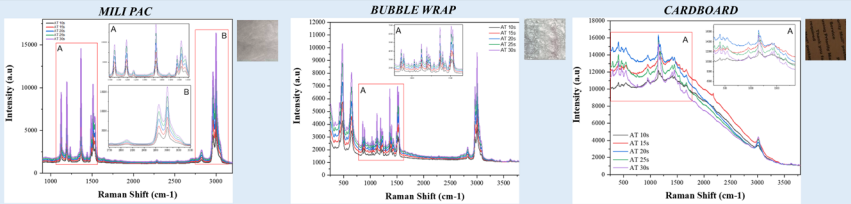
MVA for the spectral data will be carried out using The Unscrambler XTM software package (CAMO Analytics, Montclair NJ, USA). After the acquisition of the data, The Unscrambler XTM software will be used to pre-process the RS spectral data and to create multiple models for the data.



Principal Component Analysis models will be constructed using the pre-treated data with the first and second Savitzky-Golay derivatives. These derivatives will be created using a second-order polynomial fit of 15 points. Predictions models will be created using projections and soft independent modeling by class analogy (SIMCA) for the classification of unknown samples of *Bacillus thuringiensis*.

Outcomes / Results

Envelope Characterization



- ✓ The characterization of the envelopes is finished.
- ✓ The outcomes of Phase I of our research have almost been achieved.
- ✓ The Raman spectra for Phase I bacteria samples presented a high fluorescence background.
- ✓ Raman parameters are being evaluated to provide a profile for the *Bacillus thuringiensis* dilution samples.
- ✓ Still needed: to elucidate the fluorescence, evaluating whether it is endogenous or exogenous.

Conclusions

Phase I of the project is almost complete. The Bt samples showed fluorescence, so the conditions to which the sample is exposed must continue to be evaluated. This will be done to obtain a spectrum profile for Bt that provides us with crucial vibrational information. The samples analyzed contained vegetative cells and endospores for Bt. An additional step is being carried out to enhance these endospores by a selected method. Once this phase is completed, the experiment design that was carried out for Phase II, where multiple layers of envelopes will be evaluated, will follow. The progress of the project will also be dependent on Covid-19 restrictions and protocols, which have proven to be a major obstacle in the advancement of the project.

Acknowledgements

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References

1. Radnedge, L., Agron, P. G., Hill, K. K., Jackson, P. J., Ticknor, L. O., Keim, P., & Andersen, G. L. (2003). Genome differences that distinguish Bacillus anthracis from Bacillus cereus and Bacillus thuringiensis. *Applied and environmental microbiology*, *69*(5), 2755–2764. https://doi.org/10.1128/aem.69.5.2755-2764.2003
2. Kailas, L., Terry, C., Abbott, N., Taylor, R., Mullin, N., Tzokov, S. B., . . . Bullough, P. A. (2011). Surface architecture of endospores of the Bacillus cereus/anthracis/thuringiensis family at the subnanometer scale. *Proceedings of the National Academy of Sciences, 108*(38), 16014-16019. doi:10.1073/pnas.1109419108
3. Butler, H. J., Ashton, L., Bird, B., Cinque, G., Curtis, K., Dorney, J., . . . Martin, F. L. (2016). Using Raman spectroscopy to characterize biological materials. *Nature Protocols, 11*(4), 664-687. doi:10.1038/nprot.2016.036
4. Biological Attack: Human Pathogens, Biotoxins, and Agricultural Threats. (2019). [ebook] 2005: US Department of Homeland Security. Retrieved from https://www.dhs.gov/xlibrary/assets/prep_biological_fact_sheet.pdf