Activation of "Hot Spots", Aggregation and Surface Charge Modification of Borohydride Reduced-Citrate Capped Ag Nanoparticles for SERS Detection of Vegetative Cells and Endospores of Bacillus Thuringiensis

Hilsamar Félix-Rivera, Roxannie González, Oliva Primera, Carlos Ríos-Velázquez and Samuel P. Hernández*
Chemical Imaging Center / Center for Sensors Development
Department of Chemistry -University of Puerto Rico-Mayagüez

Abstract

Spectroscopic techniques such as normal Raman (NR) scattering and surface enhanced Raman scattering (SERS) are considered fast and in situ alternative methods for identification of microorganisms. These techniques provide important information about the spectroscopic signatures of cellular components of *in vitro* or *in vivo* organisms for biological warfare agents detection, microbiology, among other fields. In this research, biochemical components of the vegetative cells and endospores of *Bacillus thuringiensis* (*Bt*) were identified using surface enhanced Raman scattering (SERS) effect with silver-citrate capped nanoparticles (NPs) reduced with borohydride. Activation of "hot spots", aggregation and surface charge modification of the NPs were studied and optimized to obtain good signal enhancements of *Bt* by SERS. This also allowed the study of the interaction of the NPs and the bacteria. Principal component analysis (PCA) regression of SERS spectra has been used to classify and discriminate the bacterial samples.

Relevance

In bioterrorism, medical diagnostics, industrial microbiology and environmental areas, an improvement of detection techniques of biological samples has become a very important subject of concern in as well as in areas of national defense and homeland security. In near field or far field detection, Raman spectroscopy can be used to obtain fingerprinting information of the chemical composition of microorganisms.

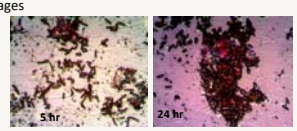
Technical Approach

Characterization of biological samples

SEM images

- Classification: Gram-positive (ATCC # 35646) → [Bt]: 10^7 cfu/mL
- soil-dwelling bacterium
- Most commonly used biological pesticide worldwide: **produces** intracellular **protein** crystals **toxic** to a wide number to **insect larvae**
- **Sporulation** as defense mechanism
- highly resistant to environmental stresses such as high temperature, irradiation, strong acids, disinfectants, etc
- *Bacillus group members: B. thuringiensis, B. cereus and B.anthracis*

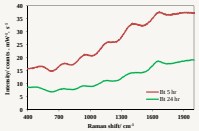
Bt as a model: similarities with *B. anthracis* which has a potential of being used for terrorist attacks



white light micrographs of *Bt* characterization at 5 and 24 hr of bacterial growth showing the vegetative cells and endospores formed (50x magnification)

* **Non-harmful toxin to humans**

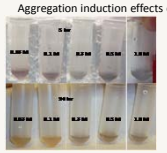
Raman experiments



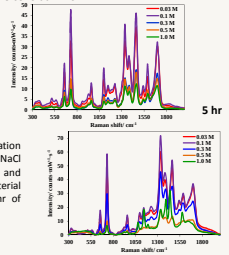
Normal Raman spectra of the bacterial sample dilution for 5 and 24 hours of bacterial growth suspended in NaCl 0.1 M. As can be observed, a highly fluorescence due to the bacterial components presents in the sample limits the chemical information.

SERS experiments

Aggregation induction effects of the colloidal NPs.



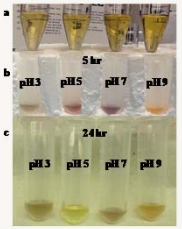
Changes in colors observed due to aggregation induced by adding different concentrations of NaCl to the mixtures of colloidal suspension and bacterial solution for 5 and 24 hr of bacterial growth (above for 5hr and below for 24 hr of bacterial growth).



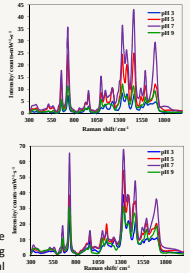
SERS results for the aggregation effect using different concentration of NaCl in mixtures of colloidal suspension and bacterial solution for 5 and 24 hr of bacterial growth. The aggregation promoted using low concentration of this aggregation agent resulted in good SERS signals.

TEM image of mix of colloidal and bacterial solutions suspended in NaCl 0.1 M using different concentrations of the aggregating agent for 24 hr.

Modifications to the surface charge of the NPs.



Changes in colors observed due to surface charge modification induced by adjusting the pH of the sols. The colloidal suspension mixtures at different pH values (3, 5, 7 and 9) with the bacterial solution for 5 and 24 hr of bacterial growth (**b** for 5hr and **c** for 24 hr of bacterial growth).



SERS results for the aggregation effect using different concentration of NaCl in mixtures of colloidal suspension and bacterial solution for 5 and 24 hr of bacterial growth.

SERS experiments: Spectral differences analysis

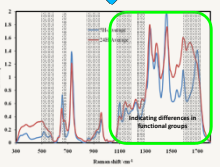
Data analysis procedure had been used to quantitatively assess the relationship between the SERS spectra of bacterial vegetative cells and endospores

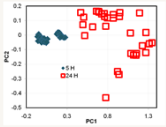
35 spectra of different batch of *Bt* for 5 and 24 hours of bacterial growth were collected and normalized

the average spectra were obtained

principal components were obtained

The principal component was used to discriminate spectra of 5 & 24 hours





Discriminant function with p-value obtained is statistically significant with a confidence level of 95%

95% CL
P value=0.0000

Conclusions

- Parameters need to be optimized to obtain reproducible SERS signals of *Bt*
- *Bt* detection resulted in good SERS substrates with a slight activation of "hot spots" using low concentrations of NaCl in which the bacteria were suspended
- SERS signal was obtained for NPs with **slightly positive charge surface was in contact with this Gram-positive bacteria (*Bt*) containing the negatively charged** phosphate groups of the teichoic acids transverse in peptidoglycan layer and the carboxylic **groups in the dipicolinic complex of the exosporium layer of endospores**
- Vegetative cells and endospores of *Bt* could be spectrally differentiated by principal component analysis

Future Work

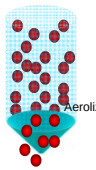
Application: Detection of *Bt* endospores as bioaerosol particles using SERS

Normal Raman Spectroscopy (NRS) and Surface Enhanced Raman Spectroscopy (SERS) can be used as quick methods for liquid bacterial detection in suspension and as bioaerosol particles with **great interest on standoff detection**.

Experimental Raman set up for bioaerosol detection

Broadband Ultrasonic Generator

Model 11 Virtually Pulse Free Syringe Pump 55-2111



Aerolized sample

Metallic Nanoparticles suspension solution

Opportunities for Transition to Customer

- SERS: as a biosensor for vegetative and endospores of *Bacillus thuringiensis*
- Food Technologies Industries
- Defense and Security
- Biomedicine and Hospitals Safety

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- Hilsamar Felix-Rivera, Roxannie Gonzalez, Gabriela Del Mar Rodriguez, Oliva M. Primera-Pedrozo, Carlos Rios-Velazquez, and Samuel P. Hernandez-Rivera, "Improving SERS Detection of *Bacillus thuringiensis* Using Silver Nanoparticles Reduced with Hydroxylamine and with Citrate Capped Borohydride", *International Journal of Spectroscopy*, Volume 2011, Article ID 989504, doi:10.1155/2011/989504, 2011.
- Hilsamar Felix-Rivera and Samuel P. Hernandez-Rivera, "Raman spectroscopy techniques for the detection of biological samples in suspensions and as aerosol particles: A review", *Sensing and Imaging: An International Journal.* DOI 10.1007/s11220-011-0067-0, 2011.

Other References

For more information, please go to:
http://academic.uprm.edu/ccsde/

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