MICROBIAL SPECTRAL SIGNATURES: IS A MICROBE MORE THAN THE SUM OF ITS PARTS?

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Abstract

MOBIUS is a deep UV Raman/fluorescence spectroscopic instrument comparable to the Scanning Habitable Environments with Raman & Luminescence for Organics and Chemicals (SHERLOC) instrument, which will be employed on the Mars 2020 rover. MOBIUS is used to compare and contrast how microbes and their various components react to deep UV Raman/fluorescence excitation in solid and dry states. This is done by growing microbial cultures in liquid defined media, scanning the microbes and various microbial components in dry and solid states using MOBIUS, and understand and interpret the data collected.

Introduction

This Summer I utilized spectroscopic approaches for life detection. This was done by establishing the similarities and differences between a signature of microbial cells and a mixture of organics using the Deep UV Raman spectroscopy instrument, MOBIUS. The microbial cells were Shewenella Oneidensis, and the mixture of organics included: amino acids, carbohydrates, and proteins.

With this data, we sought to determine how reliable Deep UV Raman spectroscopy method was for detecting microbes using organics in their environments. We also sought to collect the spectra corresponding to each microbial component, add them together, and compare this data to the microbial signature first collected.

The outcome of the project led us toward a better understanding of how reliable the deep UV Raman spectroscopy method is for detecting microbes using organics in their environments. Additionally, the data collected assisted, and will continue to assist in the future, in advancing the spectral library that was designed this summer by a fellow intern.

Methods

There are a couple ways of establishing the similarities and differences between a signature of microbial cells and a mixture of organics. The first requires growing up whole microbial cells and collecting the related spectral data, then breaking them up into their various components, collecting the related spectra, adding this spectra together to see if we get back the whole microbial cell signature back. The second path constitutes creating a fake microbe. This requires creating the various microbial components using chemicals in the lab, collecting the related spectra, adding this spectra together, and comparing this data to the real whole microbial cell signature first obtained.

The microbe I used for this project was Shewenella Oneidensis; which is a gram-negative anaerobic bacteria that can be found in lakes across the planet. Shewanella was the ideal microbe for this project as it is easy to grow in a laboratory setting. Subsequently, I made the defined media necessary for microbial survival. Defined media is a growth medium in which all the chemical components are known. Some of the chemical components consist of a carbon source - ideal for bacterial cell growth, various salts, and water. In addition, I made stock solutions of amino acids, carbohydrates, and proteins, and collected the spectral data for the various microbial components in dried and crystallized forms using the Deep UV Raman spectroscopy instrument to collect the necessary data used to understand the similarities and differences between a signature of microbial cells and a mixture of organics. Finally, I used an ultrasonic cell disruptor to break up the whole Shewanella cells into their components, this liquid being called homogenate. I centrifuged the homogenate to separate the heavier cell components - called a pellet,

from the lighter cell components - called the supernatant.

2.1 Microbial Growth and Survival

The task of making defined media required making three separate solutions (amino acid, mineral, and vitamin), which are added to a fourth solution made of additional chemical compounds. Defined media is important when growing up microbes as it provides the essential nutrients for microbial survival while in a laboratory setting.

The next part of this project required placing the Shewenella Oneidensis microbes (grown with the assistance of a JPL collaborator from Photon Systems) into the previously mentioned defined media in a sterile test tube. This test tube was placed into an incubator to aid microbial growth. Every 3-4 days the Shewanella needed to be transferred to new defined media, as they needed more nutrients to survive. This transfer required approximately 20-30 mL of fresh defined media and 10 micro-liters of Shewanella to be put into a sterile test tube. Subsequently, I took 10 micro-liters of Shewanella in defined media and dropped it onto a stainless steel coupon. When the microbe sample was dry, I ran it using the Deep UV Raman spectroscopy instrument.

2.2 Amino Acids, Carbohydrates, and Proteins

In addition, the next part of this project involved using solid amino acids and 40 mL of water to make one milli-molar amino acid stock solutions. I filtered and transferred the solutions to sterile test tubes, at which time I took 10 micro-liters of each solution and placed these on stainless steel coupons. I then waited until the samples were dry and ran them using the Deep UV Raman spectroscopy instrument. After running the dry samples I proceeded to run the aqueous and crystallized samples. The aqueous samples required taking 1 mL of each of the one micro-molar stock solutions and dropping these into the wells of a heating block. The crystallized samples required taking approximately 5 milligrams of solid amino acids and dropping these onto a shallow metal well plate. Also, four of the amino acids (Tyrosine, Phenylalanine, Tryptophan, and Histidine) were examined using fluorescence spectroscopy in a dry and crystallized form.

Next, I used solid carbohydrates and 40 mL of water to make one milli-molar carbohydrate stock solutions. I filtered and transferred the solutions to sterile test tubes, then placed 10 micro-liters of the stock solutions onto stainless steel coupons. I waited for these samples to dry before running them using the Deep UV Raman spectroscopy instrument. Due to errors with the aqueous data collected from the Shewanella Oneidensis microbes and amino acids, I ran the carbohydrates using only the dry and crystallized forms.

Finally, I used various solid proteins and 5 mL of water to make one milli-molar stock solutions. Once again, I filtered and transferred the solutions to sterile test tubes, then placed 10 micro-liters of the solutions onto stainless steel coupons. I waited for these samples to dry before running them. Similar to the carbohydrate samples, I ran the proteins using the dry and crystallized forms only.

2.4 MOBIUS-Deep UV Raman Spectroscopy

The following steps detail how the Deep UV Raman spectroscopy instrument is used:

1) For each area analyzed: a Deep UV Laser illuminates the surface of a sample with 40 microsecond pulses.

2) During these pulses, Fluorescence emissions or Raman scatter photons are collected by the instrument.

3) This collected light is diffracted through a grating and imaged onto a detector.

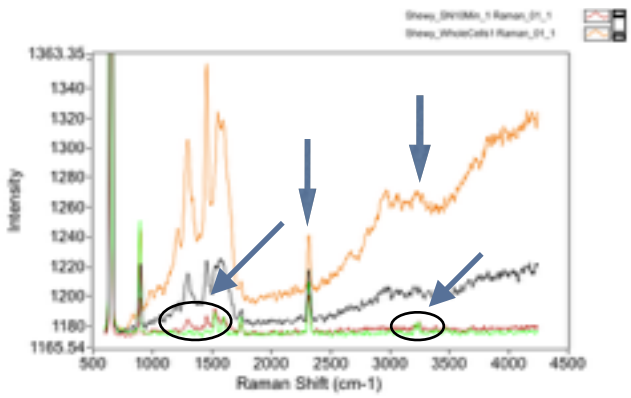
MOBIUS runs Deep UV Raman and fluorescence, but I have focused most of my attention on Raman. Deep UV Raman spectroscopy is the process of measuring the amount of light scattered, or the vibrational motions of a molecule, using a monochromatic light as an illuminator device. The monochromatic light, when shone on an object or substance, yields a spectrum of scattered light due to the photonic interactions with various particles of matter.

Results/Discussion

3.1 The Real Microbes

The graph located on the following page shows four spectra I have collected. The orange spectra corresponding to the whole Shewanella cells, the black spectra corresponds to the homogenate, the red corresponds to the supernatant, and the light green corresponds to the pellet. We can see that the peaks from the whole Shewanella cells are extremely similar to the peaks found in the homogenate spectra. This is because the homogenate contains all of the same components as the whole cells, however, they are broken up. As we can see the homogenate spectra

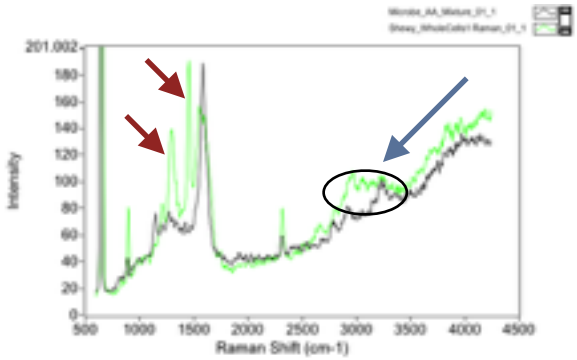
is not as intense as the whole cell spectra. This could be due to insufficient concentrations.



We can also see that the supernatant has very similar peaks to the homogenate but again much less intense. The pellet contains the cellular membrane and consists of membrane bound proteins, as well as lipids. The supernatant contains the cytoplasm and consists of free amino acids and nucleic acids. As the graph shows, the pellet does not heavily contribute to the whole Shewanella spectra. Analyzing this graph further, we can see specific peaks from 1200-1700, which correspond to the lipids found in a microbial cell. The peak located at 2300 corresponds to nitrogen found in the air, thus it is present for each spectra. Finally, at 3300 we can see peaks corresponding to a C-H stretch, this is also where the peaks for the cell membrane are located.

3.2 The Fake Microbes

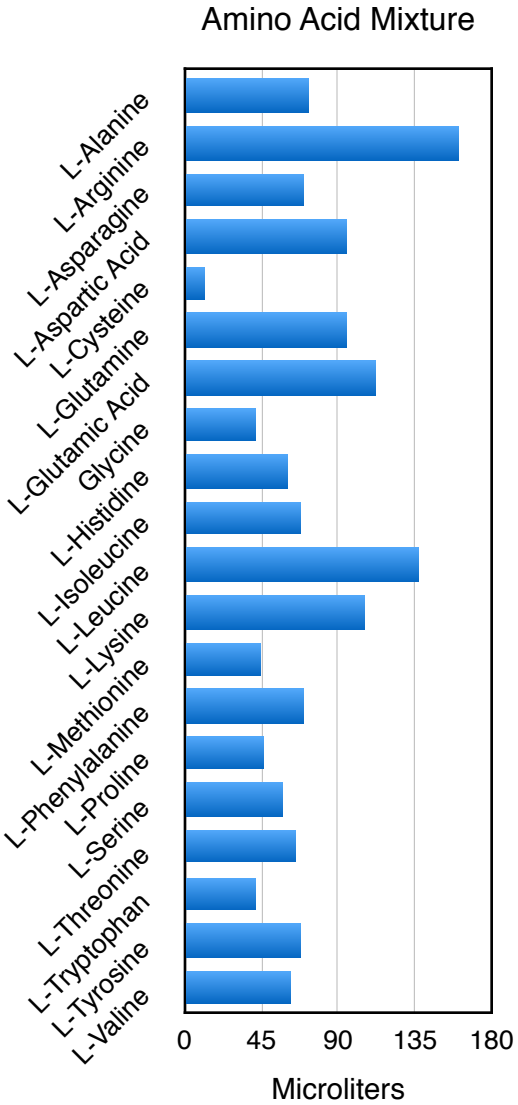
Again, the following graph is showing the whole Shewanella cells spectra in a light green color, while the black corresponds to the amino acid mixture I prepared, which mimics the amount of amino acids present in a nominal microbial cell.
Analyzing this graph, we can see a large fraction of



the whole Shewanella signal is being accommodated by the amino acid spectra. However, there are noticeable differences, which most likely correspond to a lack of nucleic acids, lipids, and cell wall within the amino acid mixture. At 1400 and 1500 we see the peaks corresponding to nucleic acids in the whole Shewanella cell spectra, 2900-3300 are the peaks corresponding to the microbial cell wall, and again at 3300 we see peaks corresponding to C-H stretch.

3.3 Amino Acid Mixture

The bar graph located below displays the various amino acids added to the mixture. I made one milli-molar stock solutions of each amino acid and the x-axis of the graph displays the amount of each amino



acid used in micro-liters to create 15 milliliters worth of the overall mixture.

There is still a large amount of work to be done before this project is complete. The next steps will include: creating stock solutions of nucleic acids, lipids, and DNA; collecting the spectra corresponding to these microbial components in dry and solid states using MOBIUS; adding the previous microbial components to the amino acid mixture; and finally sensitivity testing the amino acid mixture to discover how different ratios of amino acids correspond to the spectra obtained.

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