Bio-Detection and Chemical Fingerprinting Using the Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS) Technology

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ABSTRACT

The bioaccumulation of a large variety of inorganic trace elements in Bacillus subtilis was studied using an inductively coupled plasma mass spectrometer and compared to other related and unrelated species. Bacillus subtilis is a safe substitute for anthrax. Unique and reproducible chemical fingerprint for each species of organisms were determined. Additionally, it was found that these chemical signatures were very susceptible to laboratory and procedural effects, suggesting that this approach could be useful also as a forensic tool. In addition, quantitative analysis on these signatures have been performed to mathematically relate the chemical fingerprints of B. subtilis to the number of cells or spores present in an analyzed sample.

Keywords: icpms, spectroscopy, biodetection, chemical signatures, bacteria.

INTRODUCTION

Counterterrorism efforts have become a priority in scientific research across the globe. Presently, there has been extensive research devoted to creating bio-detectors based on our increasing understanding of the physical and biological properties of possible bio-agents. However, these detection methods typically require significant sample processing and as a result, are not capable of producing real-time data. In contrast, direct injection inductively coupled plasma mass spectroscopy (ICP-MS) is a method that can produce real-time inorganic trace element signatures specific to bioagents. By focusing on the inorganic composition or organisms, we are able to directly introduce a bio-agent into the ICP-MS, significantly reducing processing time.

The Inductively Coupled Plasma Mass Spectrometer (ICP-MS) is capable of detecting sub-nanogram/gram (ppb) levels for most elements, thereby making it a sensitive tool for the detection and characterization of biological materials.

METHODS

Simulant cultures of B. *subtilis* were streaked on Agar plates for isolated colonies and used to inoculate 50ml of LB Media. The cultures were grown for ~ 17hrs. Next, eight 500ml bottles of LB Media were inoculated, each with 1ml of the grown cultures. To obtain a growth chart for the organism, we performed hourly serial dilutions for ~10hrs, using the following rubric, and graphed the results for analysis.

Time	Procedure
0 hours	No Readings Taken
2 hours	Serial Dilution, 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *No Spinning For Pellets*
4 hours	Serial Dilution (Sample A) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
5 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
6 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
7 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
8 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
9 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*
10 hours	Serial Dilution (Sample B) 990ml →990ml → 900ml → 900ml [10^3, 10^5, 10^6, 10^7] *Spin ~250ml For Pellets*

The Plates were left to grow for ~24hrs after which, the number of cells that grew on each plat were tabulated to form a single graph.

In addition O/D readings were made using a 1-10 dilution which we ran through a Spectrophometer and graphed the results for analysis. These same protocols were repeated using B. *megaterium* and E. *coli* also. Finally, all the samples that were spun down for pellets were rinsed, dried, crushed, measured and prepped for the ICP-MS. The principle behind the operation of the ICP-MS involves the introduction of a fine aerosol of the sample into the Inductively Couple Plasma (ICP section). This is achieved using a nebulizer pump to transport minute quantities of the sample into the spray chamber, where a gas flow facilitates the formation of fine aerosol samples by dispersing the liquid into tiny droplets (*See Figure 1*).

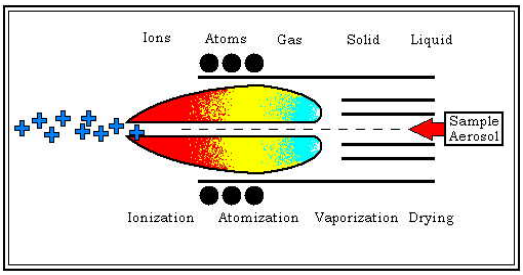

Figure 1: The Inductively Coupled Plasma forms Ions, which are then transported to the Mass Spectrometer for analysis.

Subsequently a constant amount of the aerosol is passed through to the plasma torch. In the plasma torch section argon is applied to the sample creating inductively coupled plasma that dries, vaporizes, and atomizes the aerosol. Finally, the atoms are accelerated into the mass spectrometer.

Prior to analysis, a multi-element solution containing 1 part per billion (ppb) Be, In, and Bi was used to ensure that the solution uptake and drainage are working correctly. Then 0.1 milliliters of the internal standard solution will be used to monitor any drift in signal sensitivity during the process of analyzing. The solutions will then be introduced into the ICP-MS, which has been programmed to analyze changes in concentrations as a function of time (Ion Counts Per Second). This data is then used to depict elemental ratios and proportions in the form of chemical fingerprints. The present study specified the approximate number of cells per milliliter at different growth times to develop quantitative

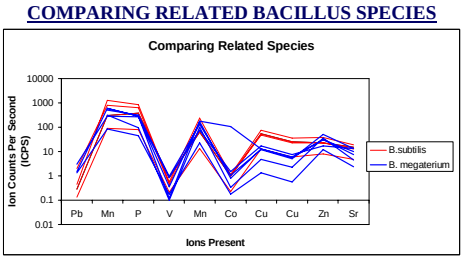

Figure 2. Comparison of B. subtilus with related species.

relationships between cell concentration and the corresponding chemical fingerprints.

RESULTS

Elemental analysis on the anthrax simulant *Bacillus subtilis* has been performed and compared to other related and unrelated species using direct injection ICP-MS [1]. Figure 1 compares signatures of species directly related to Bactillus Subtilis while Figure 2 compares non-related species. Unique and reproducible chemical fingerprints for each species of organism, whether closely related or not, have been obtained. Additionally, the chemical fingerprints also appear to demonstrate laboratory and procedural effects, suggesting that this approach could be useful also as a forensic tool. Figure 3 shows results taken on a single species of B. subtilis grown at different venues. Data taken at individual laboratories are observed to be clustered together. In addition, we have succeeded in performing quantitative analysis on these signatures to mathematically relate the chemical fingerprints of B. *subtilis* to the number of cells or

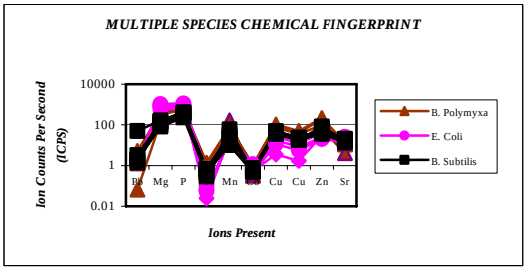

Figure 3. Chemical signature for multiple species. Fingerprints for each species clearly possesses a distinct chemical signature differentiable from the others.

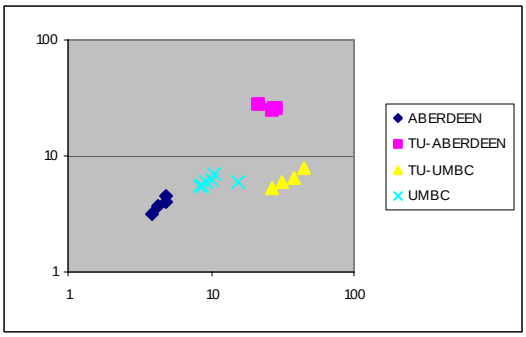

Figure 4: Elemental ratios Zn/Ba and Sr/Ba of B. subtilis grown at different sites.

spores present in an analyzed sample (*See Figure 4, 5 and 6*). With this, we hope to investigate the ability to detect from a chemical fingerprint not only the presence of a bio-agent but its abundance, approximate time of introduction and growth stage.

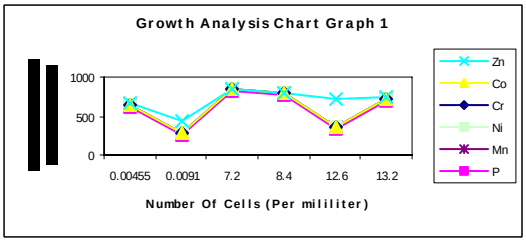

Figure 5. Displaying elements with similar uptake patterns in B. subtilis. Quantitatively relating the sample cell size to the sample's elemental concentration.

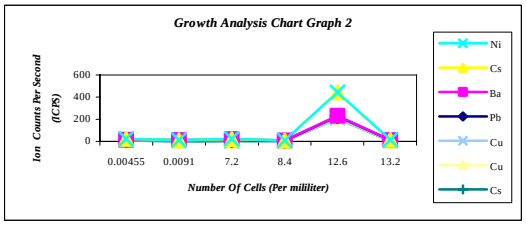

Figure 6. Displaying elements with similar uptake patterns in B. subtilis. Quantitatively relating the sample cell size to the sample's elemental concentration.

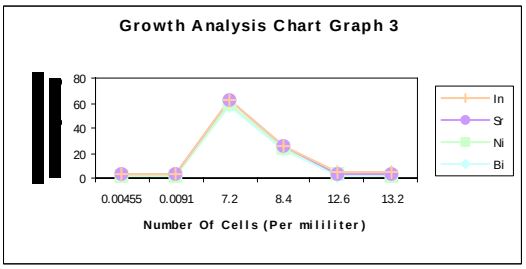

Figure 7. Displaying elements with similar uptake patterns in B. subtilis. Quantitatively relating the sample cell size to the sample's elemental concentration.

REFERENCE

[1] Gikunju C.M, S Lev. D. M. Schaefer and A. Birenzvige, "Detection and Identification of Bacteria using Inductively Coupled Plasma Spectroscopy" Talanta 2004.