Alteration of Bacterial Cell Elemental Concentrations by Environmental Influences as Determined by Laser-Induced Breakdown Spectroscopy.

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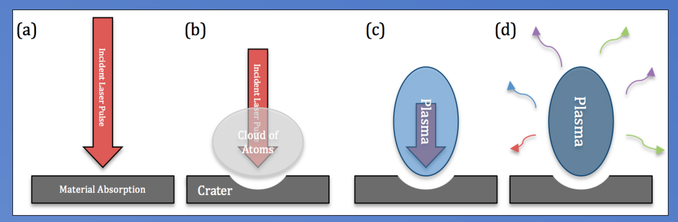
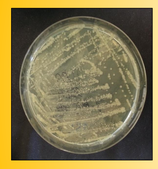
What is LIBS

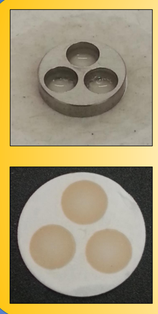
Laser-induced breakdown spectroscopy (LIBS) is a time-resolved spectroscopic technique that uses a high pulse energy laser to ablate a target of interest, forming a plasma of the constituent atomic species in the target. As this plasma cools, the excited atoms and ions emit photons that can be used to characterize their relative concentrations within the ablated target.



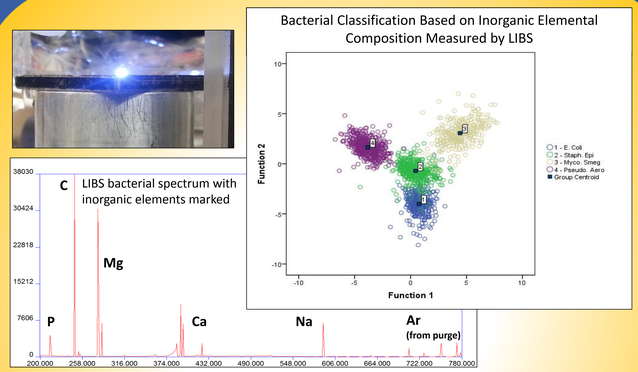
Experiments were conducted on bacteria grown on media with an excess of three chemical species: Mg, which is required for our classification and is present in hard water; Zn, which is not typically present in the cell but may be taken up in contaminated water; and glucose, which varies in the body over the course of the day and so may affect cell growth.



All tests were performed on *E. coli* grown on tryptic soy agar plates. Solutions of chemical species of interest were prepared in water to bodily (>1 ppm)[1] and environmental (>50 ppm)[2] concentrations. Bacteria was harvested into suspension in dilute H_2O and rinsed three times.



Bacterial solution was deposited onto a nitrocellulose filter paper using a metal jig with three 5 mm diameter wells to form three small lawns. The filter was mounted onto a metal plate and placed in an argon purge chamber. Ablation was performed using a Nd:YAG laser at ~5 mJ pulse energy and 8 ns pulse duration. Light was collected using matched parabolic reflectors and analyzed using an échelle spectrometer. This apparatus is further described elsewhere.[3]



Within a bacterial cell, several major elements can be consistently identified. We have shown that based on the elements C, P, Mg, Ca, and Na, a bacterium's species can be reliably identified.[4] Other groups have demonstrated specificity in these tests down to strain-level.[5] This, combined with its speed and a lack of required sample preparation, can make LIBS a powerful diagnostic tool for clinicians.

Motivation

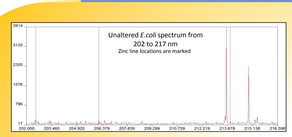
Environmental

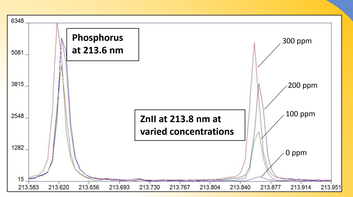
Since bacterial species take their nutrients from their environment, bacteria have been used as an indicator of environmental health, with trace metals in the cells being indicative of contamination of a water supply. [6] If this uptake is sufficiently different from normal biological conditions for the cell, it may be possible to use LIBS as a diagnostic of environmental conditions via analysis of bacterial spectra.

Clinical

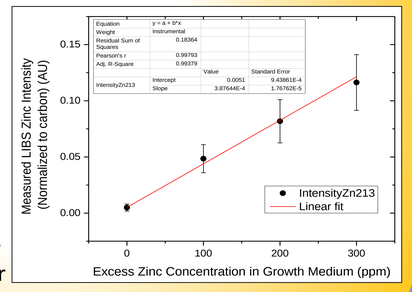
While abnormal uptake of elements may be of use for environmental assessment, certain elements are used to identify the species of bacteria. It is important to determine if any changes that may be caused by the elemental variation in the human body are drastic enough to alter the accuracy of classification.

Zinc



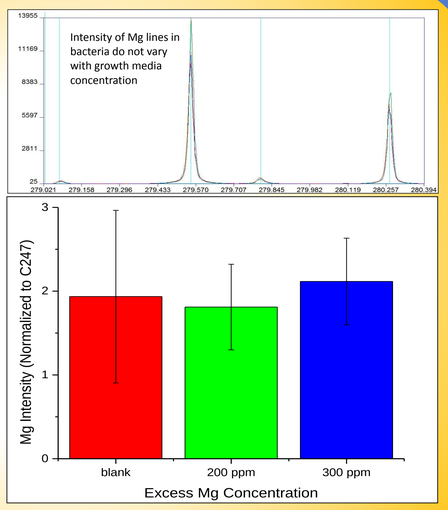


Zinc lines are not distinguishable from noise at normal growth conditions, but begin to appear as concentration in the growth medium increases. Signal from the 213.86 nm zinc line was normalized to the 247.86 nm carbon line to account for variation in mass ablated. A linear fit of zinc line intensity to the excess zinc concentration gives an adjusted r^2 of 0.994. The limit of detection (LOD) as calculated from this fit is 11 ppm. The maximum concentration allowable for drinking water is 5 ppm. [7]

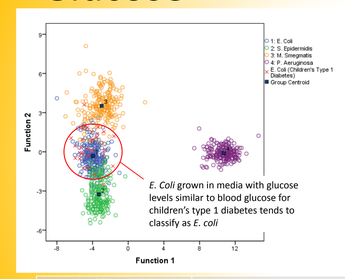


Magnesium

Magnesium lines are generally among the largest in bacterial spectra. As more Mg was added to the growth medium, the intensity of the lines was largely unchanged, but the deviation in intensity reduced as the surplus increased. A sample was prepared wherein Mg was precipitated out of the agar solution using HCl prior to autoclaving. This plate provided no bacterial growth. Water with a Mg level of >100 ppm is considered hard water. [2]



Glucose



Concentration of Glucose (g/L)	
1.35	Control
1.44	Type II diabetes
1.62	Type I diabetes
1.8	Children's type I diabetes

Glucose was added to plates in four concentrations representative of blood glucose in a healthy individual, one with type I diabetes, type II diabetes, and children's type I diabetes. While this provided increased overall bacterial growth, no shifts in spectral intensities were observed. It is not expected that this will have an impact on classification.

Conclusions

- Biological concentrations (0.6-1.3 ppm for Zn, 1.5-2.3 ppm for Mg in blood) are insufficient to influence classification and are below calculated LOD
- Bacteria take up necessary elements only up to what is needed for function. Excess reduces the observed fluctuation in emission intensity
- Uncommon elements in the environment may be tracked through bacterial spectra

References

[1]- *Reference ranges for blood tests*, Uppsala University Hospital
[2]- *Hardness in Water*, US Geological Survey School of Water Science
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[6]- F. Veglio, F. Beolichini; Hydrometallurgy, 1996
[7]- *Water Quality Assessments - A Guide to Use of Biota, Sediments and Water in Environmental Monitoring - Second Edition*, World Health Organization, 1996