Bacteria strain discrimination using nanosecond LIBS:

Escherichia coli identification
and
Pseudomonas aeruginosa membrane alteration

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Introduction

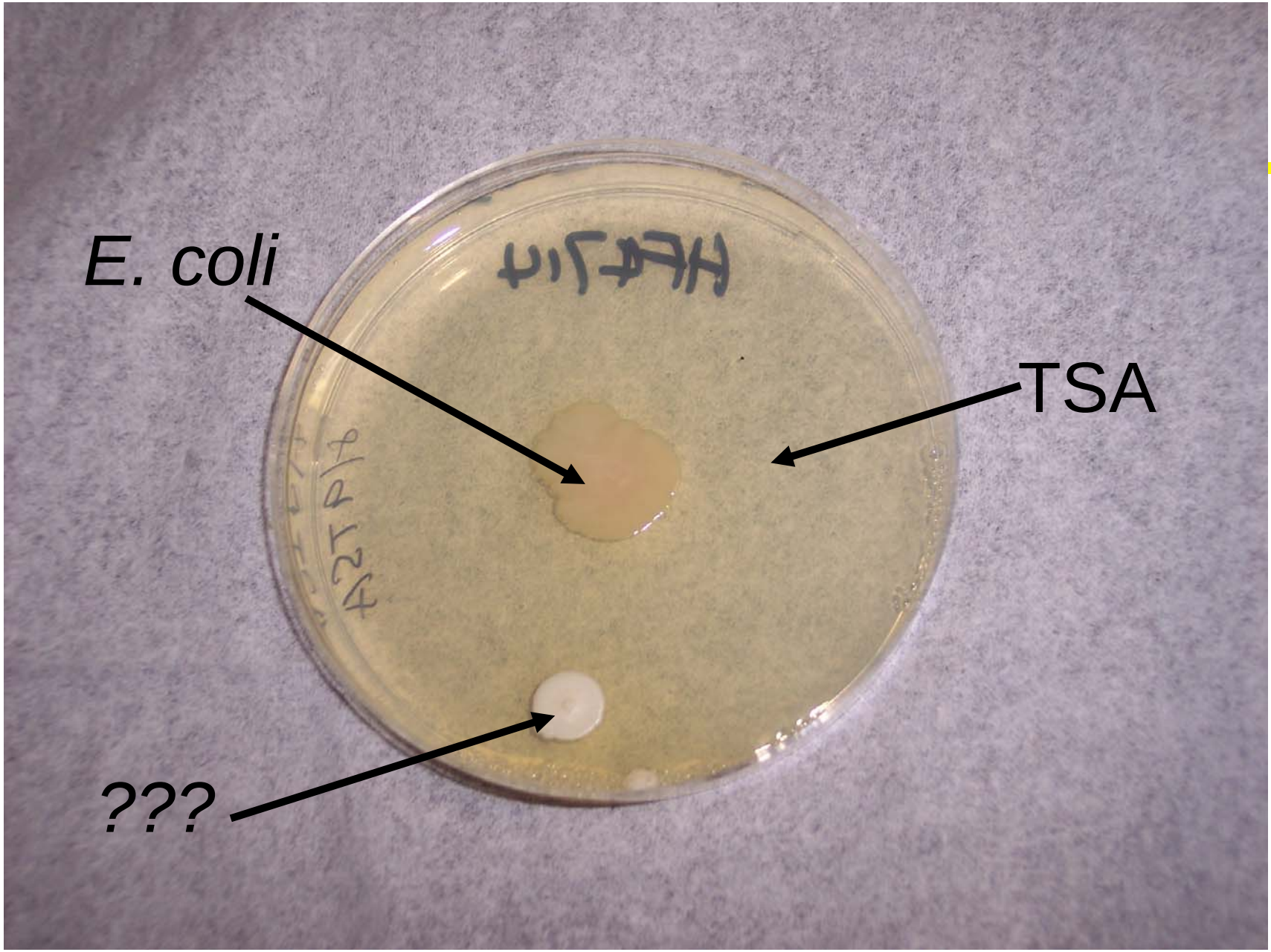
Motivating Questions:

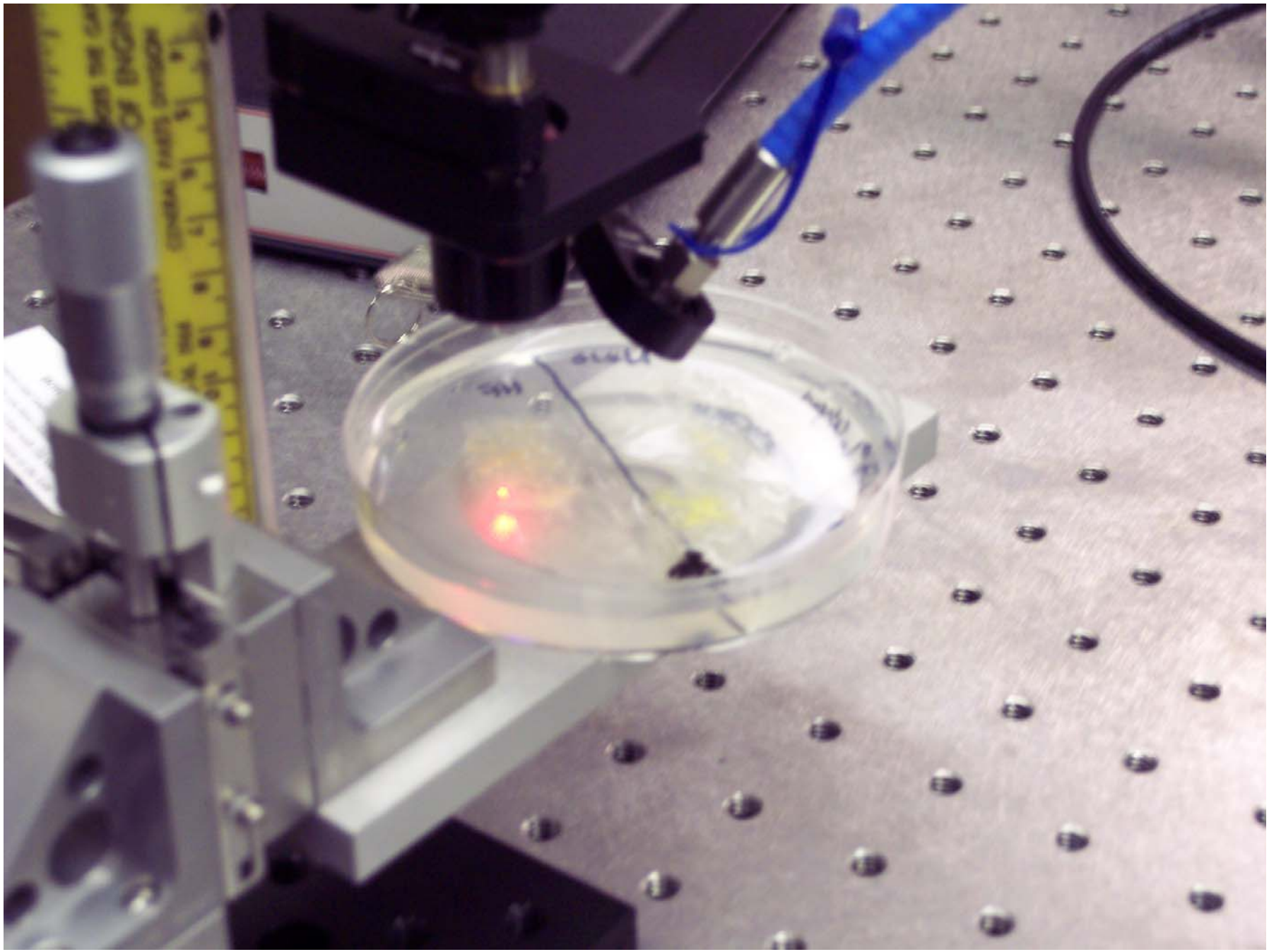
1. Can bacteria be identified from its atomic spectrum alone (using LIBS)?

   Yes! (since about 2003)

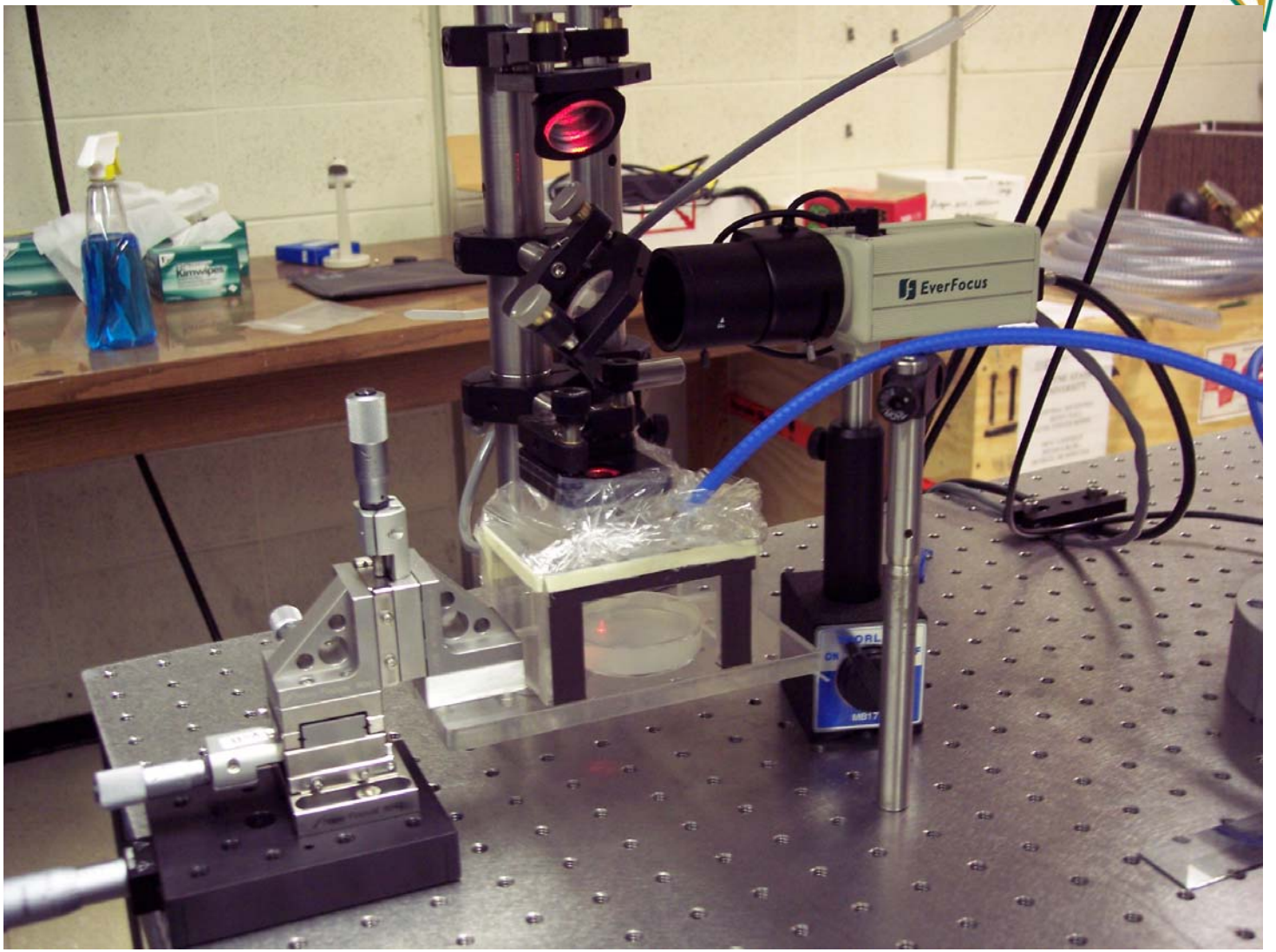
2. Can the atomic spectrum be used to do interesting science?

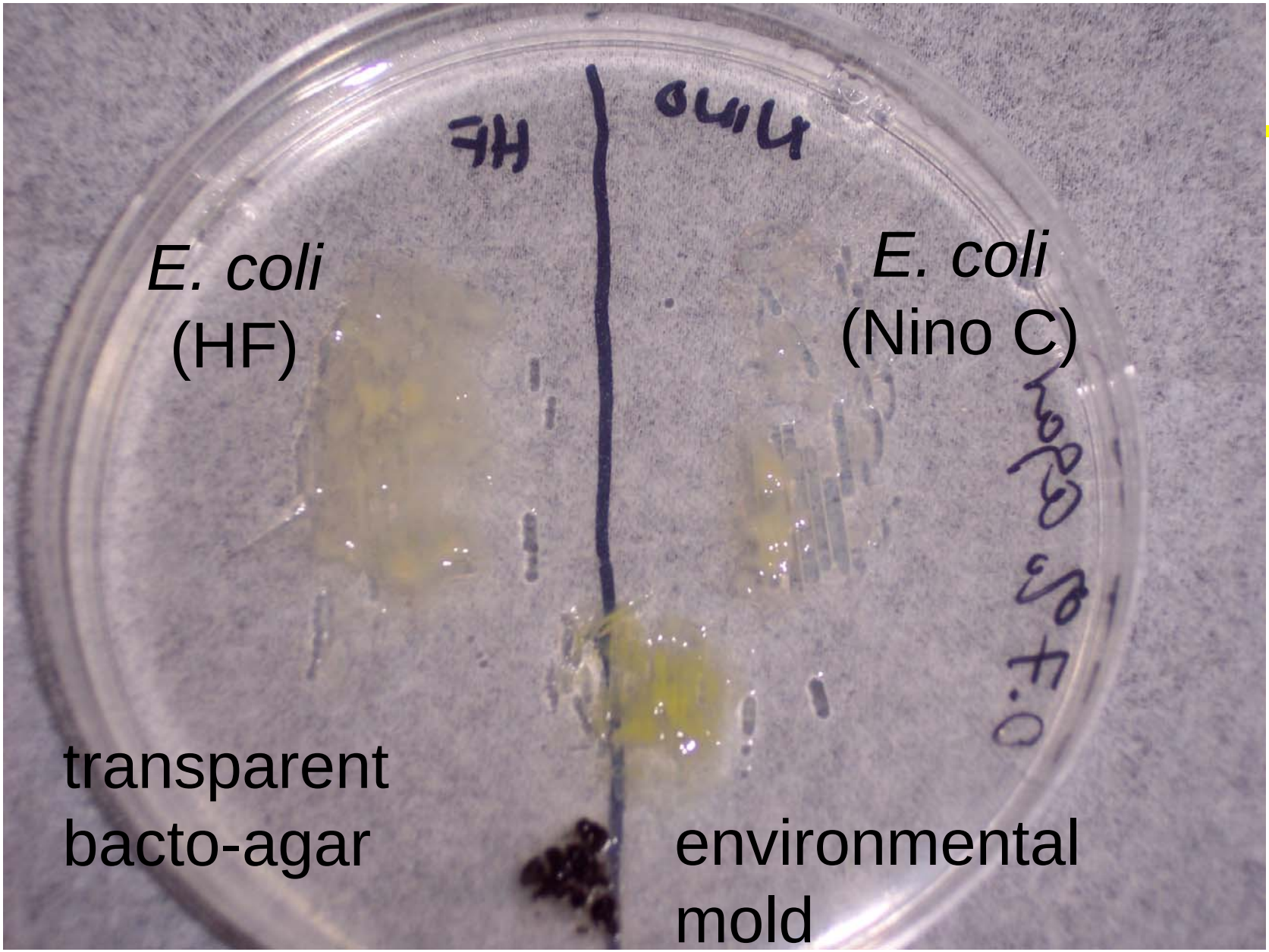
   Yes! (since ???)


E. coli
TSA
???

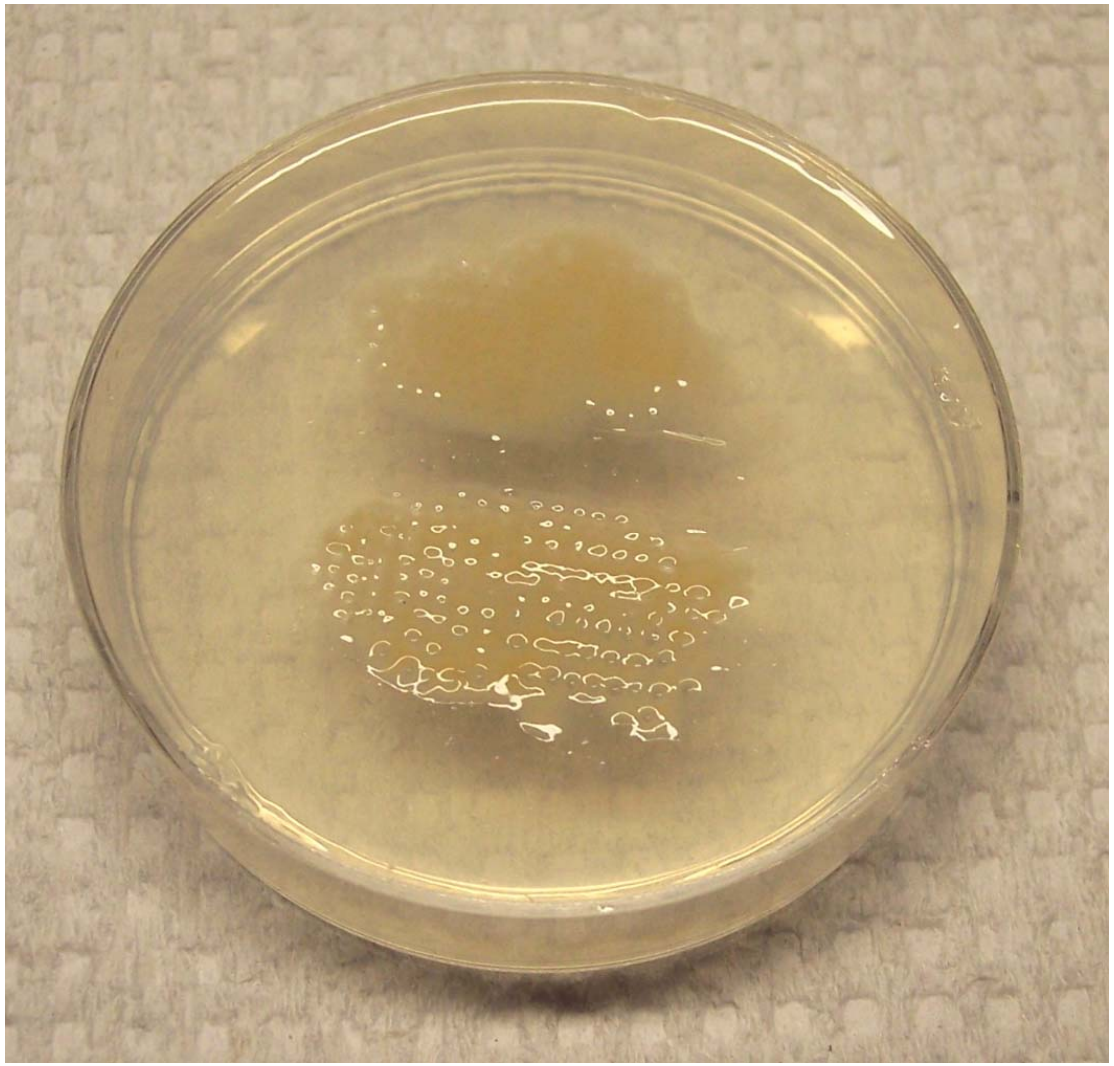


periscope / box

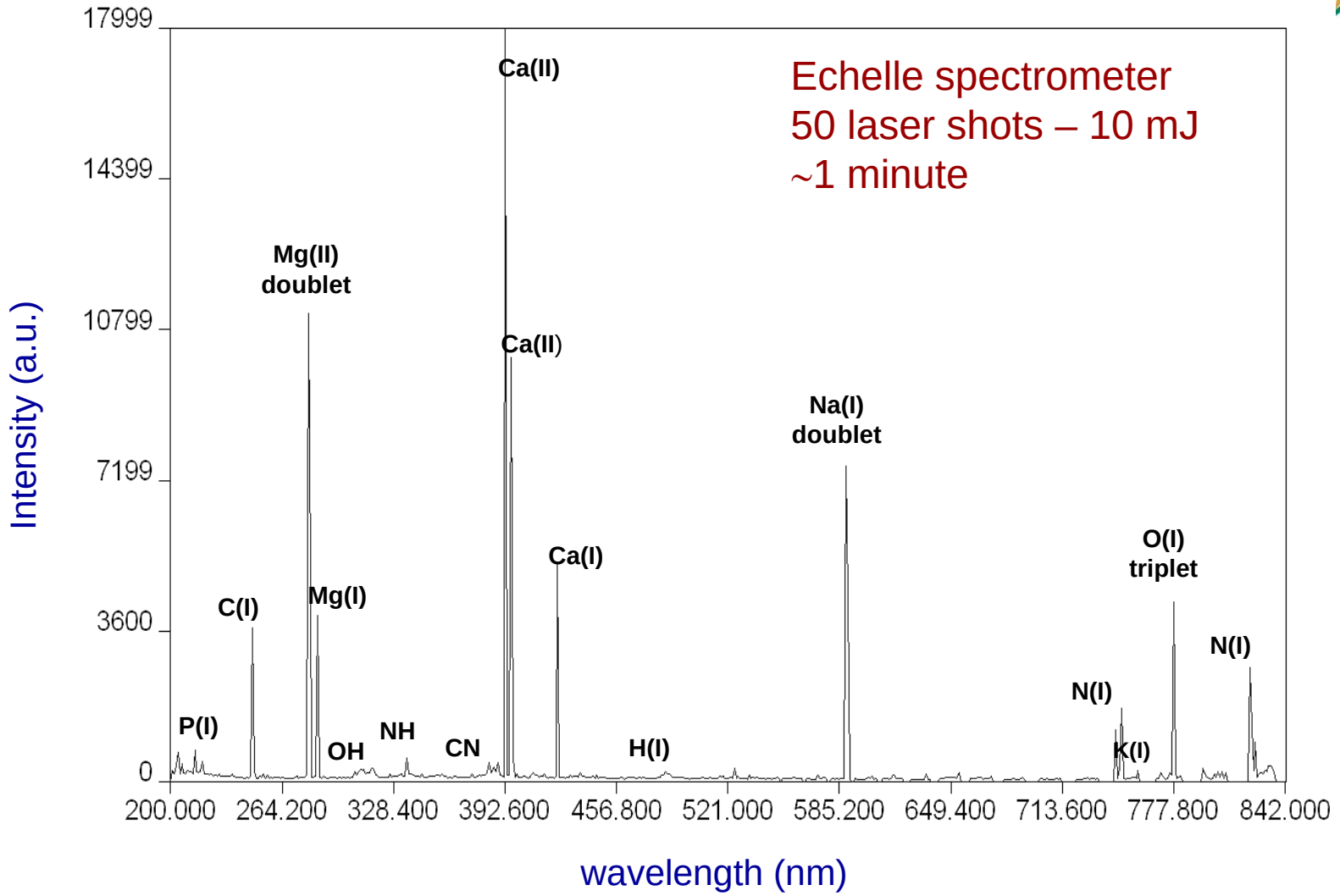



E. coli
(HF)
E. coli
(Nino C)
transparent
bacto-agar
environmental
mold

Ablated *E. coli* on Agar



LIBS Spectrum of *E. coli*



Spectral Fingerprint

The intensities of 19 spectral lines from 6 elements provides a *spectral fingerprint*

wavelength (nm)	line identification	Fraction of total spectral power	Wilks' Lambda
213.618	P I	0.034	.619
214.914	P I	0.040	.492
247.856	C I	0.099	.521
253.56	P I	0.007	.771
279.553	Mg II	0.202	.040
280.271	Mg II	0.113	.061
285.213	Mg I	0.109	.037
373.69	Ca II	0.002	.909
383.231	Mg I	0.015	.782
383.829	Mg I	0.005	.588
393.366	Ca II	0.099	.034
396.847	Ca II	0.037	.060
422.673	Ca II	0.033	.062
430.253	Ca I	0.002	.803
518.361	Mg I	0.004	.773
585.745	Ca I	0.000	.920
588.995	Na I	0.124	.020
589.593	Na I	0.067	.022
769.896	K I	0.012	.931

Discriminant Function Analysis

- Using the 19 intensities, a statistical analysis called Discriminant Function Analysis (DFA) looks for similarities and differences in spectra from different samples

1. Discriminant Functions *(eigenvectors?)* calculated.

2. Every spectrum has a canonical root *(eigenvalue?)* calculated from each function.

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Escherichia coli identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy

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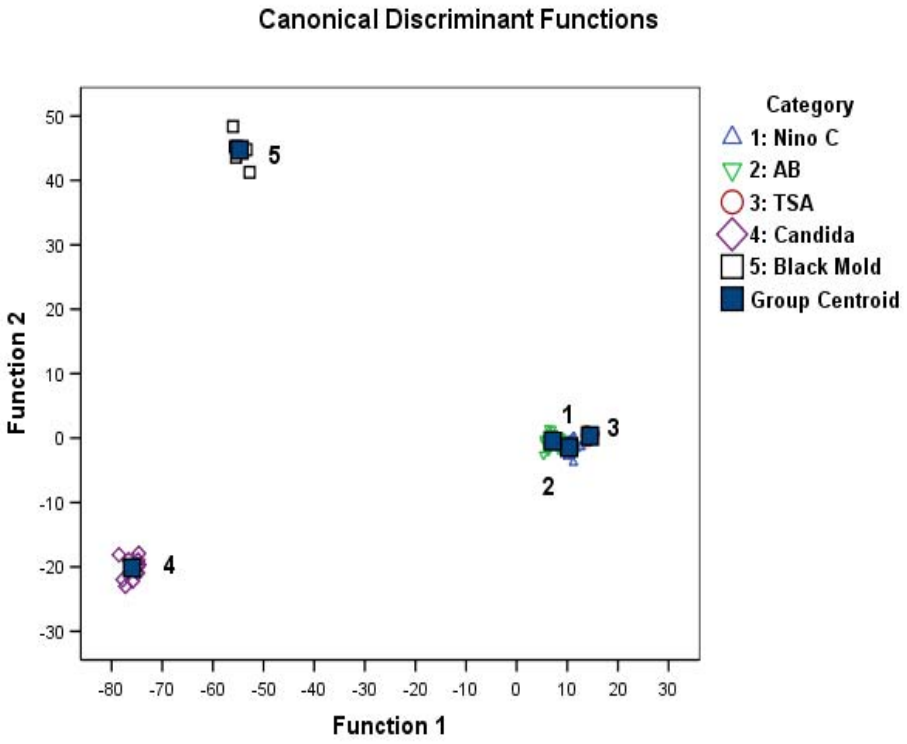
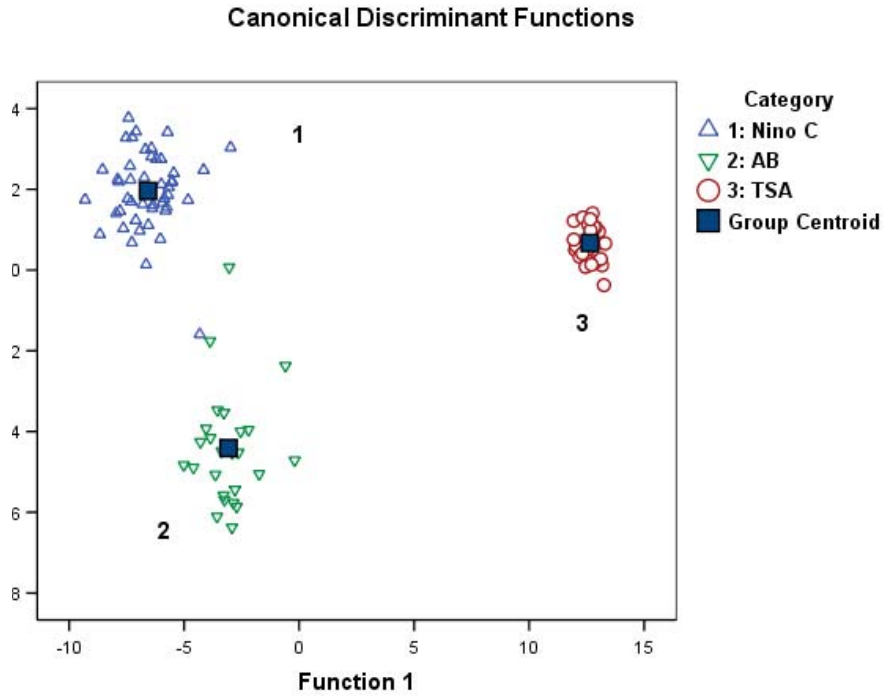
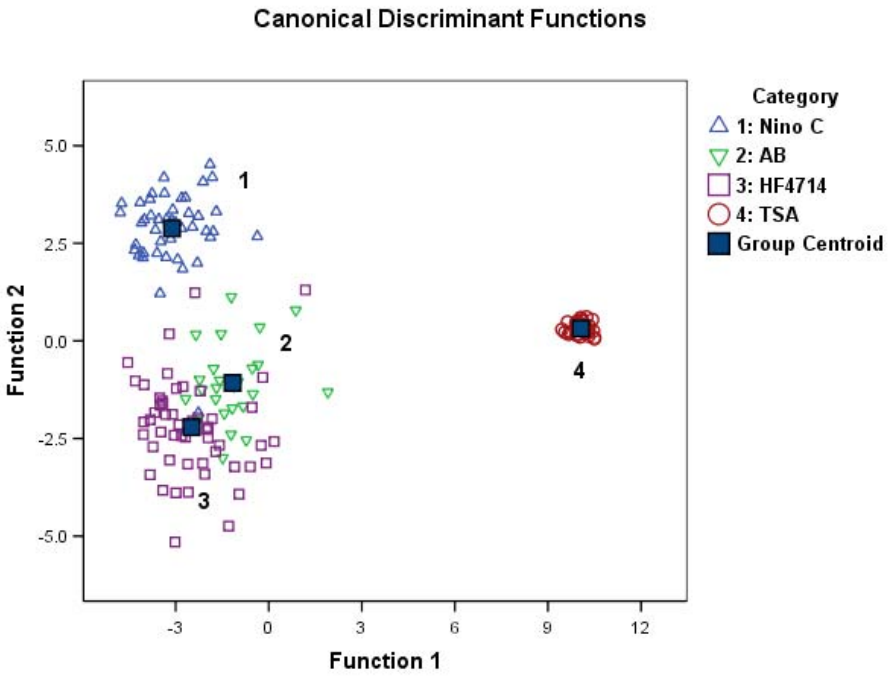
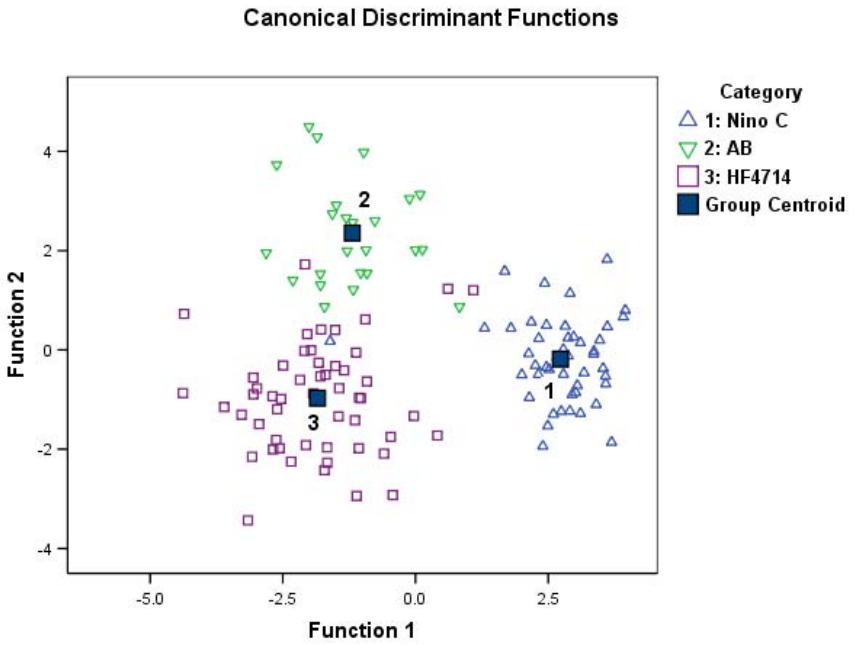




E. coli Results





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Pathogenic *Escherichia coli* strain discrimination using laser-induced breakdown spectroscopy

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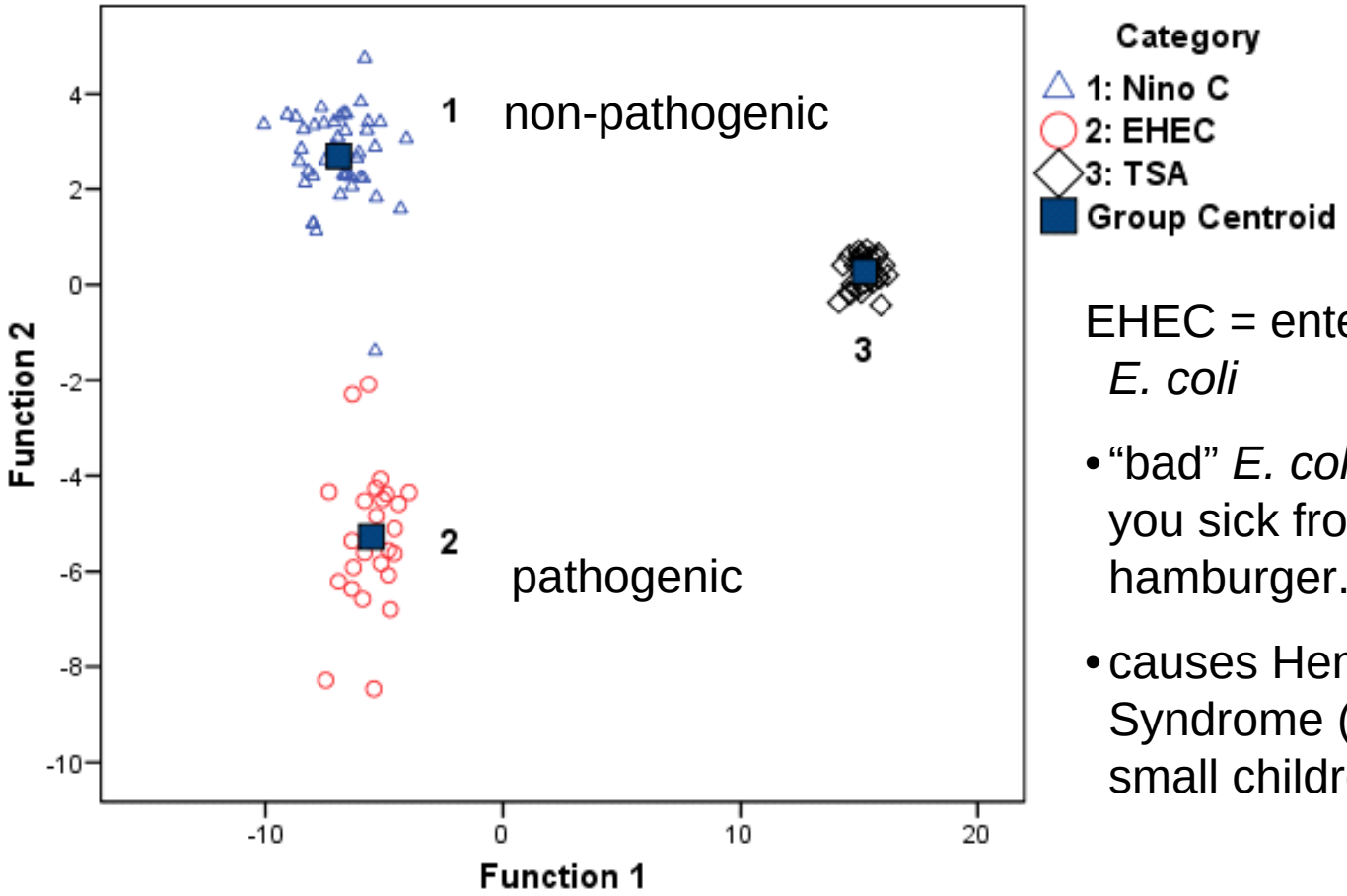
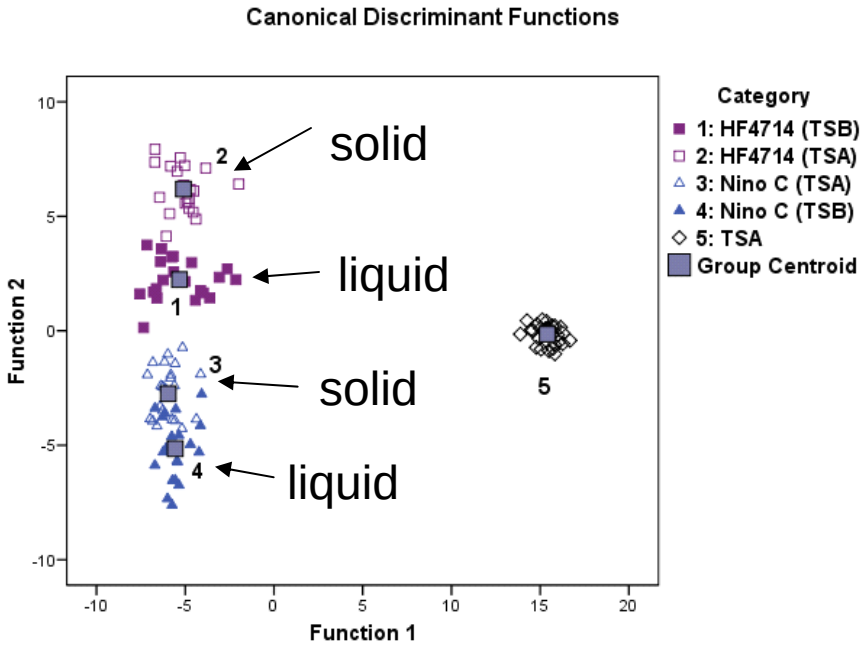


EHEC = enterohemorrhagic *E. coli*

- "bad" *E. coli* which makes you sick from eating raw hamburger.
- causes Hemolytic Uremic Syndrome (HUS) fatal to small children

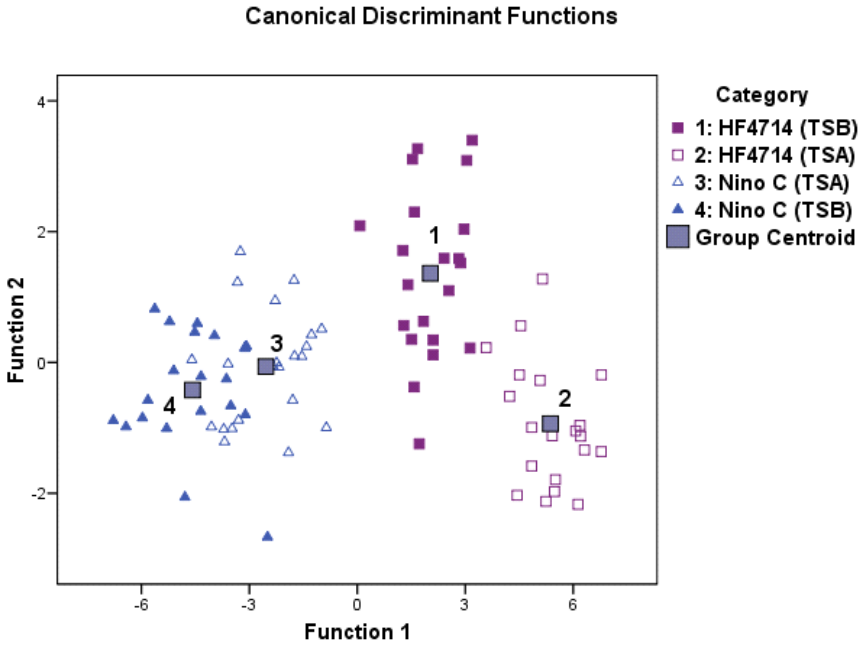
Effect of Growth Environment on *E. coli*



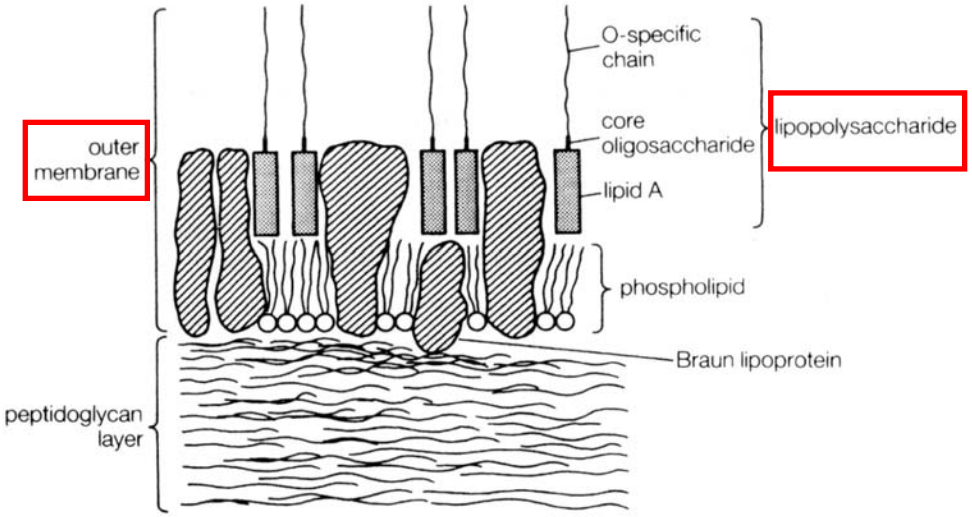
<u>two culture methods used:</u>

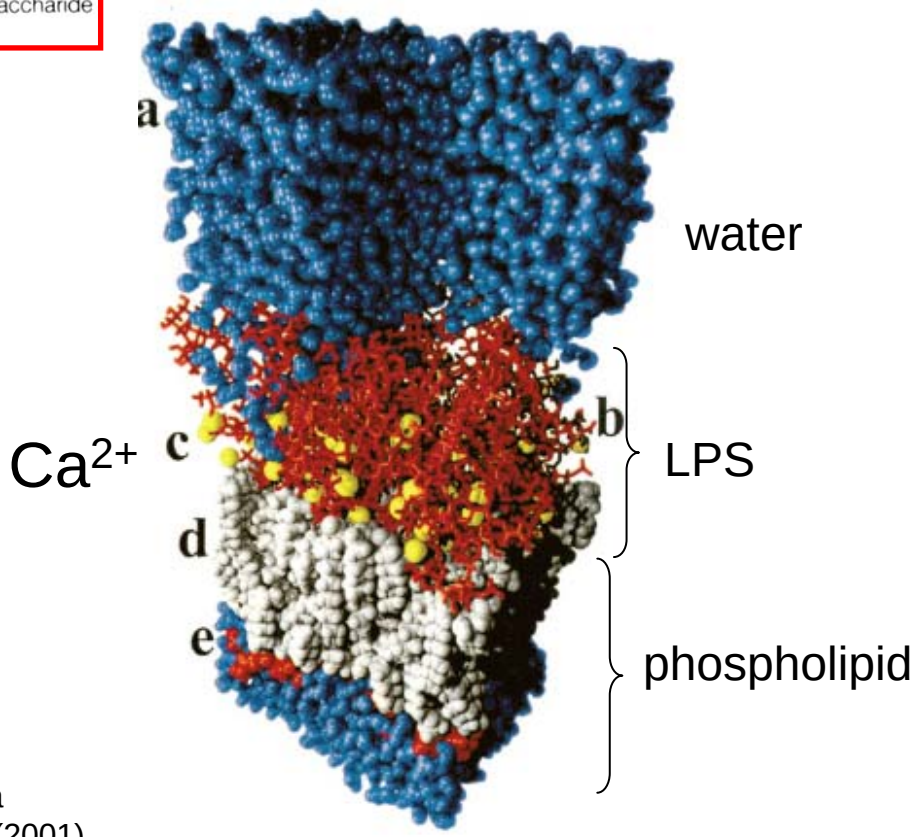
TSA gelatin (solid)

TSB broth (liquid)



Why Calcium?





Ca^{2+}

Roberto D. Lins and T. P. Straatsma
Biophysical Journal ***81,*** 1037–1046 (2001)

Effect of Growth Environment on *P. aeruginosa*

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Spectrochimica Acta Part B 62 (2007) 1169–1176

SPECTROCHIMICA ACTA PART B

www.elsevier.com/locate/sab

Identification and discrimination of *Pseudomonas aeruginosa* bacteria grown in blood and bile by laser-induced breakdown spectroscopy

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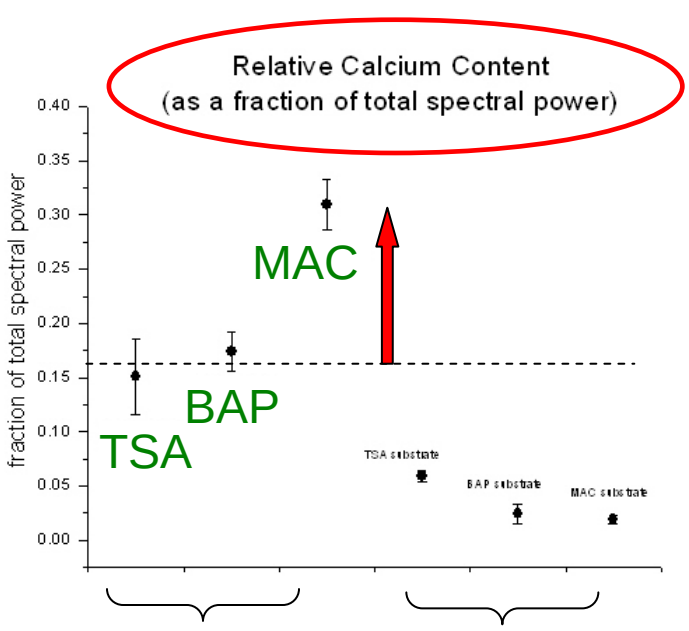
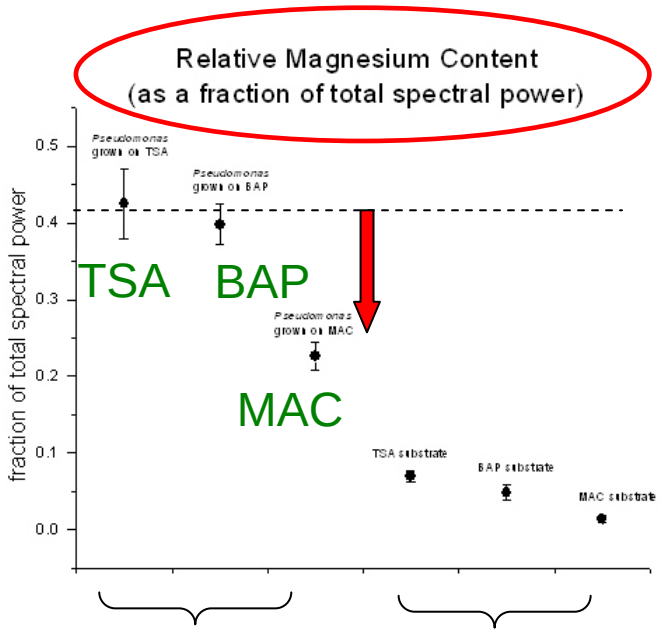
Function 1

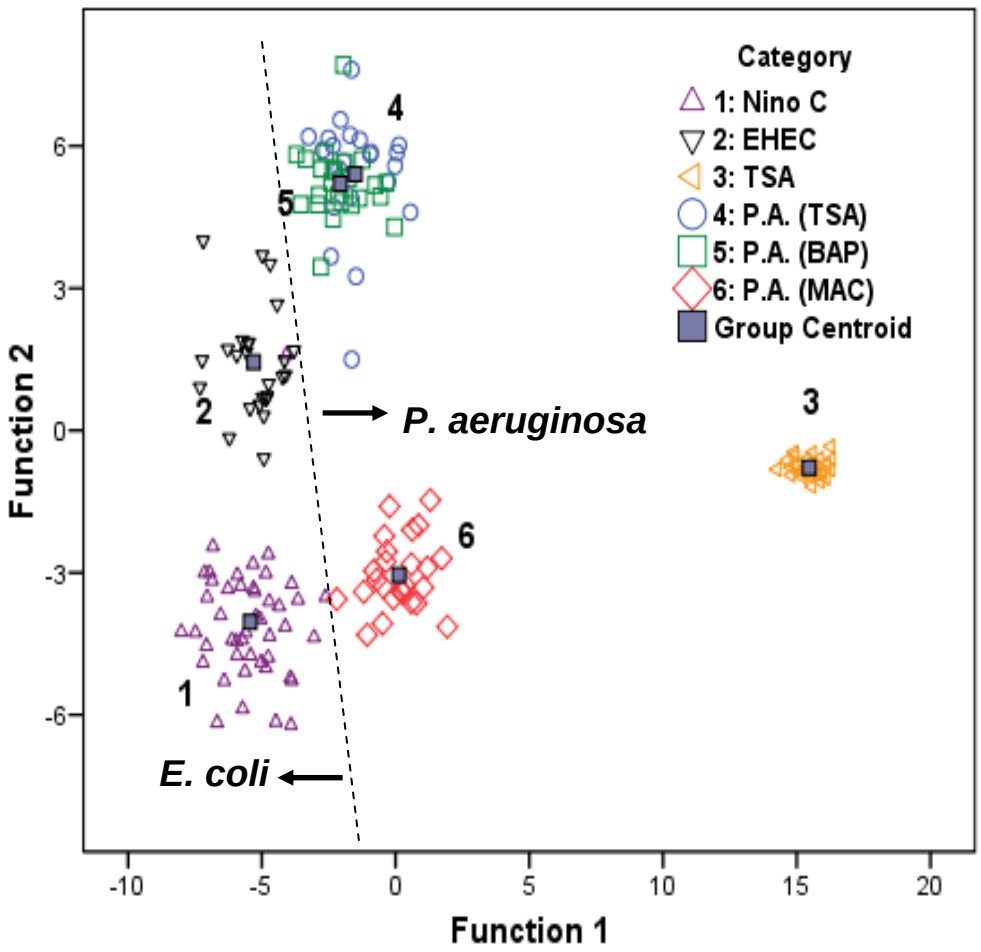
Divalent Cations (Ca^{2+}, Mg^{2+}) Concentrations Are Altered by Environment





Conclusions

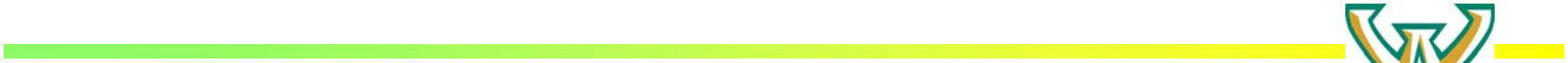
- Divalent cations (Ca^{2+}, Mg^{2+}) are integral to membrane permeability
- LIBS can be used to monitor concentrations of these elements in real-time
- Using this sensitive probe of outer membrane composition, LIBS can be used to distinguish strains of species or between species



Future

- Experiments:
  - Perform all experiments in noble gas environment (argon and helium)
  - Perform Raman spectroscopy on samples prior to ablation

- Issues to be studied
  - Stage of growth
  - Sensitivity (number of bacteria necessary)
  - Mixed cultures / contamination
  - Membrane alteration



Improvements