

2007

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## Recommended Citation

Diedrich, J.; Rehse, Steven J.; and Palchadhuri, S.. (2007). Escherichia coli identification and strain discrimination using nanosecond laser-induced breakdown spectroscopy. *Applied Physics Letters*, 90 (16).  
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Citation: *Appl. Phys. Lett.* **90**, 163901 (2007); doi: 10.1063/1.2723659

View online: <http://dx.doi.org/10.1063/1.2723659>

View Table of Contents: <http://apl.aip.org/resource/1/APPLAB/v90/i16>

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

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(Received 26 January 2007; accepted 15 March 2007; published online 16 April 2007)

Three strains of *Escherichia coli*, one strain of environmental mold, and one strain of *Candida albicans* yeast have been analyzed by laser-induced breakdown spectroscopy using nanosecond laser pulses. All microorganisms were analyzed while still alive and with no sample preparation. Nineteen atomic and ionic emission lines have been identified in the spectrum, which is dominated by calcium, magnesium, and sodium. A discriminant function analysis has been used to discriminate between the biotypes and *E. coli* strains. This analysis showed efficient discrimination between laser-induced breakdown spectroscopy spectra from different strains of a single bacteria species.

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Laser-induced breakdown spectroscopy (LIBS) is a time-resolved spectroscopic analysis technique based on optical emission following pulsed laser ablation of a sample. The technique has numerous advantages stemming from its flexibility, which has allowed it to be utilized to a wide variety of applications.<sup>1,2</sup> In the past three years, several research efforts related to the detection and identification of microbiological samples have been reported.<sup>3-5</sup> These early works were concerned primarily with investigating the ability to discriminate particular microorganisms (usually bacteria used as surrogate of pathogenic bioagents) from “background” biological organisms, often of different biotypes (pollen, molds, etc.) The underlying motivation for most of these studies was the development of a practical, real-time early-warning technology to protect against incidents of bioterrorism.<sup>6-9</sup>

Recent results have shown the utility of using LIBS with femtosecond laser pulses, specifically to identify the *Escherichia coli* bacterium.<sup>10-12</sup> *E. coli* is an ideal candidate for initial studies due to its complete genetic characterization, nonpathogenicity, and ease of preparation. These studies have mostly avoided the issue of realistic sample preparation by analyzing samples which have been harvested from culture, washed, aspirated onto filters, washed again, and then dried to ensure a high signal-to-noise ratio. Only one instance of performing LIBS on an actual *E. coli* bacterial colony has been reported.<sup>13</sup>

While *E. coli* has many nonpathogenic strains, it also has many strains which can be very harmful to humans. Different *E. coli* strains can cause an impressive variety of diseases including dysentery, hemolytic uremic syndrome (kidney failure), bladder infection, septicemia, pneumonia, and meningitis. In general, different strains are associated with different diseases. Therefore the ability to identify particular strains is very important so that outbreaks caused by a particular strain can be identified.<sup>14</sup>

In this letter, we report on the use of LIBS as a technology to differentiate not only between bacterial species and other biotypes but also between strains of a single bacterial

species. In particular, discrimination is shown between three strains of *E. coli* prepared in an identical manner and tested with no sample preparation other than transfer to a suitable ablation substrate. Discrimination is also shown between the *E. coli* strains, an environmental mold, and the *Candida albicans* yeast.

10 ns laser pulses from a Nd:YAG (yttrium aluminum garnet) laser (Spectra Physics, LAB-150-10) operating at 10 Hz at its fundamental wavelength were focused by a 5× high-damage threshold microscope objective to a beam waist diameter of 100 μm. The laser mode was made more Gaussian with an expanding telescope mode cleaner and the resulting laser pulse energy at the beam waist was 8 mJ/pulse. Optical emission from the LIBS microplasma was collected by a 1 m steel encased multimode optical fiber (core diameter=600 μm, numerical aperture=0.22) placed at a distance of 23 mm from the ablation spot with no other light collection optics. This fiber was coupled to an Echelle spectrometer equipped with an intensified charge coupled device (ICCD) camera (LLA Instruments, Inc., ESA3000), which provided complete spectra coverage from 200 to 840 nm with a resolution of 0.005 nm in the UV.

*E. coli* and *C. albicans* samples were prepared in the following way: colonies were grown on a trypticase soy agar (TSA) growth medium for 24 h, and then transferred to the surface of a 0.7% agar plate with a very thin smear. TSA is a rich bacteriological growth medium containing pancreatic digest of casein, soybean meal, NaCl, dextrose, and dipotassium phosphate. The environmental mold was prepared by exposure of a blank TSA plate to environmental conditions until a blackish-looking mold developed. Agar was chosen as a substrate for laser ablation due to its ease of preparation, lack of nutrients which would effect bacterial growth, and relatively high optical breakdown threshold due to optical transparency.

LIBS spectra were acquired for three strains of *E. coli*: a laboratory strain of K-12 (AB) which is completely characterized genetically,<sup>15</sup> a derivative of the same strain termed HF4714, and an environmental strain, *E. coli* C (Nino C), used for the assay of bacteriophage ΦX174. The purity of the cultures of all bacterial strains used in this study was rou-

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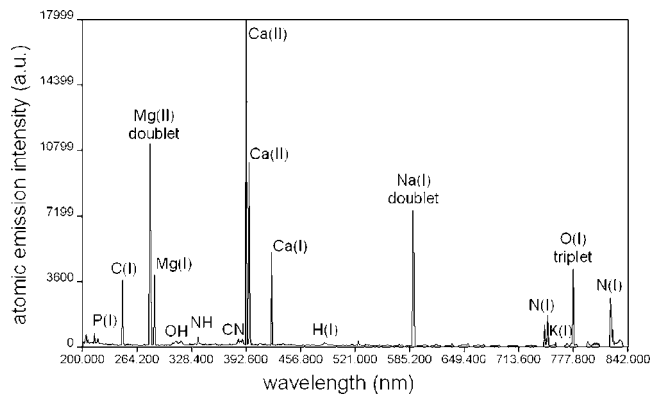


FIG. 1. LIBS spectrum of *E. coli* with the relevant atomic emissions identified.

tinely confirmed by streaking on TSA and MAC-plus lactose plates. Also, spectra were acquired from the TSA growth medium upon which all samples were cultured and the 0.7% agar plates upon which LIBS was performed.

LIBS spectra were acquired at a delay time of 1  $\mu$ s after the ablation pulse, with an ICCD intensifier gate width of 20  $\mu$ s duration. Spectra from ten laser pulses were accumulated on the CCD chip prior to readout. All of the bacteria in the focal region were ablated by these ten pulses. The sample was then translated 250  $\mu$ m and another set of ten laser pulses was averaged. Ten accumulations were averaged in this way, resulting in a spectrum comprised of 100 laser pulses that took approximately 40 s to obtain. Typically 20–30 such measurements could be made from one colony 24 h after initial streaking.

A typical LIBS spectrum from an *E. coli* sample is shown in Fig. 1. As observed by Baudalet *et al.*,<sup>12</sup> the *E. coli* spectrum is dominated by singly ionized and neutral Mg and Ca. This is due to the presence of Mg<sup>+2</sup> and Ca<sup>+2</sup> divalent cations in the outer membrane.<sup>16</sup> The intensities of 19 emission lines from Mg, Ca, P, K, Na, and C were analyzed by nonlinear least squares fitting of a Lorentzian line shape to each emission peak. The 19 areas (intensities) of the emission peaks provided a 1  $\times$  19 array that served as the “spectral fingerprint” of the bacterium. This choice of atoms emphasizes that it is not a genetic difference detected by the LIBS analysis (the analysis only utilizes one element which comprises DNA or proteins), it is rather the difference in the chemical composition of the outer membrane which is detected.

Array elements were normalized by dividing them by the sum of all array elements, the total spectral power, and were then input into a commercial program (SPSS Inc., SPSS V14.0) which performed a discriminant function analysis (DFA). DFA is a statistical methodology similar to the analysis of variance and principal component analysis (PCA) which has been utilized in the past to discriminate biological organisms interrogated with LIBS.<sup>4,5,8</sup> Like PCA, DFA is a data reduction technique that evaluates each 1  $\times$  19 array from  $N$  classification groups utilizing  $N-1$  orthogonal canonical discriminant functions. The canonical discriminant functions are optimized such that the first function (denoted DF1) provides the most overall discrimination between groups, the second function (DF2) provides the second most, etc. Results from DFA, like PCA, are typically displayed in graphical

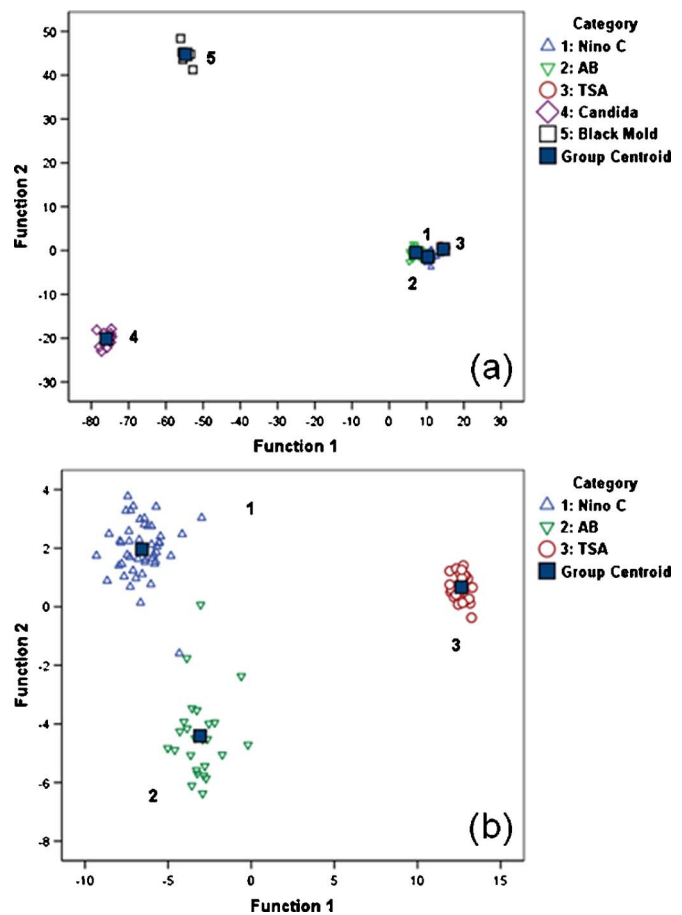


FIG. 2. (Color online) DFA plots of LIBS spectra obtained from the microorganisms in this work. (a) Discriminant function scores of two strains of the *E. coli* bacterium, an environmental mold, the *C. albicans* yeast, and the TSA growth medium. (b) Discriminant function scores of the two *E. coli* strains and the medium on which they were grown showing strain discrimination.

form with each array plotted according to its canonical discriminant function score.

Figure 2(a) shows a DFA plot for two *E. coli* strains (Nino C and AB), the *Candida albicans* yeast, the mold, and the TSA on which they were all grown. Each data array is shown as well as a “group centroid” which is the effective “center of mass” of the distribution. In this analysis, all group memberships were predicated correctly 100% of the time, with 83.3% of the variance between groups represented by DF1 and 15.3% by DF2. The two *E. coli* strains as well as the TSA show similar classification, while the yeast and mold show a discrimination many times greater than the in-group variance. The TSA data points exhibit very little variance and are all grouped under the larger symbol for the group centroid. Such an analysis demonstrates that LIBS can easily discriminate between the microorganism of interest and potential environmental contaminations.

Figure 2(b) shows a DFA plot of just the two *E. coli* and TSA groups shown in Fig. 2(a). In this plot the scale of both axes is expanded compared to Fig. 2(a), indicating the similarity of the spectra. The DFA was able to correctly predict the group membership of the Nino C spectra 97.8% of the time, the AB spectra 96.0% of the time, and the TSA spectra 100% of the time. The specific values of DF1 and DF2 for the groups are not the same as in Fig. 2(a), due to the fact that the canonical discriminant functions are composed

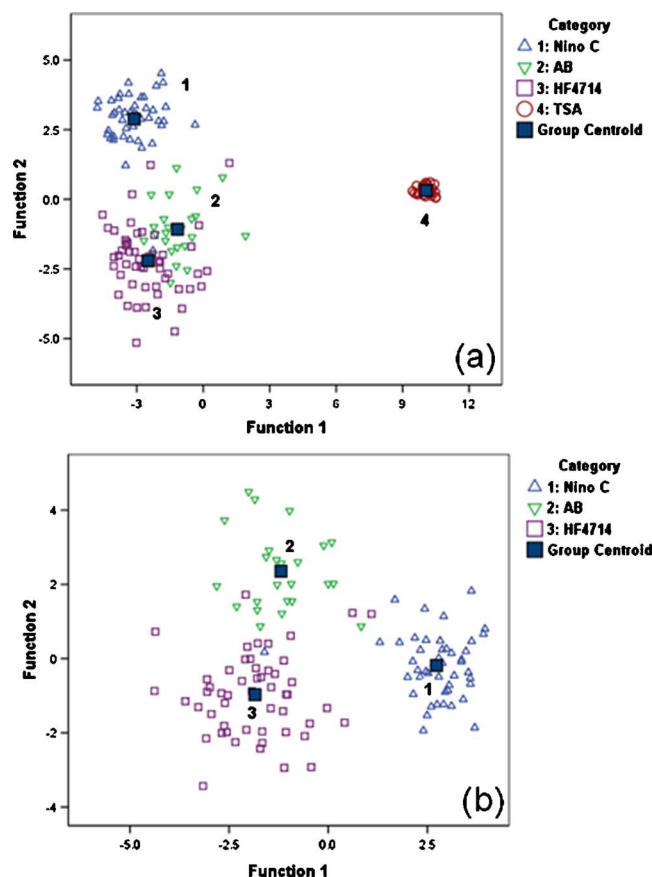


FIG. 3. (Color online) DFA plots of LIBS spectra obtained from *E. coli* bacteria. (a) Discriminant function scores of three strains of the *E. coli* bacterium and the TSA medium on which they were grown. (b) Discriminant function scores of the three *E. coli* strains alone.

solely to maximize differences between the groups used in the current analysis. Figure 2(b) demonstrates that the two different *E. coli* strains have been discriminated with near 100% accuracy.

Figure 3 shows a DFA plot containing the third *E. coli* strain, HF4714, which is a derivative of the AB (K-12) strain. This plot shows that spectra from the two K-12 strains (AB and HF4714) are very similar compared to spectra from the Nino C strain. Figure 3(a) shows the DFA plot for the three *E. coli* strains and the TSA medium on which they were grown. The DFA was able to correctly predict the group membership of the Nino C spectra 97.8% of the time, the AB spectra 96.0% of the time, and the HF spectra 92.3% of the time, with 81.7% of the variance between groups represented by DF1 and 14.2% by DF2. Figure 3(b) shows the DFA plot for the three *E. coli* strains alone. 95.1% of all the original groups were correctly classified, but only 74.6% of the variance between groups was represented by DF1 and 25.4% by DF2. Due to the omission of the TSA data points, the discriminant function scores shown in Fig. 3(b) were constructed to more effectively describe differences between bacteria strains than those shown in Fig. 3(a).

The differences observed in this work between laboratory and environmental strains indicate that it is possible that

a significant difference will exist between nonpathogenic strains of *E. coli* and pathogenic strains such as enterohemorrhagic *E. coli* 0157:H7 (EHEC). Work is currently under way to investigate this possibility. The ability to rapidly identify and discriminate between harmless and pathogenic *E. coli* strains has implications in the study of water-borne illness due to *E. coli*. Due to the difficulties in the isolation and detection of pathogens in waste water and sludge, the use of surrogate (indicator) bacteria has been a standard practice in water quality monitoring for some time.<sup>17</sup> Most *E. coli* strains are not pathogenic and are thus commonly used as an indicator bacterium. The ability to identify the actual pathogenic strain quickly, safely, and by nonmicrobiological specialists would greatly improve the efficiency of this water monitoring.

Although the ability to identify particular strains uniquely responsible for disease would be extremely useful to epidemiologists in a clinical diagnostic setting, the issues of strain identification in mixed cultures and spectrum modification due to growth conditions must first be addressed. By culturing the bacteria on a wide variety of nutrient media, current studies are under way in our laboratory to explore the effect that the growth conditions have on the LIBS spectra of not only *E. coli* but also other Gram-negative bacteria as well.

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