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

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A pathogenic strain of bacteria, *Escherichia coli* O157:H7 (enterohemorrhagic *E. coli* or EHEC), has been analyzed by laser-induced breakdown spectroscopy (LIBS) with nanosecond pulses and compared to three nonpathogenic *E. coli* strains: a laboratory strain of K-12 (AB), a derivative of the same strain termed HF4714, and an environmental strain, *E. coli* C (Nino C). A discriminant function analysis (DFA) was performed on the LIBS spectra obtained from live colonies of all four strains. Utilizing the emission intensity of 19 atomic and ionic transitions from trace inorganic elements, the DFA revealed significant differences between EHEC and the Nino C strain, suggesting the possibility of identifying and discriminating the pathogenic strain from commonly occurring environmental strains. EHEC strongly resembled the two K-12 strains, in particular, HF4714, making discrimination between these strains difficult. DFA was also used to analyze spectra from two of the nonpathogenic strains cultured in different media: on a trypticase soy (TS) agar plate and in a liquid TS broth. Strains cultured in different media were identified and effectively discriminated, being more similar than different strains cultured in identical media. All bacteria spectra were completely distinct from spectra obtained from the nutrient medium or ablation substrate alone. The ability to differentiate strains prepared and tested in different environments indicates that matrix effects and background contaminations do not necessarily preclude the use of LIBS to identify bacteria found in a variety of environments or grown under different conditions.
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I. INTRODUCTION

The rapid detection and identification of microbiological organisms, particularly bacteria, has been an active area of research in the past several years, particularly with the threat of biological warfare attacks and frequently recurring outbreaks of illness caused by food-borne pathogens. Laser-induced breakdown spectroscopy (LIBS) has recently been considered as a candidate for rapid real-time detection of the microbes responsible for such illness both in the field and in a laboratory setting. LIBS is a time-resolved spectroscopic analysis technique based on optical emission following pulsed laser ablation of a sample. The technique has numerous advantages, primarily stemming from its flexibility which allows it to be utilized in a wide variety of applications.[1,2] In the past three years, several research efforts related to the detection and identification of microbiological, particularly bacterial, samples have been reported.[3–5] These early works were concerned primarily with investigating the ability to discriminate particular microorganisms (usually bacteria used as surrogates of pathogenic bioagents) from "background" biological organisms, often of a different biotype (pollen, molds, etc.). The underlying motivation for many of these studies was the development of a practical, real-time early-warning technology to protect against incidents of bioterrorism.[6–9]

Recent results have shown the utility of using LIBS with both nanosecond and femtosecond laser pulses to identify the *Escherichia coli* bacterium.[10–12] *E. coli* is an ideal candidate for initial studies due to its complete genetic characterization, nonpathogenicity, and ease of preparation.[13] As observed by Baudelet *et al.*,[12] *E. coli* is a nonsporulating, Gram-negative bacterium with a specific outer membrane which contains divalent cations, such as Mg^{+2} and Ca^{+2}.[14] Thus the dominant spectral features in the LIBS spectrum of the *E. coli* bacterium are ionized and neutral Mg and Ca emission lines. The creation of a "spectral fingerprint" or full spectral identification of a particular bacterium results from the measurement of emission lines from these and other trace inorganic elements, such as iron, potassium, sodium, manganese, and phosphorus, among others. It is important to note that it is not a genetic difference detected by the LIBS analysis (the typical LIBS analysis does not rely heavily on the elements which comprise DNA or proteins such as carbon, nitrogen, hydrogen, and oxygen), it is rather the differences in the chemical composition of the outer membrane which is detected and which varies between bacterial species as a function of the genetic variation between those species.

These studies performed hitherto have mostly avoided the issue of realistic sample preparation, instead relying on techniques which ensure high-repeatability and high signal-to-noise spectra. For example, *Bacillus globigii* has been grown as spores, harvested by suspension, followed by centrifugation to pelletize the spores then vacuum filtrated

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through silver membrane filters.[4] *E. coli* and *Bacillus subtilis* have been prepared by growth in a nutrient medium and harvested from culture followed by subsequent washing, aspiration onto cellulose nitrate membrane filters, washing again, and then drying to ensure uniform layers of bacteria.[11] Only one instance of performing *in situ* LIBS on an *E. coli* bacterial colony prepared on a nutrient medium has been reported.[15]

While the detection of *E. coli* via the LIBS technique has been studied previously as a bacterial surrogate (e.g., for the pathogen *Yersinia pestis*, which causes plague)[3,15] the use of LIBS to detect and identify *E. coli* is a relevant and important diagnostic problem in its own right. 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 identity particular strains is very important so that outbreaks caused by a particular strain can be identified.[16]

E. coli is also an important indicator of water contamination. High levels of water borne fecal bacteria are associated with an increased risk of disease for people living in coastal areas. Representative genera of bacterial pathogens found in domestic water and sludge include *Escherichia, Salmonella, Shigella, Vibrio, Campylobacter*, and *Yersinia*. Because of 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.[17] Because most strains of *E. coli* are not pathogens (and live in the intestines of healthy humans and animals) it is commonly used as an indicator bacterium. For example, beach water in Michigan showing 300 or more *E. coli* per 100 ml on a single day is considered out of compliance and results in beach closures. The USEPA has recorded that approximately 40 000 km of streams and coastal waters nationally have levels of bacteria that exceed health standards. Clearly, the ability to identify and discriminate between the harmful strains of *E. coli* and the near-ubiquitous nonpathogenic strains would be a great tool for health scientists.

Since current methods for the detection and identification of pathogenic *E. coli* can be time consuming (on the order of 24 to 48 h) due to the need to culture the bacteria to confirm a detection, multiple studies are underway to exploit rapid optical detection and identification strategies which could detect the presence of the pathogenic bacteria, e.g., with a fiber-optic probe.[18,19] Such studies ultimately require the use of a genetic assay (polymerase chain reaction) after optical detection to confirm the identification.[20] As well, these types of fluorescent optical detection techniques require the use of pathogen-specific fluorescently tagged antibodies, which are therefore restricted to the detection of only one pathogen and can have a limited active shelf life. LIBS, in contrast, is not a target-specific optical identification technique and has the potential to discriminate between a wide variety of bacteria with equal efficiency. Rapid LIBS analyses could be performed in concert with, or in place of, these competing optical technologies.

Our group has recently used the LIBS technique to directly compare emission spectra from two laboratory strains of *E. coli* K-12 with an environmental strain.[21] This analysis, which utilized a statistical package similar to principal component analysis known as discriminant function analysis (DFA), proved conclusively the ability of the LIBS technique to discriminate common environmental strains from several laboratory strains of a single species. In this paper we describe the use of LIBS to discriminate between the pathogenic strain of *Escherichia coli* O157:H7 (enterohemorrhagic *E. coli* or EHEC) and the three nonpathogenic strains characterized in the earlier work: a laboratory strain of K-12 (AB) which is completely characterized genetically,[13] 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 EHEC strain, which contains the virulence factors in a lysogenic bacteriophage,[22] closely resembled the laboratory K-12 strains in the LIBS analysis but was easily distinguished from the environmental Nino C strain.

We also report on the effect that changing the nutrient medium used to culture the bacteria had on the LIBS discrimination analysis for two nonpathogenic strains. Significant spectral similarities allowing positive strain identification were observed between spectra from a single strain cultured in two different media (a solid nutrient agar plate and a liquid broth). These spectra were more similar than spectra from the second strain cultured in either medium. This analysis indicates that the use of LIBS to identify bacteria which may have grown in a variety of environments (or hosts) or may have suffered environmental contamination may be practical. While the growth environment does have an effect on the observed bacterial LIBS spectrum in some way, growth in a variety of environments does not preclude the use of the LIBS technique to identify and classify these bacteria.

II. EXPERIMENTAL SETUP

In all the LIBS experiments, 10 ns laser pulses from a Nd:YAG (yttrium aluminum garnet) laser (Spectra Physics, LAB-150-10) operating at its fundamental wavelength were used to ablate the bacteria. The infrared laser pulses were focused by a high-damage threshold 5× infinite-conjugate microscope objective (LMH-5X-1064, OFR). This objective had an aperture of 10 mm, an effective focal length of 40 mm, a numerical aperture of 0.13, and a working distance of 35 mm. This large working distance allowed easy sample manipulation and plasma light collection while the fast focusing provided by the objective's short confocal region allowed extremely precise placement of the beam waist within the bacterial target but not substantially in the substrate material below. Visual examination of single-shot ablation craters on a variety of substrates yielded an average diameter of 100 μm in the objective's focal plane. Typical pulse energies at the ablation surface were 8 mJ/pulse.

Optical emission from the LIBS microplasma was col-

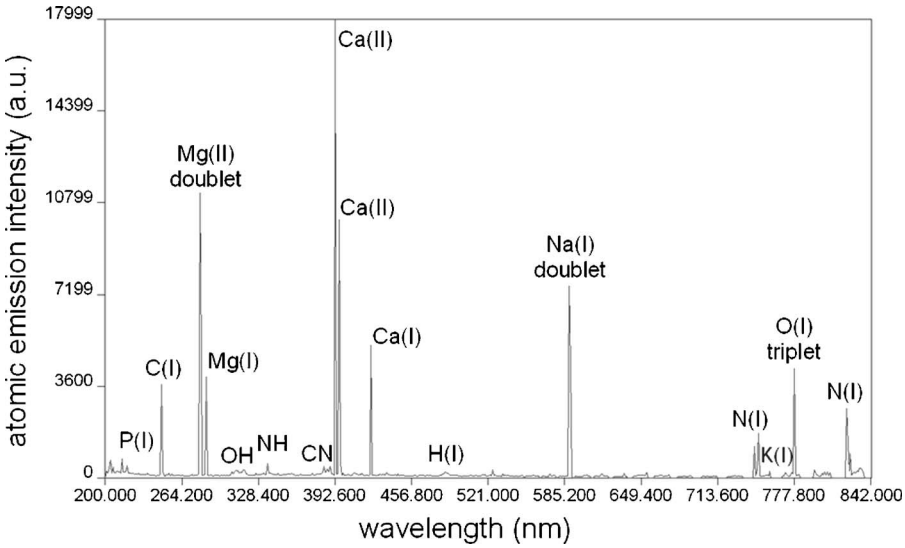

FIG. 1. A LIBS spectrum of *E. coli* with the relevant atomic, ionic, and molecular emissions identified. The spectrum is dominated by emission from inorganic elements.

lected by a 1 m steel encased multimode optical fiber (core diameter=600 μm, numerical aperture (NA)=0.22) placed at a distance of 23 mm from the ablation spot and angled at 30° relative to sample normal with no other light collection optics. This fiber was coupled to an Echelle spectrometer equipped with a 1024×1024 (24 μm^2 pixel area) intensified charge coupled device (CCD) array (LLA Instruments, Inc., ESA3000) which provided complete spectra coverage from 200 to 840 nm with a resolution of 0.005 nm in the UV. The Echelle spectrometer was controlled by a personal computer (PC) running manufacturer-provided software. The PC controlled not only the gating (shuttering) of the intensified ccd (ICCD) but also controlled operation of the pulsed laser via an on-board fast pulse generator to eliminate jitter in the time between laser pulse and plasma observation.

LIBS spectra were acquired at a delay time of 1 μs after the ablation pulse, with an ICCD intensifier gate width of 20 μs duration. This delay time was chosen to maximize emission from the strongest, most obvious emission lines while minimizing the contribution from the broadband background emission. Spectra from ten laser pulses were accumulated on the CCD chip prior to readout. The sample was then manually translated 250 μm 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.

E. coli samples were prepared in two ways. In the first method, all four strains were cultured as colonies on trypticase soy agar (TSA) nutrient medium for 24 h at 37 °C. TSA is a rich bacteriological growth medium containing pancreatic digest of casein, soybean meal, NaCl, dextrose, and dipotassium phosphate. Bacteria were then transferred to the surface of a 0.7% agar plate with a very thin smear. The choice of agar as an ablation substrate was detailed in a previous work and provided a flat, large area substrate with a high-breakdown threshold.[21] The specific advantage of utilizing this highly watery agar was that it did not contribute directly or indirectly to the LIBS spectra of the bacteria. The optical emission from this agar was an order of magnitude smaller than from the bacteria and lacked many of the elements present in the bacteria. Ablation on blank agar substrates was immediately identifiable as lacking bacteria and provided a convenient method for determining when the bacteria target had been "missed" by the ablation laser. But when using such highly watery or liquidlike substrates, it is advisable to use a LIBS delay time on the order of at least 1 μs or even longer to avoid potential complications from the broadband background inherently observed with such substrates.

In the second method, two nonpathogenic strains (HF4714 and Nino C) were cultured in a liquid TS broth (TSB) for 24 h at 37 °C and were then transferred to the surface of an agar plate with a micropipette. No centrifugation or pelletization of the sample was performed prior to the transfer of the liquid samples. A small puddle of bacteria-containing broth was created on the surface of the agar, and after drying of the sample for 5 min, more bacteria-containing broth was deposited on the puddle. Several subsequent depositions resulted in a very thin smooth film of bacteria and dehydrated broth about a centimeter in diameter on the surface of the agar. The purity of the cultures of all bacterial strains used in this study was routinely confirmed by streaking on L-agar plates and MAC-plus lactose plates.

III. EXPERIMENTAL RESULTS AND DISCUSSIONS

A. Pathogenic strain discrimination

Figure 1 shows a typical LIBS spectrum from *E. coli* bacteria. The spectrum is dominated by emission from Ca, Mg, and Na. To a lesser extent, emission from K, P, C, and atmospheric O and N is visible. Molecular emissions from OH, NH, and CN are also visible. The identification of all spectral features observed in the LIBS spectra from the *E. coli* bacteria, the TSA growth medium, and the agar substrate is given in Table I. The observed molecular bands are ascribed to the recombination of ablated atomic species (often with atmospheric oxygen and nitrogen) rather than "native" bonds as has been observed in femtosecond LIBS on

TABLE I. Resolved spectral emission lines observed in LIBS spectra acquired from *Escherichia coli* (Strain Nino C), the nutrient-rich growth medium TSA, and the nutrient-free 0.7% agar substrate on which the bacterial samples were ablated. The atomic and molecular lines/bands are identified and categorized by whether they were visible in the bacteria only (1), in the bacteria and TSA (2), or in the bacteria, TSA, and agar (3).

Wavelength (nm)	Line identification	Source
213.618[a]	P I	1
214.914[a]	P I	1
247.856[a]	C I	3
253.560[a]	P I	1
279.553[a]	Mg II	3
280.271[a]	Mg II	3
285.213[a]	Mg I	2
306.3, 306.7, 307.8, 308.9	OH: $A\,^2\Sigma^+ - X\,^2\Pi$ (0-0)	3[b]
315.887	Ca II	1
317.933	Ca II	1
336.0	NH: $A\,^3\Pi - X\,^3\Sigma^-$ (0-0)	3[b]
337.0	NH: $A\,^3\Pi - X\,^3\Sigma^-$ (1-1)	3[b]
373.690[a]	Ca II	1
383.231[a]	Mg I	1
383.829[a]	Mg I	1
386.19	CN: $B\,^2\Sigma - X\,^2\Sigma$ (2-2)	1[b]
387.14	CN: $B\,^2\Sigma - X\,^2\Sigma$ (1-1)	1[b]
388.34	CN: $B\,^2\Sigma - X\,^2\Sigma$ (0-0)	1[b]
393.366[a]	Ca II	3
396.847[a]	Ca II	3
410.175	H I (H_δ)	3[c]
422.673[a]	Ca II	2
430.253[a]	Ca I	1
434.047	H I (H_γ)	3[c]
436.826	O I	3[b]
445.478	Ca I	1
486.134	H I (H_β)	3[b]
518.361[a]	Mg I	1
585.745[a]	Ca I	1
588.995[a]	Na I	3
589.593[a]	Na I	3
744.229	N I	3
746.832	N I	3
777.194	O I	3[b]
777.417	O I	3[b]
777.539	O I	3[b]
769.896[a]	K I	2
821.634	N I	3[b]
822.314	N I	3[b]
824.240	N I	3[b]

[a]The 19 emission lines used in the discriminant function analysis.
[b]Spectral line enhanced by, or attributed to, ablation under atmospheric (oxygen- and nitrogen-rich) conditions.
[c]Significantly Stark-broadened spectral lines.

bacteria.[11] This spectrum represents one measurement of the bacterial colony and is comprised of 100 laser shots accumulated in sets of ten acquired at 10 different locations. The intensities of 19 emission lines from Mg, Ca, P, K, Na, and C were analyzed in every spectrum by nonlinear least squares fitting of a Lorentzian line shape to the area under the line. These 19 lines are identified in Table I. The 19 areas (intensities) of the emission peaks were called "independent variables" which composed a 1×19 array that served as the spectral fingerprint of the bacterium.

These arrays of independent variables were normalized by dividing by the sum of all array elements, which is called the total spectral power, and were then input into a commercial program (SPSS Inc., SPSS V14.0) which performed a DFA. DFA is a statistical methodology similar to analysis of variance (ANOVA) and principal component analysis (PCA) which has been utilized in the past to discriminate biological organisms interrogated with LIBS.[4,5,8] Like PCA, DFA is a data reduction technique. It evaluates each 1×19 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. The canonical discriminant functions which are also arrays with 19 coefficients (and a constant offset) are then multiplied by the array containing the independent variables for each spectrum. The resulting vector products are called the canonical roots or "discriminant function scores" and are given by $\mathrm{DF}^j = b_0^j + \Sigma_{k=1}^{19} b_k^j x_k$, where DF^j is the jth discriminant function score, b_0^j is the constant offset of the jth canonical discriminant function, b_k^j are the coefficients of the jth canonical discriminant function, and x_k are the independent variables (the emission line intensities). In this way, $N-1$ canonical discriminant function scores were calculated for each spectrum and these scores reflected specific, statistically significant, and quantifiable differences in atomic emission intensities which were related to differences in the elemental composition of the bacteria targets.

Results from DFA, like PCA, are typically displayed in graphical form with each array or spectrum plotted according to its canonical discriminant function scores. Discriminant function 1, representing the most distinct or obvious differences between spectra, is used as the abscissa, while discriminant function 2 is used as the ordinate. Discrimination among many groups will possess more than two canonical discriminant function scores, but these typically perform very little discrimination and are not usually utilized [to construct three-dimensional (3D) plots of results, for example].

Figure 2(a) shows a DFA plot for all four *E. coli* strains and the TSA on which they were all cultured. Each measurement is shown as well as a "group centroid" which is the effective "center of mass" of the distribution of measurements. In this analysis, although four canonical discriminant functions were used to characterize the five groups, most of the discrimination was performed by the first two functions. In particular, 81.1% of the variance between groups was represented by DF1 and 14.4% by DF2. Therefore it is neither necessary nor particularly illustrative to show plots utilizing the higher discriminant functions, although such can certainly be done. DF1 provided the primary discrimination between growth medium and bacteria, while DF2 provided the primary score for discrimination between the bacteria. An analysis of the coefficients of discriminant function 1 indicated that it was the sodium concentration that provided the primary discrimination between the nutrient medium and the bacteria. Typically, most nutrient media have considerably

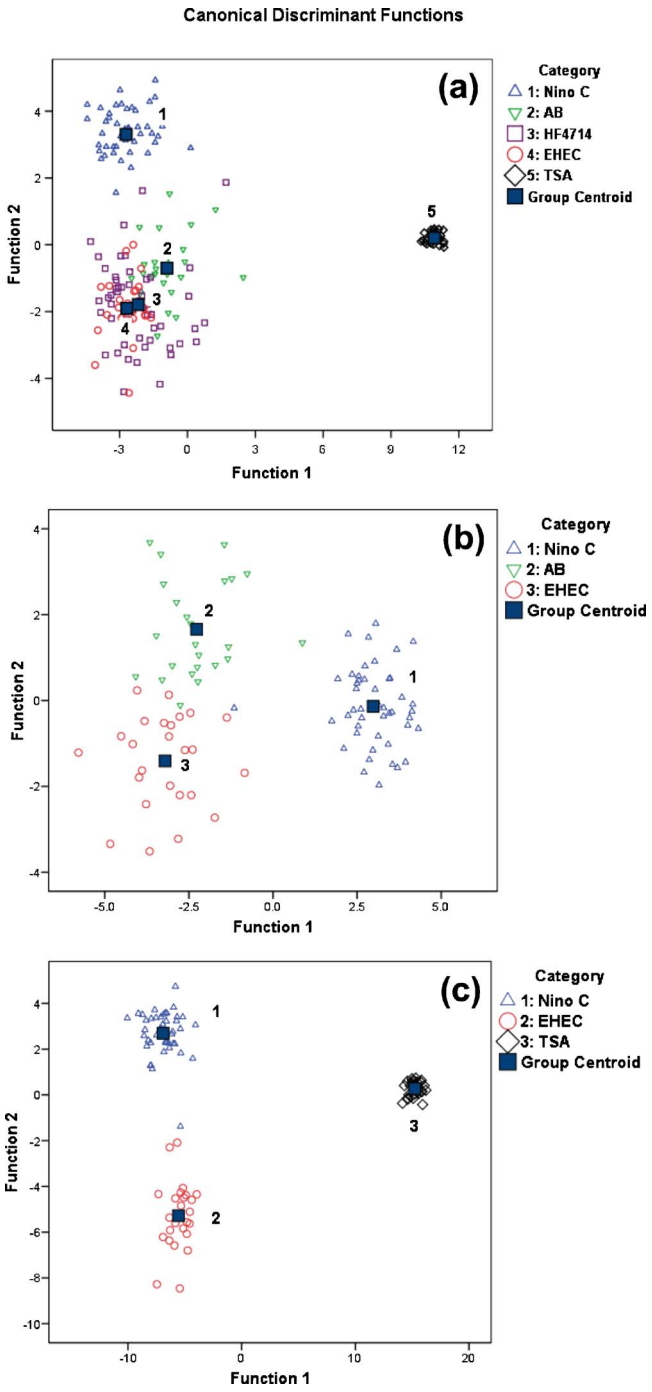

FIG. 2. (Color online) DFA plots of LIBS spectra obtained from various strains of *E. coli*. (a) Canonical discriminant function scores of four strains of *E. coli* and the TSA nutrient medium on which they were prepared. (b) Canonical discriminant function scores of three *E. coli* strains (strain HF4714, a K-12 derivative, has been omitted). (c) Canonical discriminant function scores of the TSA nutrient medium and two strains of *E. coli*: pathogenic EHEC and the environmental strain Nino C. Almost complete discrimination is achieved between these two strains using DF2.

higher sodium concentrations than the bacteria. The bacteria all have very similar DF1 scores, as their relative concentrations of sodium are all similar (smaller). The coefficients of DF2 indicated that it was primarily a difference in the relative concentration of calcium, and to a lesser extent potassium and phosphorus, which provided the discrimination between the bacteria. In Fig. 2(a) the environmental Nino C strain (group 1) as well as the TSA nutrient medium (group 5) were easily discriminated, with 97.8% and 100%, respectively, of the original grouped cases being correctly classified. The pathogenic EHEC strain and the two laboratory K-12 strains, however, showed highly similar DF1 and DF2 scores. In their original groups, AB, HF4714 and EHEC were correctly classified only 84%, 73.1%, and 88% of the time. This similarity was unsurprising given that both AB and HF4714 are variants of the K-12 strain and that EHEC has recently been found to be highly related genetically to the laboratory strain K-12.[23]

Because the centroids for EHEC and HF4714 possessed nearly identical DF1 and DF2 scores, all the data for HF4714, as well as for the TSA medium, were omitted from the analysis presented in Fig. 2(b). When only the EHEC, Nino C, and AB strains were evaluated using DFA, the results improved significantly. In this analysis 86.9% of the variance between spectra was represented by DF1 and 13.1% by DF2. Of the original grouped cases of Nino C, AB, and EHEC, 97.8%, 92.0%, and 100%, respectively, were correctly classified. This represented a significant improvement over the analysis presented in Fig. 2(a). In Fig. 2(b), DF1 provided the discrimination between the Nino C spectra (positive DF1 scores) and the more similar AB and EHEC strains (negative DF1 scores). This discrimination was based primarily on small differences in the calcium concentrations in these three strains. All but one Nino C sample possessed a positive DF1 score, while all but one of the samples in the other two groups possessed a negative DF1 score. DF2 was then used to discriminate between the similar EHEC and AB spectra. An analysis of the coefficients of discriminant function 2 revealed that it was primarily differences in the amount of magnesium that provided the discrimination between EHEC and AB. Only one AB sample possessed a negative DF2 score, while all but two EHEC samples possessed a negative DF2 score. Based on these two canonical discriminant functions, a near complete identification and discrimination between these three strains was achieved.

Lastly, an analysis was performed utilizing only the nutrient medium, strain EHEC, and strain Nino C. This was perhaps the most realistic test, as the original intent of the experiment was to investigate the ability to discriminate a pathogenic strain (which would occur in food or water contamination) with a commonly occurring environmental *E. coli* strain which also might be present, but harmless, in food or water. The results of this DFA are shown in Fig. 2(c). In this analysis, 90.6% of the variance between spectra was represented by DF1 and 9.4% by DF2 (only two canonical discriminant functions can be formed from three data groups). Again, it was the relative sodium concentration which provided the discrimination of DF1. The Nino C was correctly classified 97.8% of the time and the EHEC was correctly classified 100% of the time. These two strains showed the greatest absolute difference in canonical DF score of any two bacterial strains, indicating strong spectral dissimilarities which could form the basis of a discrimination test when only these two strains are suspected of being present. All but one of the Nino C samples (a clear outlier) possessed a positive DF2 score, with a group centroid DF2

score of 2.69. All EHEC samples possessed a DF2 score below zero with a group centroid DF2 score of −5.28. The difference in scores between groups is several times greater than the intragroup variations in DF2 score, allowing for a unique identification and discrimination. An analysis of the DF2 coefficients revealed that the concentration of calcium, followed by the concentrations of potassium and phosphorus, again contributed most significantly to the discrimination provided by DF2.

B. Identification of nonpathogenic strains grown in different media

Figure 3 shows the results of the DFA performed on LIBS spectra acquired from *E. coli* HF4714 and Nino C prepared on solid (open symbols) TSA and in liquid (closed symbols) TSB as described above. Group 1 is HF4714 cultured in TSB. Group 2 is HF4714 cultured on a TSA plate. Group 3 is Nino C cultured on a TSA plate. Group 4 is Nino C cultured in TSB. Figure 3(a) shows the canonical discriminant function scores of the two strains grown in these two media as well as the TSA nutrient medium itself. In this analysis, 85.1% of the variance between spectra was represented by DF1 and 13.9% by DF2. All other functions contributed less than 1% to the variance. Of the original grouped cases, 95.6% of all data sets were correctly classified. While DF1, based on the sodium concentrations, discriminated between the bacteria and the medium, the discrimination between strains was provided by DF2, with all HF4714 samples having a positive DF2 score and all Nino C samples having a negative DF2 score. The DF2 scores of the group centroids for the grouped cases 1–4 as shown in Fig. 3(a) were 2.23, 6.19, −2.75, and −5.15, respectively. It is important to note that because all four bacteria samples possessed a highly similar DF1 score (approximately −5) it was not possible to interpret these results as being due to contamination of the spectra by the nutrient media. If there had been residual contribution from the TSA and the TSB media, it would have been reflected in differences in the DF1 score for the four samples. Specifically, the two samples grown in TSB would be expected to have the same DF1 score, which would be much more similar to the TSA medium DF1 score (+15) than the bacteria DF1 score (−5). The fact that the samples cultured in TSB possessed identical DF1 scores to the samples cultured on TSA indicates that contamination by the medium did not occur. Such sample contamination would also have manifested itself in the discrimination based on DF2 scores, where one would expect the samples cultured in TSB to have identical scores. This was clearly not the case.

While the Nino C strain samples possessed a similar DF2 score regardless of whether they were cultured in TSB or on a TSA plate, HF4714 samples possessed different scores when prepared in these ways, showing some discrimination. This difference was not unanticipated, given the varying ability of these two strains to interact with their environment. It is known that strain HF4714 possesses a greater ability to interact with its surrounding environment, while Nino C is a much more inert strain, as indicated by the DFA.

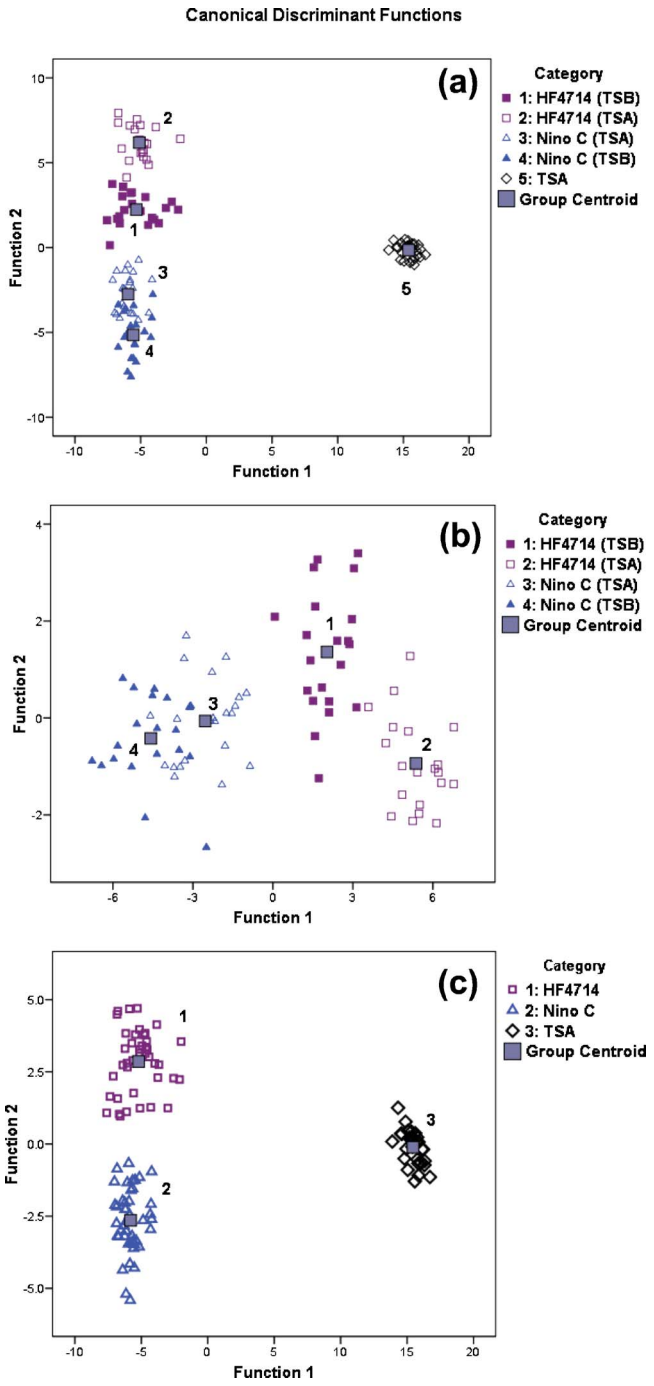

FIG. 3. (Color online) DFA plots of LIBS spectra obtained from *E. coli* strains HF4714 and Nino C cultured on a TSA plate (open symbols) and in a TSB (liquid) medium (closed symbols). (a) Canonical discriminant function scores of the two strains and the TSA nutrient medium. DF2 scores provided discrimination between strains regardless of what medium the bacteria were cultured in. (b) Canonical discriminant function scores of the two strains alone. DF1 scores provided the discrimination between strains regardless of what medium the bacteria were cultured in. (c) Canonical discriminant function scores of the two strains grouped together regardless of nutrient medium as well as the TSA.

Figure 3(b) shows the same analysis with the TSA nutrient medium data omitted. In this analysis, 92.2% of the variance between spectra was represented by DF1, 4.5% by DF2, and an almost equal 3.3% by DF3. A complete discrimination between strains was provided by DF1, which was constructed primarily from differences in the relative intensities

of calcium and magnesium between strains. All HF4714 samples possessed a positive DF1 score and all Nino C samples possessed a negative DF1 score. The DF1 scores of the group centroids for the four grouped cases 1–4 were 2.03, 5.37, −2.54, and −4.58, respectively. Of the original HF4714 grouped cases, 100% of the samples cultured in TSB and 95% of the samples cultured on TSA were correctly classified. Of the original Nino C grouped cases, only 91.3% of the samples cultured on a TSA plate and 95.0% of the samples cultured in TSB were correctly classified. Again, this indicates that the Nino C spectra were much more self-similar than the HF spectra were.

Figure 3(c) shows the discriminant function analysis when all the spectra were grouped together by strain, regardless of what medium the samples were cultured in. In this analysis, 93.9% of the variance between spectra was represented by DF1 and 6.1% by DF2. 100% of the original grouped cases were correctly classified, with no spectra being misidentified. DF1 provided clear discrimination between the bacteria strains and the nutrient medium, while DF2 provided a clear discrimination between the two strains regardless of preparation in the liquid broth or on the TSA plate. Analysis of the coefficients used by DF2 indicated that it was again primarily differences in the calcium and magnesium intensities that provided such efficient discrimination. Use of these canonical discriminant functions would thus correctly identify the strain of the bacteria no matter which medium it was cultured in, showing an insensitivity to the initial state of the bacteria (a positive quality for a widespread discrimination and identification technique).

Figure 3 demonstrates that a discrimination between strains was possible even for bacteria cultured in (or on) different media. As evidenced by the canonical discriminant function scores for the group centroids, the difference between samples prepared in liquid or on solid plates was not as great as between different strains. As well, Fig. 3 demonstrates explicitly that spectral "background contamination" due to ablated residual growth medium (which was present in both samples grown in TSB) did not obscure the identification and discrimination between strains. Further studies are underway to investigate the effects that culturing the bacteria on alternate nutrient media (such as in blood, sputum, or bile) has on the LIBS spectra.

IV. CONCLUSIONS

Nanosecond laser-induced breakdown spectroscopy has been used to identify and discriminate between four strains of the *E. coli* bacteria, including one pathogenic to humans (EHEC). A discriminant function analysis was used to analyze the LIBS spectra from bacteria colonies cultured on TSA plates and in a liquid TS broth nutrient medium. The DFA clearly showed a discrimination between a common environmental strain of *E. coli* (Nino C) and the pathogenic EHEC. One nonpathogenic laboratory strain of *E. coli* K-12 (AB) possessed spectra more similar to EHEC than the Nino C but was still distinguishable, while a derivative of the laboratory K-12 strain (HF4714) was almost indistinguishable from EHEC. The ability to discriminate an environmental strain from a pathogenic strain suggests the possibility of using LIBS as a practical diagnostic test to identify strains obtained from environmental assays.

The DFA of LIBS spectra obtained from two nonpathogenic strains grown in two different media demonstrated the ability to discriminate between two different strains regardless of the growth environment. While spectral differences were, in fact, seen between samples cultured on TSA and in the liquid TS broth, these differences were smaller than that seen between the different strains. The ability to discriminate strains even when prepared in different environmental conditions and tested with differing amounts of residual nutrient medium contamination is a strong indicator of the robustness of the LIBS technique when used for bacterial analysis. Different strains of bacteria may be more or less responsive to the environment in which they are grown, which may alter the level of discrimination possible or the ability to uniquely identify bacteria without knowing its growth environment. However, this result suggests that growth in a different environment or host does not necessarily preclude the use of LIBS as a diagnostic technique. Much progress needs to be achieved before LIBS can be considered a practical clinical diagnostic technique, however. The issues of sample dilution and limits of detection, sample mixing and contamination, as well as changes of the LIBS spectrum due to growth in a myriad of environments need to be addressed. Experiments are currently underway in our laboratory utilizing several different species of bacteria grown on a variety of nutrient media to address these issues.

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