Spectral signature of native CN bonds for bacterium detection and identification using femtosecond laser-induced breakdown spectroscopy

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(Received 30 August 2005; accepted 8 December 2005; published online 7 February 2006)

A sample of *Escherichia coli* has been analyzed by laser-induced breakdown spectroscopy (LIBS) using femtosecond pulses. The spectrum shows strong CN molecular bands due to the direct ablation of native CN molecular bonds from the bacteria in contrast with weak atomic lines from carbon. The native nature of the observed CN bonds is supported by the kinetic behavior of the CN band head which rapidly decays with a time constant of 94 ns, while for a pure graphite sample the CN band head increases with a delay of 450 ns due to recombination with the ambient air. Moreover, about hundred resolved lines belonging to 12 atomic or molecular species are recorded, providing a valuable spectral signature to identify the bacterium. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2170437]

Laser-induced breakdown spectroscopy (LIBS) is a laser-based analysis method that exhibits numerous advantages.[1–3] However, only a few works related to analysis of biological samples by LIBS have been reported so far. Detection of microbiological samples (for example bacteria) has become urgent because of the threats of biological warfare[4–7] and epidemic spreads. Apart from technical improvements dedicated to the increase of sensitivity in order to detect single bioaerosol particles,[8–10] major efforts have been drawn to extract relevant information from spectroscopic data for either biological samples being detected from background or a targeted biological species being distinguished from the others.[4–6]

Application of the LIBS technique to organic and *a fortiori* biological samples is indeed far from a straightforward extension of the well-established analysis method for inorganic samples. The main reason is that a biological sample, such as bacteria, cannot be totally characterized by elemental atomic particles which compose the material. One more relevant observable would be a signature from a functional group which characterizes the biomolecules constituting the sample.[11] For amino acids, for example, a characteristic functional group is the amino group which consists of one atom of nitrogen and two atoms of hydrogen. Attached to a carbon atom, an amino group provides a CN molecular bond in a molecule of amino acid. The observation of CN molecular bands in LIBS spectra would therefore provide a reliable marker for biological samples. However, it has been established in previous works using nanosecond LIBS analysis of bacteria that the observed CN bands were due to the formation of CN molecular radicals by recombination between C_2 available in the plasma plume and N_2 from the ambient air.[4,5] Although easily observable in a LIBS spectrum of a biological sample, the CN bands could thus not be considered as a reliable spectral marker so far. Another group of elements that would provide a characterization of a microbiological sample consists of mineral species, since they participate in metabolism of bacteria.[11]

In this letter, we report LIBS analysis using femtosecond pulses (Femto-LIBS)[12,13] of a bacterial sample: *Escherichia coli*. We demonstrate that the CN molecular bands observed in the femtosecond ablation regime are due to CN bonds directly ablated from the bacteria and can be distinguished from the contribution due to recombination with ambient air using a kinetic analysis of the molecular band head intensity. Furthermore, the lower initial value and the quicker decay of the temperature of a femtosecond laser-induced plasma[14,15] allows the detection time window to be opened earlier after

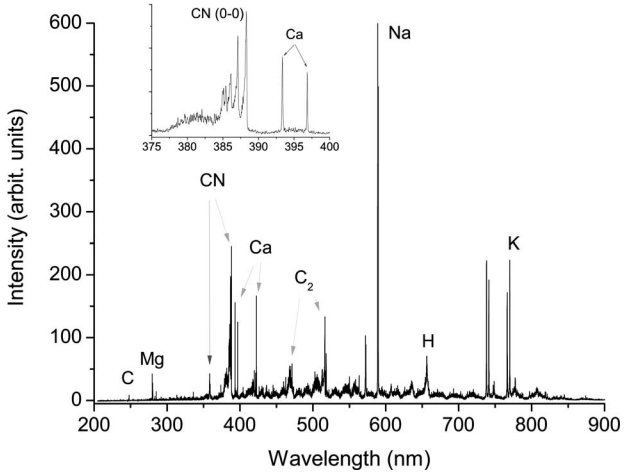

FIG. 1. Time-integrated femtosecond LIBS spectrum of *Escherichia coli*.

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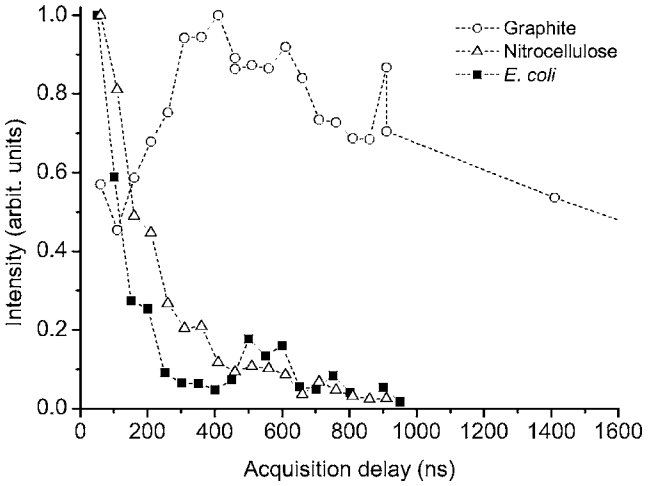

FIG. 2. Kinetics of the CN (0-0) band head intensity for graphite, nitrocellulose filter, and *Escherichia coli*. The signals are normalized by the maximum in each sample.

the laser shot, which provides high contrast for the emission lines of trace mineral elements from the bacteria.

In our experiments, a chirped pulse amplification laser system provided 4.5 mJ, 120 fs, and 810 nm pulses at a repetition rate of 20 Hz. Laser pulses were focused by a single lens of 30 mm focal length on the surface of the samples. The resulted beam waist was about 100 μm, resulting in a fluence of 14 J/cm^2. Samples were prepared according to the following process: Growth of bacteria overnight in liquid Luria Broth at 37 °C under aeration; impact by aspiration of 20 mℓ solution of bacteria on a filter; washing of the charged filter by 20 mℓ distilled water with the same aspiration process; indoor drying for about an hour. Nitrocellulose filters were finally used instead of silver filters,[4] to avoid low-photon excited plasma emission. We observed in the same experimental conditions spectra of a filter charged with bacteria, a filter charged with the nutrition medium and an unexposed filter. The samples were fixed on a rotation stage, allowing a fresh sample spot for each laser shot. Optical emission was collected by a lens of 50 mm focal (f No.=2), and coupled, via an optical fiber, to an Echelle spectrometer equipped with an intensified charge coupled device camera (Andor Technology, Mechelle and iStar).

Figure 1 shows a time-integrated spectrum from a bacteria-charged filter. The spectrum is obtained with an accumulation of 1000 laser shots, with a time detection window of 5 μs and a delay of 100 ns after each laser pulse. One should note the predominance of CN and C_2 molecular bands, and the relatively weak emission from atomic carbon. A detailed spectrum of a CN molecular band around 388 nm is shown in Fig. 1. The predominance of CN and C_2 molecular bands over relatively weak carbon atomic lines suggests significant ablation of native CN and C_2 molecular bonds from the bacterial sample. However, as has been pointed out in previous works on bacterial analysis using LIBS in the nanosecond regime,[4,5] this molecular band could also be due to recombination on the interface of the plasma plume in contact with ambient air.[16] In this case, the observed CN band head intensity increased with a time constant of several hundred of nanoseconds after the laser shot. A kinetic study has thus been carried out to distinguish the different mechanisms for CN molecular bond formation.

TABLE I. Resolved spectral lines in a time-integrated spectrum of *Escherichia coli* and identified atomic and molecular species. A specific spectral line can be observed either: (1) Only in the bacteria (*N.B.*: 1) (2) in the bacteria and in the nutritional medium (*N.B.*: 2), (3) in the bacteria, in the nutritional medium and in the filter (*N.B.*: 3), or (4) in the bacteria and in the filter (*N.B.*: 4).

λ(nm)	Species	NB
247.86	C I	3
279.54	Mg II	2
280.28	Mg II	2
283.43	-	1
285.17	Mg I	2
313.18	Fe II	1
313.43	Fe II	1
317.94	Ca II	1
336.00	O II	3
358.26	CN	4
358.34	CN	3
358.57	CN	3
358.99	CN	3
373.78	Fe I	1
385.10	CN	3
385.39	CN	3
386.13	CN	3
387.08	CN	3
387.80	Fe I	1
388.29	CN	3
393.35	Ca II	3
396.83	Ca II	3
404.42	K I	1
415.74	CN	3
416.70	CN	3
418.07	CN	3
419.65	CN	3
421.52	CN	3
421.05	Fe I	1
421.39	Fe I	1
422.66	Ca I	3
426.24	-	1
430.28	Ca I	1
432.04	-	1
441.49	O II	3
443.52	Fe I	1
444.70	N II	3
445.51	Ca I	4
459.90	Fe I	1
462.63	P II	1
467.81	C2	3
467.96	C2	3
468.46	C2	3
469.71	C2	3
471.45	C2	3
473.65	C2	3
500.58	N II	3
512.87	C2	3
516.45	C2	3
516.71	Mg I	1
517.23	Mg I	1
517.37	Mg I	1
550.10	Fe I	1
558.46	C2	3
563.47	C2	3
572.15	-	2
572.72	-	2

TABLE I. (*Continued.*)

λ(nm)	Species	NB
574.37	-	1
589.01	Na I	3
589.59	Na I	3
607.18	-	1
625.94	-	1
635.59	-	3
656.08	H I	3
711.83	Fe I	1
720.16	Ca I	4
738.30	-	2
741.36	-	2
742.44	N I	3
744.33	N I	3
746.94	N I	3
747.35	Fe I	1
748.62	N I	3
766.55	K I	3
769.92	K I	3
777.47	O I	3
795.26	O I	3
797.12	-	1
818.37	Na I	3
818.86	N I	3
819.44	Cl I	3
824.34	N I	3
833.62	-	3
844.73	O I	4
854.20	Ca II	3
856.68	N II	3
859.63	-	1
863.14	N I	3
868.15	N I	3
870.46	N I	3
871.28	N I	3
871.95	N I	3
874.06	P I	1
881.93	O I	3

Three different types of samples have been used for this study: Bacteria containing native CN bonds, a nitrocellulose filter that contains nitrogen and carbon atoms as elementary constituents, and a pure graphite sample containing exclusively carbon rings. Time-integrated spectra of these samples showed similar spectra of the molecular bands of interest. The results of a time-resolved kinetic study on the intensity of CN (0-0) band head are presented in Fig. 2. The series of kinetic spectra have been recorded with an automatically shifted detection gate of 50 ns width. For the graphite sample, CN bond formation due to a recombination with ambient air is clearly observed. The band head intensity increases and reaches a maximum at a delay of 450 ns. Dramatically different behavior has been observed for either *Escherichia coli* or nitrocellulose filter. The band head intensity decays rapidly for both of these two samples, with an exponential decay time constant of (94 ± 20) ns for bacteria and (185 ± 35) ns for the filter. These results show that a carbon containing graphite sample can be distinguished unambiguously from an organic component containing simultaneously carbon and nitrogen or a biological sample which contains inevitably native CN bonds. The specific distinction between the last two types of samples needs more precise quantitative comparison between decay time constants. Different samples ablated with different laser parameters could present *a priori* different time constants. However, as shown by our result, we expect a shorter decay time constant for a biological sample than for an organic carbon and nitrogen containing sample since recombinations in the plasma between atomic carbon and nitrogen lead to a longer decay time constant.

A detailed analysis of the spectrum allowed us to isolate 94 spectral lines. The identification of these lines using atomic and molecular spectral data basis[17,18] results in a total number of 12 different emitters belonging to three groups of atomic or molecular species: Mineral elements (neutral or ions); organic elements; and molecular bonds. A summary of the spectral identification is given in Table I.

In conclusion, we have reported the LIBS analysis of *Escherichia coli* using femtosecond pulses. We demonstrated that a kinetic study of the CN band head intensity allows an identification of the contribution of native CN molecular bonds from the biological medium. The ensemble of the spectral information, atomic lines, as well as molecular bands due to native CN bonds, provides spectral signatures of the studied bacterium. Our observation on the kinetics of an intramolecular bond as the spectral marker of a bacterial sample should be also applied to other biospecies or even to other organic materials in general, since as we pointed out above such media are characterized by molecular functional groups. The association of significant and relevant molecular spectral information with atomic spectral information makes femtosecond LIBS a powerful analytical tool to address organic or biological samples.

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