Spectral Fingerprints of Bacterial Strains by Laser-Induced Breakdown Spectroscopy

T. Kim, Z. G. Specht, P. S. Vary,† and C. T. Lin*

Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, Illinois 60115-2862

Received: November 25, 2003; In Final Form: February 4, 2004

Laser-induced breakdown spectroscopy (LIBS) is used to record the plasma emission for the colonies of vegetative cells or spores of five bacterial strains: *Bacillus thuringiensis* T34, *Escherichia coli* IHII/pHT315, *Bacillus subtilis* 168, *Bacillus megaterium* QM B1551, and *Bacillus megaterium* PV361. The major inorganic components of the bacterial samples, including Ca, Mn, K, Na, Fe, and phosphate, are clearly identified from the breakdown emission spectra. The bacterial spores accumulate a lot of calcium that shows strong LIBS emission at 393.7 and 396.9 nm. The diverse emissions from the phosphate component at 588.1 and 588.7 nm provide a fingerprint for bacterial strains. The relative change of inclusions in the bacteria is clearly distinguished by two-dimensional charts of the bacterial components. The results demonstrate the potential of the LIBS method for the rapid and low false-positive classification of bacteria with minimum sample preparation.

Introduction

Technologies developed for detection and identification of microorganisms are of great concern in the pathogenic microbiology field.[1] Rapid determination of microbials is desirable for a wide range of military, agriculture, and medical applications. The chemical compositions, both inorganics and organics contained in the protein, nucleic acid, and cell components of the microorganisms, may be determined and used for fingerprinting of bacterial strains.

In the standard mass spectrometric technique, charged species from bacterial samples may be generated and then detected for identification of the strain of microorganisms. A strong ionization source is used to open up the bacterial cells, but the ion detectors must be in close contact with the species in question. Other techniques of spectrophotometric analysis, employing a fluorescent binder[2] or gold nanoparticles,[3] have been used to distinguish the extracted DNA or RNA from microorganisms. The major problems with these spectrophotometric methods are the long sample preparation time and the restriction that analysis can only be performed in a biospecialized laboratory.

In immunology, a specifically designed enzyme or binder molecule is required to accomplish any sensitive immunoassay test for the targeted microorganisms. These immunological methods can have fewer sample preparation steps by utilizing small-sized biosensors.[4] Three major problems still remain when the immunological methods are used in the analysis of bacterial strains. First, the sample analyte must be drawn into direct contact with the detector unit, similar to mass spectroscopy. Second, the process of analysis is still a "wet" chemistry technique that utilizes enzymatic agents. This means that the chemicals used in the bacterial identification procedures are likely to have a shelf life which may be dramatically shortened depending on the test environments. Third, immunological methods are only effective for identifying some well-known bacterial strains.

The recent development of laser-induced breakdown spectroscopy (LIBS) has motivated us to apply this technique to the fingerprinting of bacterial strains. Bacteria require nitrogen, carbon, water, and energy sources to grow. Essential mineral salts such as phosphorus, sulfur, potassium, and iron are also required for the life cycle of bacteria. It is known that some of inorganic elements such as Na, K, Ca, and Mg are essential and play regulatory roles for cell functions.[5] The inorganic and organic constituents in bacteria can thus be probed by the LIBS technique. In LIBS, a focused laser beam is directed on the bacterial colony on a plastic plate to produce a plasma plume, which may contain the excited atomic and molecular species of the microbial constituents. The light emission of these excited species can be assigned for a fast identification of bacterial strains. In LIBS, no additional sample preparation or extraction of bacterial colonies is required. Both the laser source and spectroscopic detector employed in LIBS are positioned at a remote distance from the bacterial colony.

The practical applications of the LIBS technique have been reported previously for metal samples.[6,7] Most of the metallic elements in the sample can produce a strong emission by laser-induced breakdown. Light elements such as Li and B, which are usually difficult to observe using X-ray methods, have also been successfully detected by the atomic emission technique in the visible spectral range. In addition to the inorganic/metallic components, LIBS is also capable of characterizing organic gas samples, including specific functional groups in the organic compounds.[8] In LIBS, the laser-targeted spot size on the sample is very small, less than 50 μm in diameter.[9] The analytical target can be on a solid surface, in a liquid stream, or within a gas cloud. One of the major advantages of LIBS over other convenient methods, when applied to the rapid identification of bacterial strains, is the simple sample preparation. All these advantages suggest the feasibility of LIBS to the fingerprinting of microorganisms.

In this paper, the applicability of LIBS in fingerprinting microorganisms is illustrated. Five strains of nonpathogenic bacteria were cultured in colonies containing vegetative or spore forms. A pulsed Nd:YAG-laser light at 532 nm was focused

* To whom correspondence should be addressed. Phone: (815) 753-6861. Fax: (815) 753-4802. E-mail: ctlin@niu.edu.
† Department of Biological Sciences.

10.1021/jp031269i CCC: $27.50
Published on Web 03/31/2004

on the bacterial colony area to generate breakdown emission spectra of the microorganisms. The major inorganic components of the bacterial samples, including Ca, Mn, K, Na, Fe, and phosphate, were considered in the spectral analysis. A two-dimensional chart of the bacterial components, that is, Ca versus phosphate, was constructed for distinguishing five different bacterial strains. In addition, we discuss the feasibility of establishing a library of LIBS spectra that contain the analytical information of microorganisms and bacterial toxins. This library would form the basis for the signal-processing algorithms necessary for high-confidence identification of specific strains of bacteria or bacterial toxins from an unknown source.

Experimental Section

Preparation of Bacterial Strains. Five strains of bacterial samples were prepared in a microbiology lab at Northern Illinois University. The laboratory stock stains used were *Bacillus thuringiensis* T34, *Escherichia coli* IHII carrying pHT315,[10] *Bacillus subtilis* 168, *Bacillus megaterium* QM B1551 (containing seven indigenous plasmids), and *Bacillus megaterium* PV361 (QM B1551 with all plasmids removed).[11] The strains of *B. megaterium* are therefore closely related but are divergent, as is *B. thuringiensis* from *B. subtilis*.[12] *E. coli* is the only one among the five bacterial strains that belongs to a Gram-negative genus, and it has a more antibiotic and enzyme resistant cell wall. Moreover, *E. coli* does not sporulate in contrast to the *Bacillus* species studied.

All bacterial strains used in this investigation have been classified as biosafety level 1 (nonpathogenic). Each strain was streaked on Luria−Bertani (LB) plates (10.0 g tryptone, 5.0 g yeast extract, and 5.0 g of NaCl, 15% agar, in 1 L of double-distilled H_2O) and grown overnight at 37 °C for the streak-plate tests.[13] Single colonies were inoculated into 5 mL of LB broth (no agar) and grown overnight for broth tests. LB plates were then spread with 0.1 mL of culture and grown for 24 h for the confluent plate test. These vegetative forms of the bacterial strains were observed to differentiate into their spore forms after growing for 5 days or longer at room temperature.

Laser-Induced Breakdown Spectroscopy. A pulsed Q-switched Nd:YAG laser (Continuum, Powerlite 8050, 10 ns pulse width) was focused on the bacterial sample plate using a 20 cm focal length lens. The frequency-doubled laser output at 532 nm was used for plasma generation. The laser power used at 532 nm for the analysis and fingerprinting of bacterial strains was 50 mJ/pulse. A light-collecting optical fiber was placed near the sample surface to detect plasma emission, which was sent to a spectrometer (Acton research, 1200 grooves/mm grating). The LIBS spectra were captured with a photodiode array detector (OMA4 CCD, EG&G) with a spectral resolution of 0.061 nm. The OMA output was processed and stored using a personal computer.

Results and Discussion

LIBS Spectra of Bacterial Strains. All five bacterial strains were cultured separately on LB medium in plastic Petri dishes and used for the LIBS experiments without further sample pretreatment. The bacterial colonies on the culturing medium (LB) were grown to roughly 0.5 mm thickness. The areas of the colonies were wide enough to be manually mounted on the sample stage and allow a focused laser beam ($\sim$50 μm diameter or less) to generate the breakdown emission. It is important to note that the observed LIBS spectra (in both the UV and visible spectral ranges) from bacterial samples and culturing medium controls displayed a higher background level than those recorded for solid metallic samples (such as aluminum, copper, and steel).[14] This background light is the result of the soft nature of the bacteria and culture medium (mostly agar), which contain 50−80% water and scatter a lot of material fragments or vapor during the breakdown process. The bacterial sample and LB were semitransparent. The lack of light absorption at 532 nm on the sample required a more intense laser beam for the breakdown. The threshold intensity of the laser pulse for a stable and sufficient breakdown of the bacterial samples was measured as 40 mJ. This value is 2−4 times higher than the 10−20 mJ required normally for LIBS application on solid samples.[15,16]

A complete LIBS spectrum, from the UV to the visible range, was initially scanned for all five bacterial samples. It was experimentally determined that three spectral ranges (at around 275, 400, and 600 nm) contained most of the emission peaks of possible interest. Table 1 lists the 11, 20, and 14 emission peaks for five bacterial strains, obtained from three broader spectral scans around 275, 400, and 600 nm, respectively. The peaks with high intensity are marked with "S", those with medium intensity are marked with "M", and those emission bands with no mark are weak peaks. For all five bacterial strains (columns 2−6 in Table 1), there are eight strong emission peaks identified at 252.8, 279.7, 393.7, 396.9, 398.3, 578.8, 588.1, and 588.7 nm that display similar spectral distributions of LIBS in each bacterial sample. The results suggest that despite the different outward appearance and life cycle of the bacterial species, they all share a similar elemental composition. The LIBS peaks listed in Table 1 can be assigned tentatively using the atomic emission data available in the NIST database.[17] The possible spectral assignments are given in the columns 7−16 of Table 1. It is expected that chlorine, sulfur, phosphorus (in phosphate form), calcium, sodium, and potassium should be the major elements contained in the microorganisms. Other elements in trace amounts are also expected, such as zinc, magnesium, manganese, cadmium, nickel, cobalt, and strontium. On the basis of the spectral assignments in Table 1, the elements, Mg, Cd, Ni, Co, and Sr were not observed. The complexity of the iron emission bands has resulted in some difficulties in spectral assignments to distinguish them from other elemental peaks of interest.

Spectral Fingerprints of Bacterial Strains. The use of a high spectral resolution (a 1200gr/mm grating at 50 nm blocks) enables four major LIBS peaks of interest for Ca and phosphate contained in the bacterial strains to be identified. Figure 1 displays the LIBS spectra from 380 to 410 nm for *B. megaterium* PV361 (top-1), *B. thuringiensis* (top-2), *B. megaterium* QM B1551 (top-3), *B. subtilis* (bottom-3), E. *coli* (bottom-2), and LB (bottom-1, culture medium). The emission peaks at 393.7 and 396.9 nm are attributed to the calcium atomic transitions, $4s\ ^2S_{0,1/2} \rightarrow 4p\ ^2P_{1,1/2}$ and $4s\ ^2S_{0,1/2} \rightarrow 4p\ ^2P_{0,1/2}$, which are the two strongest emissions from calcium used in many other types of atomic spectroscopy. These calcium emissions were further verified by using $CaCl_2$ and $Ca_3(PO_4)_2$ as spectral references. The calcium emissions are strong for *B. megaterium* and *B. thuringiensis* as shown in the top three spectra of Figure 1, and no calcium emission is detectable for the LB medium. The emission peak at 398.3 nm in Figure 1 is strong for LB and *E. coli* (Gram-negative) and relatively weak for PV361, *B. thuringiensis*, QM B1551, and *B. subtilis* samples (all four are Gram-positive bacteria). This peak is overlapped with the Mn emission listed in the NIST database.[17] However, the strong intensity at 398.3 nm could not be assigned to a Mn emission because of the relative emission intensity of Mn at other possible wavelengths. A strong emission peak at 398.3 nm with a similar band

TABLE 1: Observed LIBS Peaks for Five Bacterial Strains and Their Assignments

wavelength	bacterial strains					assignments based on NIST data								
(nm)	B1551	*B. sub*	*B. thur*	PV361	*E. coli*	Na	K	Ca	Mn	Fe	Zn	N	PO*x*	S
252.8	S	S	S	S	M					yes	yes			
254.7				M				yes		yes				
262.9						yes				yes				yes
266.2	M	M	M	M		yes				yes				yes
270.2	M								yes					yes
272.2								yes	yes	yes				yes
279.7	S	S	S	S	S					yes				yes
279.9	M							yes	yes	yes	yes	yes		yes
286.9								yes		yes		yes		
288.2						yes		yes						yes
294.2							yes			yes		yes		
377.1										yes		yes		
379.4										yes		yes		
381.5										yes				yes
383.0									yes	yes		yes		
386.0										yes				yes
387.6								yes	yes	yes				
393.7	S	S	S	S				yes						
395.3		M	M						yes	yes				
396.9	S	S	S	S				yes						
398.3	S	S	M	M	M				yes					
398.8		M												
400.3												yes		
401.8						yes			yes	yes				
403.8									yes					
405.2					M				yes	yes				
407.7	M		M	M						yes				
412.2						yes			yes	yes				
414.4										yes		yes		
416.6									yes	yes				
418.8							yes			yes				
576.0										yes				
578.9	S	S	S	S	S					yes				
581.7									yes					
584.3														
588.1	S	S	S	S	S					yes				yes
588.7	S	S	S	S	S									
590.3										yes				
594.0										yes		yes		
595.1												yes		yes
602.9														
610.5										yes	yes			
614.3													yes	
617.2														
621.8									yes			yes		

shape as that shown in Figure 1 is also observed in the LIBS spectra for samples containing only organic components, such as the LB medium (bottom-1 spectrum in Figure 1), cellulose, and many organic polymers. Thus, the band at 398.3 nm is assigned to the excited organic species that was generated during laser breakdown of the bacterial strains.

Another way to positively identify calcium and fingerprint bacterial strains is to follow the natural transformation of bacteria from the vegetative to the spore form. At the spore stage of the bacteria, after remaining for 5 days at room temperature, most of the surface water evaporated. It is known that the water content of spores is only about 10−30% of the water content of vegetative cells (actively dividing for the survival of the bacteria); spores are at levels of dehydration that would kill vegetative cells. The low water content also provides the spore with chemical resistance (e.g., resistance to hydrogen peroxide) and causes the remaining enzymes of the spore cell to become inactive. This dormancy of the spores makes it difficult for immunological detection to achieve sufficient sensitivity. One of the chemicals produced during the transformation of spores is dipicolinic acid, which is thought to lend to their high resistance to the environment.[18] Dipicolinic acid interacts with calcium ions to form calcium dipicolinate, which is the main substance for protecting spores and represents about 10% of the dry weight of a spore. Therefore, the intensity distribution of LIBS spectra for calcium at 393.7 and 396.9 nm may be closely related to the spore formers and may provide a sensitive way for fingerprinting bacterial strains.

Figure 2 shows the LIBS spectra of overnight cultures versus 5-day cultures for bacterial samples, *B. megaterium* PV361 (top two spectra), *B. subtilis* (middle two spectra), and *E. coli* (bottom two spectra). The organic emission peak at 398.3 nm is strong for the 1-day cultures, and it remains strong for the 5-day cultures of *E. coli* but becomes relatively weak for the 5-day cultures of PV361 and *B. subtilis* and also of QM B1551 and *B. thuringiensis* (not shown). The experimental result shows that the intensity of calcium peaks at 393.7 and 396.9 nm is stronger on the 5-day (spore) samples as compared to their 1-day (vegetative) forms for *B. megaterium* (both PV361 and QM B1551), *B. subtilis*, and *B. thuringiensis* (not shown) colonies. *B. megaterium* is more efficient at sporulation than *B. subtilis*, which may explain the higher peaks at 393.7 and 396.9 nm in the second spectrum as compared to the fourth spectrum in Figure 2. *E. coli* colonies, on the other hand, have low calcium

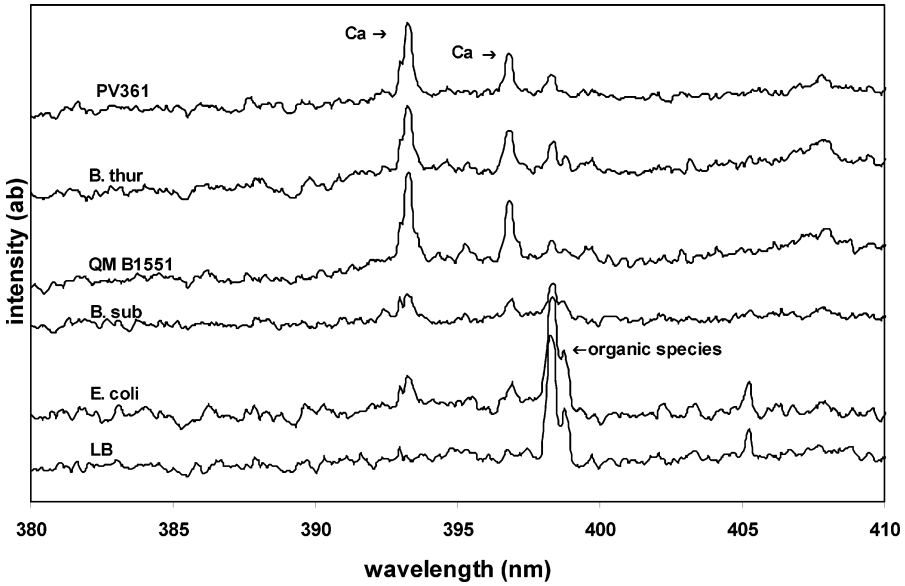

Figure 1. LIBS spectra representing calcium in five bacterial strains and the LB medium.

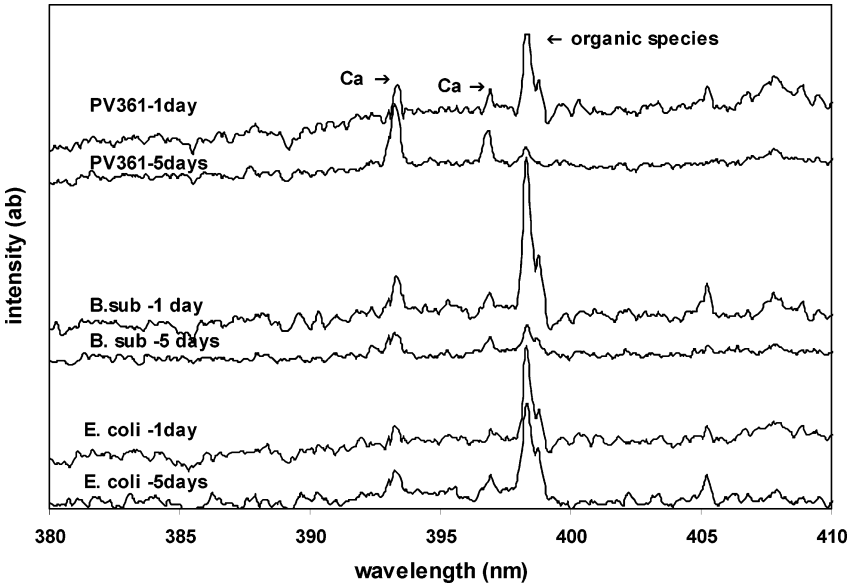

Figure 2. LIBS spectra representing calcium in *B. megaterium* PV361, *B. subtilis*, and *E. coli*: 1-day cultures vs 5-day cultures.

content and do not change their compositions after aging. The observation is consistent with the fact that *E. coli* does not make spores.

The LIBS technique is particularly capable of analyzing the spore forms of bacterial strains. The spore coat contains a complex structure of as many as 30 proteins. These protective coat structures are highly resistant to heat desiccation, chemical disinfecting, and radiation. The major resistance of spores is in the core of the spore (the primordial cell) and is the result of the complexing of proteins with calcium dipicolinate and dehydration and also the complexing of the DNA with small acid soluble proteins.[19] The highly resistant spore coat in spores as well as specific small acid soluble proteins can protect DNA from a harsh environment and can also interfere with chemical characterization by conventional spectroscopy. In LIBS, the laser breakdown beam is intense enough to penetrate the protective spore coat of the spores and is able to probe the inner components of the cell.

Figure 3 shows the LIBS spectra from 575 to 600 nm for five bacterial colonies and the LB medium. The doublet peaks at 588.1 and 588.7 nm are due to phosphate functional groups in the bacterial samples. These emission doublets do not match with the elemental emission of phosphorus (P) listed in the NIST database[17] but are associated with the emission of the phosphate functional group (PO_x) as observed from the LIBS spectra of known phosphate compounds examined. *B. megaterium* PV361 gives the strongest phosphate emission peaks (top-1 spectrum in Figure 3), followed by *B. thuringiensis* and *B. subtilis*, whereas *E. coli* and the LB medium display a weak phosphate emission (bottom 1 and 2 spectra in Figure 3). *B. megaterium* QM B1551 has an intermediate peak height of phosphate emission. The LIBS spectra in the spectral range of the phosphate emission doublets have also been examined for the overnight (vegetative) cultures versus the 5 day (spore) cultures (similar to Figure 2 for the calcium emission). The results indicate that the amount of phosphate contained in the bacterial samples is strain specific and is very sensitive to their transformations from vegetative to spore forms. The LIBS band intensities of phosphate in all bacterial strains are weak at the vegetative stage and then increase as the spores form. The phosphate emission is the most intense for *B. megaterium* PV361. In Figure 3, the LIBS peak at 578.9 nm displayed a

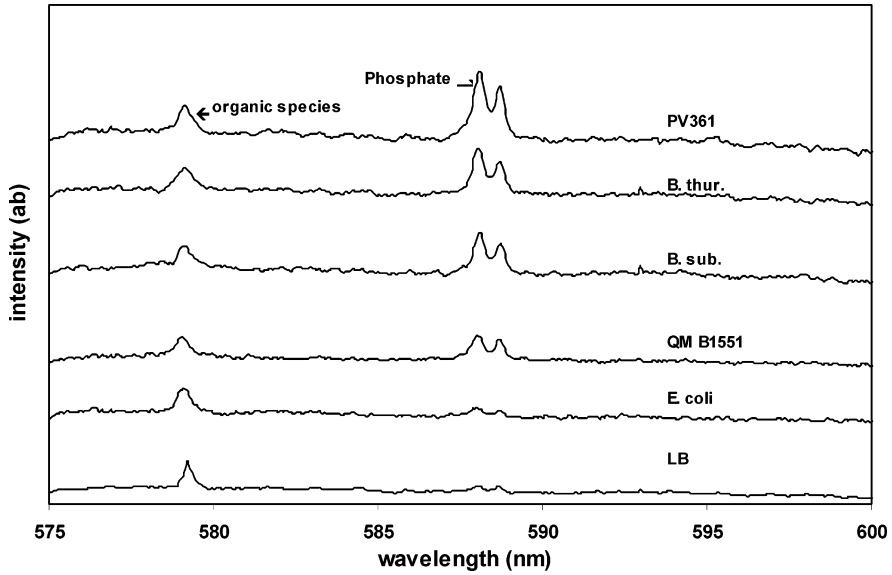

Figure 3. LIBS spectra representing phosphate in five bacterial strains (after aging for 5 days) and the LB medium.

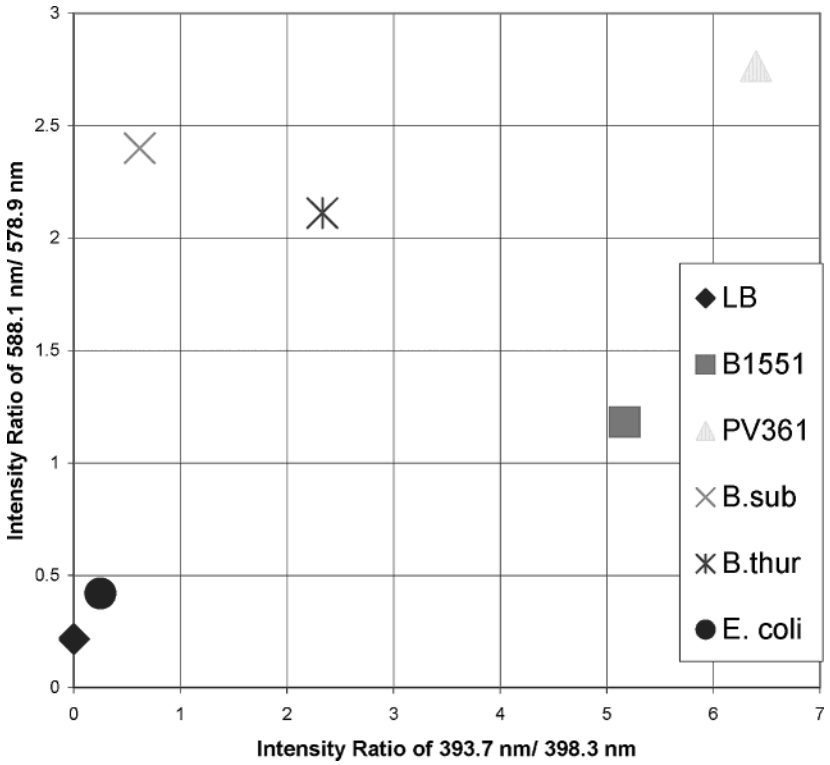

Figure 4. A two-dimensional spread chart representing the distribution of bacterial strains with a normalized LIBS intensity of calcium in the *X*-axis and that of phosphate in the *Y*-axis.

similar intensity for every bacterial sample and the LB medium without bacteria. The band at 578.9 nm, based on the same experimental data as that at 398.3 nm in Figure 1, is assigned to the excited organic species generated during the laser breakdown of bacterial strains.

The LIBS spectra of calcium at 393.7 and 396.9 nm in Figure 1 and of phosphate at 588.1 and 588.7 nm in Figure 3 are used as fingerprints for mapping these bacterial strains. In both figures, each has one LIBS peak that corresponds to an excited organic species: 398.3 nm in Figure 1 and 578.9 nm in Figure 3. The organic composition in each bacterial strain is large and may be considered as constant. So, the variation of calcium composition in each bacterial strain is taken as the intensity ratio of calcium bands at 393.7 and 396.9 nm over the emission peak at 398.3 nm and that of the phosphate doublets at 588.1 and 588.7 nm over the peak intensity at 578.9 nm. The organic peak intensity at 398.3 nm is used to normalize the calcium emission in Figure 1 and that at 578.9 nm is used to normalize the phosphate emission in Figure 3. The normalized band intensity of calcium versus that of phosphate is used to construct a two-dimensional spread chart, representing the distribution of bacterial strains as illustrated in Figure 4. The *X*-axis and *Y*-axis are the normalized spectral intensity of calcium and phosphate, separately, representing the relative amount of calcium and phosphate in the bacterial samples. Each data point in Figure 4 is an average value of 15 independent measurements (including three preparations and five bacterial colony areas in each sample). The experimental results are reasonably reproducible, and the uncertainty is marked by the size of the data points in Figure 4. The data points in Figure 4 are widely distributed,

indicating that the LIBS technique is suitable for the fingerprinting of bacterial strains. Both *B. megaterium* QM B1551 (containing seven indigenous plasmids) and *B. megaterium* PV361 (QM B1551 with all plasmids removed) have a high amount of calcium. The calcium composition decreases in the following order: *B. megaterium* PV361 > *B. megaterium* QM B1551 ≫ *B. thuringiensis* > *B. subtilis* > *E. coli*. *E. coli* does not store calcium in vegetative or aged colonies as discussed in Figure 1. High phosphate content is observed for the *B. megaterium* PV361, *B. subtilis*, and *B. thuringiensis* colonies. It is interesting to note that the phosphate content in *B. megaterium* PV361 is higher than that in *B. megaterium* QM B1551. Both strains are *B. megaterium*, where PV361 has all seven indigenous plasmids removed. The plasmid-free *B. megaterium*, that is, PV361, seems to store more phosphate in the spore cells. It is not understood at this time why the lack of 11% of cellular DNA that is plasmid DNA in QM B1551 would cause more intense phosphate peaks in PV361. However, several metabolic genes have been found on the four sequenced plasmids[20] which may influence the phosphate content.

The LIBS spectra of the culture medium (LB) have also been recorded in Figures 1 and 3. The calcium and phosphate contents in LB are very low; in particular, the calcium level is lower than the LIBS detection limit as shown in Figures 1 and 4. The results indicate that as bacteria grow, they can selectively take up certain elements from the culture medium, especially during sporulation.

Conclusion

Five nonpathogenic bacterial strains have been analyzed, depending on their major chemical components, by laser-induced breakdown spectroscopy (LIBS). The bacterial spores accumulated a lot of calcium within the spore which exhibited strong emissions at 393.7 and 396.9 nm in the LIBS spectrum. The diverse emission from phosphate at 588.1 and 588.7 nm also provided a fingerprint for the bacteria. The difference in bacterial strains was clearly distinguished by two-dimensional charts of the bacterial components, calcium versus phosphate. This work demonstrates the potential of the LIBS method for rapid and precise classification of bacteria with minimum sample preparation.

Of special interest are the results obtained from the spectra of *B. thuringiensis* which is very closely related to anthrax. Some of our future work in this research will be to apply LIBS to differentiate anthrax from other bacterial strains. Tests of several genera of Gram-positive and Gram-negative bacteria to establish the LIBS technology are currently in progress in our lab. Technically, a UV laser source (355 and 266 nm from a Nd:YAG laser or 308, 248, and 193 nm from an excimer laser) can be used in LIBS to enhance breakdown emission for those weak peaks, corresponding to the organic species, listed in Table 1. A critical requirement of an effective microbial sensor is a real time, low false-positive rate of identification and fast detection before possible exposure occurs. To achieve this goal, we plan to establish a library of LIBS spectra for a complete set of microbials or specific pathogens. The measured spectral data from an unknown bacterial strain will be converted and fit into the GRAMS program and compared with those in the library using a SPECTRAL ID software. This technique has the potential for establishing an extensive library of microbial fingerprints.

Acknowledgment. Financial support from ChemNova Technologies, Inc. (the first spin-off company of Northern Illinois University) via a research contract from Green Products, Inc., Taiwan is acknowledged.

References and Notes

(1) Burrow, W. D.; Renner, S. E. *Environ. Health Perspect.* **1999**, *107*, 975.

(2) Reynolds, D. T.; Fricker, C. R. *J. Appl. Microbiol.* **1999**, *86*, 785.

(3) Storhoff, J. J.; Lazarides, A. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L.; Schatz, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4640.

(4) Stratis-Cullum, D. N.; Griffin, G. D.; Mobley, J. M.; Vass, A. A.; Vo-Dinh, T. *Anal. Chem.* **2003**, *75*, 275.

(5) Seydel, U.; Lindner, B. *Fresenius' Z. Anal. Chem.* **1981**, *308*, 253.

(6) Davies, C. M.; Telle, H. H.; Mongomery, D. J.; Corbett, R. E. *Spectrochim. Acta, Part B* **1995**, *50*, 1059.

(7) Wang, X. T.; Man, B. Y.; Wang, G. T.; Zhao, Z.; Liao, Y.; Xu, B. Z.; Xia, Y.; Mei, L. M.; Hu, X. Y. *J. Appl. Phys.* **1996**, *80*, 1783.

(8) Anglos, D.; Solomidou, M.; Zergioti, I.; Zafiropulos, V.; Papazoglou, T.; Fotakis, C. *Appl. Spetrosc.* **1996**, *50*, 1331.

(9) Locke, R. J.; Morris, J. M.; Forch, B. E.; Miziolek, A. W. *Appl. Opt.* **1990**, *29*, 4987.

(10) Arantes, O.; Lereclus, D. *Gene* **1991**, *108*, 115−119.

(11) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Press: Cold Spring Harbor, NY, 1989.

(12) Priest, F. G. In *Systematics and Ecology of Bacillus*; American Society for Microbiology: Washington, DC, 1993; pp 3−16.

(13) Vary, P. S.; Tao, Y. P. In *Genetics and Biotechnology of Bacilli*; Ganesan, A. T., Hoch, J. A., Eds.; Academic Press: New York, 1988; pp 403−407.

(14) Sizemore, C.; Neuder, H.; Kim, T.; Lin, C. T. In *Advancing Materials in the Global Economy: Applications, Emerging Markets and Technologies*, Proceedings of the 48th International SAMPE Symposium, Long Beach, CA, May 11−15, 2003; Cohen L. J., Ong, C.-L., Arendt, C., Eds.; Society for the Advancement of Material and Process Engineering: Covina, CA, 2003; pp 2267−2280.

(15) Kim, T.; Yoon, Y.; Lee, G. *J. Korean Phy. Soc.* **1999**, *35*, 198.

(16) Kim, T.; Lin, C, T.; Yoon, Y. *J. Phys. Chem. B* **1998**, *102*, 4284.

(17) NIST Atomic Spectra Database—Lines Holdings. http://physics-.nist.gov.

(18) Setlow, P. *Annu. Rev. Microbiol.* **1988**, *42*, 319−338.

(19) Kunnimalaiyaan, M.; Stevenson, D. M.; Zhou, Y.; Vary, P. S. *Mol. Microbiol.* **2001**, *39*, 1010−1021.

(20) Vary, P. S.; Kunnimalaiyaan, M.; Scholle, M. S. *Plasmid* **2002**, *48*, 284.