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Non-contact bacterial identification and decontamination based on laser-induced breakdown spectroscopy

Jiahui Liang [a,b], Shuqing Wang [c], Xuebin Luo [d], Yan Zhang [e], Fei Chen [a,b], Ziqi Mi [a,b], Lei Zhang [a,b,*], Gang Wang [d], Wanfei Zhang [d], Zhenrong Liu [d], Xiaofei Ma [d], Zefu Ye [f], Zhujun Zhu [f], Wangbao Yin [a,b,*], Suotang Jia [a,b]

[a] State Key Laboratory of Quantum Optics and Quantum Optics Devices, Institute of Laser Spectroscopy, Shanxi University, Taiyuan, China
[b] Collaborative Innovation Center of Extreme Optics, Shanxi University, Taiyuan, China
[c] SINOPEC Research Institute of Petroleum Processing Co., Ltd., Beijing, China
[d] Shanxi Xinhua Chemical Defense Equipment Research Institute Co., Ltd., Taiyuan, China
[e] School of Optoelectronic Engineering, Xi'an Technological University, Xian, China
[f] Shanxi Gemeng US-China Clean Energy R&D Center Co., Ltd., Taiyuan, China

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ABSTRACT

As a new kind of modern military biological weapon, bacterial agents pose a serious threat to the public health security of human beings. Existing bacterial identification requires manual sampling and testing, which is time-consuming, and may also introduce secondary contamination or radioactive hazards during decontamination. In this paper, a non-contact, nondestructive and "green" bacterial identification and decontamination technology based on laser-induced breakdown spectroscopy (LIBS) is proposed. The principal component analysis (PCA) combined with support vector machine (SVM) based on radial basis kernel function is used to establish the classification model of bacteria, and the two-dimensional decontamination test of bacteria is carried out using laser-induced low-temperature plasma combined with a vibration mirror. The experimental results show that the average identification rate of the seven types of bacteria, including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens*, Bacillus megatherium, *Pseudomonas aeruginosa*, *Bacillus thuringiensis* and *Enterococcus faecalis* reaches 98.93%, and the corresponding true positive rate, precision, recall and F1-score reaches 0.9714, 0.9718, 0.9714 and 0.9716, respectively. The optimal decontamination parameters are laser defocusing amount of −50 mm, laser repetition rate of 15–20 kHz, scanning speed of 150 mm/s and number of scans of 10. In this way, the decontamination speed can reach 25.6 mm^2/min, and the inactivation rates for both *Escherichia coli* and *Bacillus subtilis* are higher than 98%. In addition, it is confirmed that the inactivation rate of plasma is 4 times higher than that of thermal ablation, meaning that the decontamination ability of LIBS mainly relies on the plasma rather than the thermal ablation effect. The new non-contact bacterial identification and decontamination technology does not require sample pretreatment, and can quickly identify bacteria in situ and decontaminate the surfaces of precision instruments, sensitive materials, etc., which has potential application value in modern military, medical and public health fields.

1. Introduction

Bacteria, fungi, mycoplasma, viruses and other microorganisms can all be used as modern military bioweapons, threatening public security and national defense equipment. Rapid identification and decontamination of harmful bacteria has become an urgent need for the development of new equipment in various countries. Currently, bacterial identification mainly employs biological methods such as polymerase chain reaction (PCR) amplification and conventional physicochemical morphological characterization, but these take a long time. For decontamination, chemical methods of water-soluble reagents or physical methods such as ultraviolet, gamma ray, X-ray, ultrasonic and microwave radiation are usually used, but they may cause secondary pollution or radiation hazards, especially for surfaces of precise electronic

* Corresponding authors at: State Key Laboratory of Quantum Optics and Quantum Optics Devices, Institute of Laser Spectroscopy, Shanxi University, Taiyuan, China.
E-mail addresses: k1226@sxu.edu.cn (L. Zhang), ywb65@sxu.edu.cn (W. Yin).

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instruments or sensitive materials. Therefore, it is urgent to develop new non-contact, non-destructive and "green" bacterial identification and decontamination technology. Compared with the existing inactivation mechanisms, laser-induced breakdown spectroscopy (LIBS) [1] uses high-energy laser beams to ablate target and generate plasmas, which can not only be used to classify and analyze their radiation spectra, but also to clean the target surface by using their shock waves. The shock wave of plasma generated by LIBS is characterized by slight loss, high efficiency and strong penetrability. It has shown good prospects in mineral classification [2], waste treatment [3–5], rust cleaning [6,7], etc. In this way, LIBS can not only be used for bacterial identification, but also has obvious destructive effect on the cellular structure and protein activity of microorganisms [8], showing certain application potential in non-contact bacterial decontamination.

In terms of LIBS bacterial identification, due to the extremely high similarity of spectra among different categories of microorganisms, and the spectra may vary with the environment, growth stage, concentration, etc., how to extract features from these similar spectra to establish an efficient classification model is the key to bacterial identification [9]. Rao et al. enriched the concentration of bacteria through filter paper, and used a random forest combined with principal component analysis (PCA) to identify 10 types of bacteria, such as *Escherichia coli* and clostridium, with an identification rate of >90% [10]. Mohaidat et al. used discriminant function analysis (DFA) to identify nonpathogenic *Escherichia coli* and non-toxic derivatives of pathogens deposited on agar culture medium under three metabolic conditions, with an identification rate reached 100% [11]. Manzoor et al. employed a neural network algorithm to identify 40 strains of bacteria from different species such as *Escherichia coli* in a petri dish with an accuracy of 95% [12]. Marcos et al. prepared samples of *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* at different times in three days and divided them into three groups. The accuracy of using a neural network algorithm to identify these samples also reached 95% [13]. However, these studies require sample pretreatment such as thawing, rejuvenation, culturing, separation and drying, which is not convenient for in situ bacterial identification.

For laser based bacterial decontamination, it is mainly focused on the use of ultraviolet lasers to explore the inactivation efficiency of bacteriostatic substrates of different materials on bacteria. For example, Alexander et al. used an ultraviolet laser to irradiate pathogenic viruses in water, and up to 98% of bacterial walls were destroyed [14]. Hanbyeol et al. used the ultraviolet laser to irradiate spores of Bacillus atrophia on ceramic tiles and found that the sterilization efficiency increased with the increase of laser intensity and irradiation times [15]. However, the efficiency of ultraviolet laser sterilization for spore bacteria is relatively low, so researchers tried to use high power pulsed laser. For example, Hiroharu et al. studied the inactivation efficiency of yeast and spores by generating plasmas on different metal targets with pulsed lasers, and found that the silver target was the best [16]. Shishir et al. verified that nanosecond CO_2 laser can be used to ablate cells on glass and silicon oxide through dry laser cleaning and wet laser cleaning [17]. Sasa et al. attempted to remove the biofilm of *Enterococcus faecalis* attached to the titanium surface with an ultrashort pulse laser, significantly reducing the number of remaining live bacteria [18]. It is necessary to further explore the damage to the substrate caused by pulsed laser, and to correlate the identification with the decontamination.

This paper mainly focuses on the direct identification and decontamination of bacteria using LIBS without sample pretreatment, exploring the methods of extracting the characteristics of bacterial spectra and establishing identification models, and verifying the efficiency of laser induced plasma for decontamination of various bacteria.

2. Experiment

2.1. Sample Preparation

Seven types of bacteria were investigated in the experiment, including *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), *Pseudomonas fluorescens* (*P. fluorescens*), Bacillus megatherium (B. megatherium), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus thuringiensis* (*B. thuringiensis*) and *Enterococcus faecalis* (*E. faecalis*), and ferroalloy plates were selected as the substrates for LIBS detection. The test tubes, inoculation rings, metal substrates and other experimental instruments were sterilized at 394 K for 20 min, The bacteria in the slanted solid medium were inoculated into another solid medium using the inoculation ring on the sterile table. The medium was then placed into a constant temperature incubator and purified for 48 h at 310 K. A single colony of *E. coli* and *B. subtilis* were inoculated into the liquid medium and placed into a shaking box with a constant temperature of 310 K and a rotating speed of 220 $r \cdot min^{-1}$ to enrich and culture for 12 h, so that the bacteria were in the growth exponential phase, and thus the bacteria samples were obtained. The bacteria were placed into the visible light spectrometer, and the OD_{600} measured at the wavelength of 600 nm was adjusted to 1.0, so that the bacterial concentration used each time was relatively constant. 100 μl of bacterial solution was pipetted onto the surface of the metal substrate measuring 20 mm × 20 mm, which was evenly spread and dried for subsequent experiments.

2.2. Experimental Setup

The experimental LIBS setup for bacterial identification and decontamination is shown in Fig. 1. The high repetition rate pulsed laser was divided into two beams by the polarization beam splitter (PBS) after passing through the half-wave plate and the beam expander. One of the beams entered the power meter to monitor the real-time laser power, and the other beam was focused on the sample surface after passing through the lens, vibration mirror and field mirror. Here, the diode-side-pumped Nd: YVO_4 laser (AUT-ONDA) is used as the excitation source, with the output laser wavelength of 1064 nm, pulse energy of 200–800 μJ, repetition rate of 1 Hz ~ 100 kHz, energy jitter of $\leq \pm 5\%$ and beam diameter of 2 mm. The wavelength range of the four-channel spectrometer (AvaSpec-ULS2048L) is 200–1040 nm, the exposure delay and integration time are set to 300 ns and 1 ms, respectively, and every five spectra are automatically averaged. The vibration mirror (SG7210) has a maximum beam incidence diameter of 10 mm, a maximum scanning speed of 8000 mm/s, and a damage threshold of 9.1 J/cm^2. The detection range of the power meter (Nova II) is 0–30 W. It is worth mentioning that the vibration mirror was used to adjust the scanning speed of the laser and plan the scanning path, while the field mirror was used to ensure that the focal points of the laser from the vibration mirror with different incident angles were in the same focal plane. The simulation with Zemax showed that when the laser spot is scanned from the center of the sample surface to a distance of 10 mm, its diameter correspondingly increases from 241 μm to 415 μm.

The overall experimental scheme was to identify the bacteria with LIBS first. The average spectrum of each point on the sample ablated by five laser pulses was taken as a group, and 200 groups of spectra were collected for each sample. Then, the parameters of the laser and vibration mirror were optimized for the subsequent experiment of bacterial decontamination.

3. Results and Discussion

3.1. Identification of Bacteria

By taking into account the similarities between bacterial components and the potential interference of the nutrient solution in the culture medium, this study focused on spectral pretreatment, information

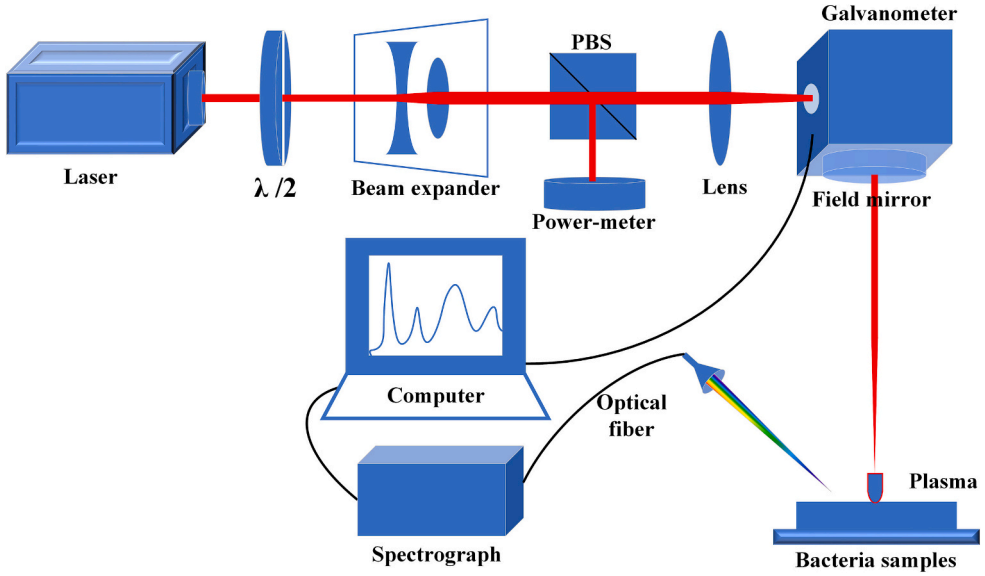

Fig. 1. Experimental LIBS setup for bacterial identification and decontamination.

extraction, and classification modeling methods to accurately identify bacteria.

3.1.1. Spectral Pretreatment

In spectral classification, the most common pretreatment methods include maximum and minimum normalization, standardization, mean centralization, standard normal transformation, moving average

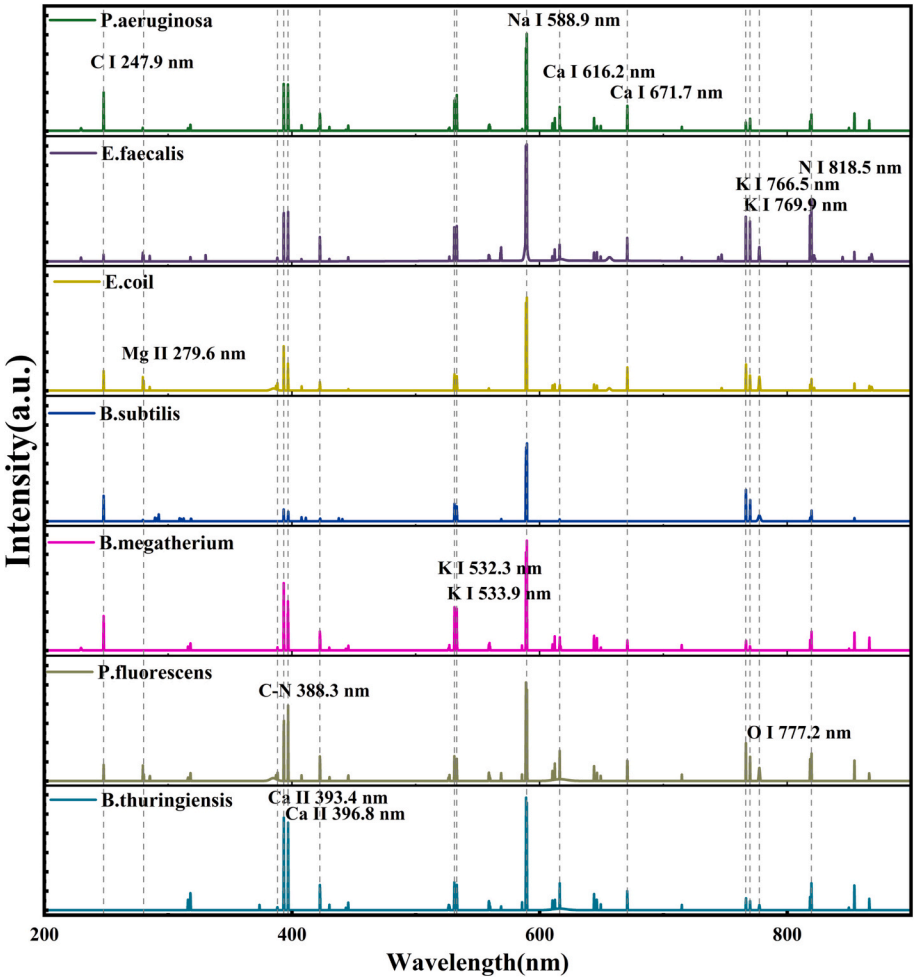

Fig. 2. Comparison of the LIBS spectra of seven types of bacteria after pretreatment.

smoothing, smoothing filtering, first derivative, second derivative, trend correction, multivariate scattering correction, and so on. In this study, the bacterial spectra of Na 588.9 nm line with signal-to-noise ratio (ratio of standard deviation to spectral line intensity) lower than 5 dB were screened out first, then background subtraction and Savitzky-Golay smoothing with five-window were performed, and finally all spectral lines were fitted. Fig. 2 displays the spectra of seven types of bacteria after the above pretreatment, featuring 46 visible spectral lines. Characteristic spectral lines are highlighted in the figure and enumerated in Table 1. For instance, in the case of the K I line at 766 nm, the normalized line intensity of seven bacterial strains exhibited a relative standard deviation (RSD) of <10%, with a mean value of 7.18%, thus enhancing the stability of the line intensity. It is noteworthy that the bacteria mainly consist of elements like C, H, O, N, Na, Mg, K, Ca, which are also the primary components of most bacterial microorganisms. Furthermore, the spectral lines of C, H, O and N also had contributions from the atmosphere.

3.1.2. Identification by PCA

PCA is a linear dimensionality reduction algorithm that can effectively eliminate redundant information from the spectrum. In this case, PCA was applied to 46 visible characteristic lines from 21 sets of spectra of seven different types of bacteria following pretreatment, The cumulative contribution rate of the obtained principal components is depicted in Fig. 3. It can be seen that the cumulative contribution rate of the first three principal components is over 90%, which is able to effectively represent the composition information of bacteria. Fig. 4a and b provide examples of *P. aeruginosa* - *B. thuringiensis* and *E. coli* - B. megatherium, respectively, demonstrating that this method can distinguish at least between two types of bacteria. However, the example of *B. thuringiensis*, *E. coli* and B. megatherium in Fig. 4c reveals that this method is unable to accurately differentiate multiple types of bacteria simultaneously, as well as provide quantitative analysis and a rate of identification [19]. Therefore, it is necessary to further combine other algorithms for identification.

3.1.3. Identification by SVM

Support Vector Machine (SVM) is a machine learning statistical method proposed by Vapnik. Because they are not limited to the dimension of data, they can project high-dimensional vectors that are linearly non-separable and achieve multi-class identification with the help of a hyperplane. It is known from both linear and nonlinear classification principles that not only can SVM handle binary classification problems, but they also exhibit good results for multi-classification problems. Its kernel functions include linear, polynomial, radial basis function (RBF) and other types. Here, RBF was selected to better apply to nonlinear multi-class identification, so as to transform it into a linear separable form. In addition, cross-validation was used to automatically find the parameters with the highest classification accuracy without test labels, thereby ensuring good generalization ability for the bacterial spectra training and modeling.

A total of 1400 valid bacterial spectra were gathered in the experiment, 1120 of them were randomly chosen to be the training set and the other 280 were allocated to the testing set. Using the classification model obtained by 46 spectral lines and SVM with RBF kernel, Fig. 5 shows the prediction results for seven different types of bacteria for both the training and testing sets, with the color indicating the type of bacteria. The data analysis revealed that 5 samples of B. megatherium were misclassified in the training set, while 1 and 2 samples of B. megatherium and *P. fluorescens* were misclassified in the testing set. The overall identification rate of the training and testing sets reached 99.55% and 98.93%, respectively. In the reliability verification, multiple training and validation sets were randomly chosen for repeated tests, and the accuracy rates were higher than 97% in all cases, demonstrating the generalizability and robustness of the SVM-built classification model. The evaluation results of the identification performance of the classification model using true positive (TP) rate, precision, recall and F1-score are shown in Fig. 6, where support represents the number of samples in the testing set for each type of bacteria. The TP rate, precision, recall and F1-score were 0.9714, 0.9718, 0.9714 and 0.9716, respectively.

In addition, Fig. 7 displays the identification outcomes of *E. coli* and *B. subtilis* as research subjects utilized in subsequent decontamination experiments using the same method. It is evident that the identification rates for both the training and testing sets are 100%, while the TP rate, precision, recall, and F1-score all possess a value of 1. As a result, it can be inferred that these two types of bacteria can be completely and accurately identified from one another.

Table 1
Several strong elemental lines in LIBS spectra of bacteria.

No.	Element	Wavelength (nm)
1	C I	247.856
2	N I	818.487
3	O I	777.194
4	Na I	588.995
5	Mg II	279.553
6	C-N	388.300
7	K I	532.328, 533.969, 766.490, 769.896
8	Ca I	422.673, 612.222, 616.217, 643.907, 671.770
	Ca II	317.933, 393.366, 854.209

3.2. Decontamination of Bacteria

The low-temperature plasma induced by LIBS is an efficient, clean and homogeneous method of non-contact, dry decontamination of microorganisms. Its sterilization mechanism can be classified into four distinct categories, as illustrated in Fig. 8: 1) The pulsed laser with high power density can instantly deform and crack various internal structures of bacteria, killing them through ablation [20]. Additionally, differences in thermal conductivity, melting point and thermal expansion coefficient between the bacteria and substrate materials can generate varying degrees of thermal stress, which can induce dehydration and deformation of the bacteria, ultimately causing them to detach from the surface [21]. 2) The copious high-velocity particles present within the transient plasma can engage in elastic collisions with bacteria through physical impact [22], vibration or vaporization mechanisms, ultimately causing them to detach from the substrate surface. 3) The oxygen free radicals produced within the plasma exhibit exceptional reactivity and can readily engage in chemical reactions with the organic molecules present within bacteria, such as proteins, ribosomes, and nucleic acids. This can result in the generation of volatile particles and gaseous substances (such as CO_2 and H_2O, $O_2 + e^- \rightarrow 2O + e^-, O^* + organics \rightarrow CO_2 + H_2O$). 4) The shockwave of plasma imbalances the biochemical environment inside and outside the cell through impact and gasification, resulting in the generation of positively and negatively charged particles, the bacteria are inactivated under the influence of electrostatic interference. Although the mechanism of inactivation during the laser decontamination process may differ [23,24], the aforementioned physical and chemical processes undoubtedly cause varying degrees of destruction to the structure and activity of bacteria.

In this experiment, *E. coli* and *B. subtilis* were selected as model bacteria for the decontamination research. A vibration mirror and field mirror were incorporated into the LIBS setup. To achieve rapid and efficient decontamination of bacteria on the substrate surface, a rectangular scanning path was chosen, and the optimal decontamination parameters of the device were selected based on the different identification results of the bacteria. Here, the decontamination efficacy of LIBS on bacteria under different number of scans, scanning speed, laser repetition rates and pulse energies was preliminarily explored to obtain the optimal decontamination parameters. The process for determining the total number of viable bacteria remaining on the surface of the

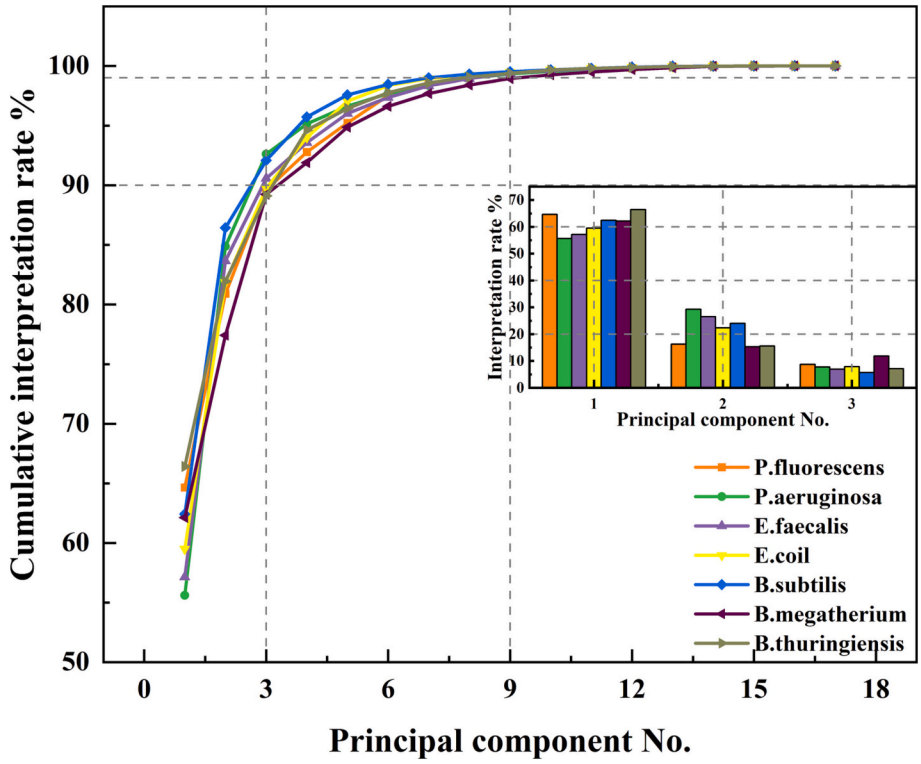

Fig. 3. Cumulative contribution rates of different number of principal components.

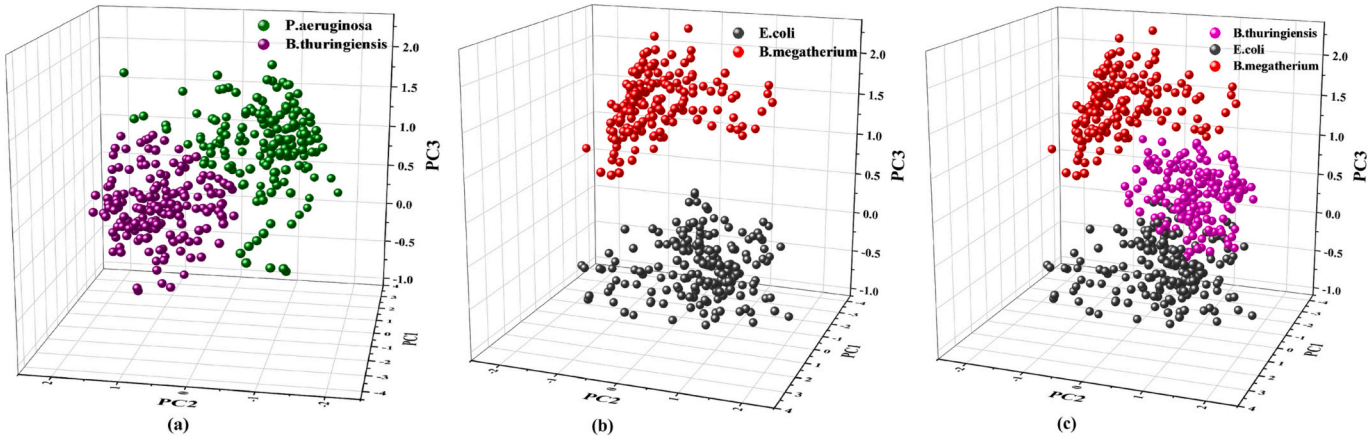

Fig. 4. Identification of (a) *P. aeruginosa* and *B. thuringiensis*, (b) *E. coli* and B. megatherium, (c) *B. thuringiensis*, *E. coli* and B. megatherium using PCA.

decontaminated substrate is as follows: 1) Place the substrate into 1 ml of deionized water for 30 min, then measure the absorbance of the bacterial solution with a spectrophotometer and observe the bacterial death under a microscope. 2) Dilute the bacterial solution several times using the dilution coating plate method, and then place it into an incubator at 37 °C to facilitate resuscitation and culture. 3) Ensure that all culture media are not contaminated by other bacteria, and that *E. coli* is yellowish-white, round and smooth, while *B. subtilis* is grayish-white and rough. Try to select plates with 30–300 colonies for counting, and repeat the experiment three times. 4) A threshold with statistical significance of $P < 0.05$ is taken as the threshold, indicating that the overall mean is significantly different at the 0.05 level, ensuring confidence in the calculated inactivation rate compared to untreated bacteria.

In Fig. 9, the distribution of colonies of *E. coli* and *B. subtilis* on the surface of the culture medium were shown under varying laser number of laser scans, while Fig. 10 illustrates the correlation between scanning speed, number of scans and the inactivation rate of the two types of bacteria. It is observed that the inactivation rate decreases as the scanning speed increases, and exhibits a sharp rise initially followed by a plateau as the number of scans increases. When the number of scans is set to 10 and the scanning speed to 150 mm/s, the inactivation rate reaches a high of 98%, indicating swift decontamination and a high level of bacterial inactivation. One noteworthy point is that the inactivation rate witnesses a slight dip when the number of scans is 3, which is speculated to be due to the bacteria being split into 2–5 active daughter cells after the decontamination process.

Under optimal conditions of scanning speed and number of scans, the relationship between laser repetition rate, laser energy and the inactivation rate of the two types of bacteria is presented in Fig. 11. It can be observed that the inactivation rate is positively correlated with laser energy, meaning that an increase in pulse energy enhances thermal ablation, leading to a higher inactivation rate of bacteria. The

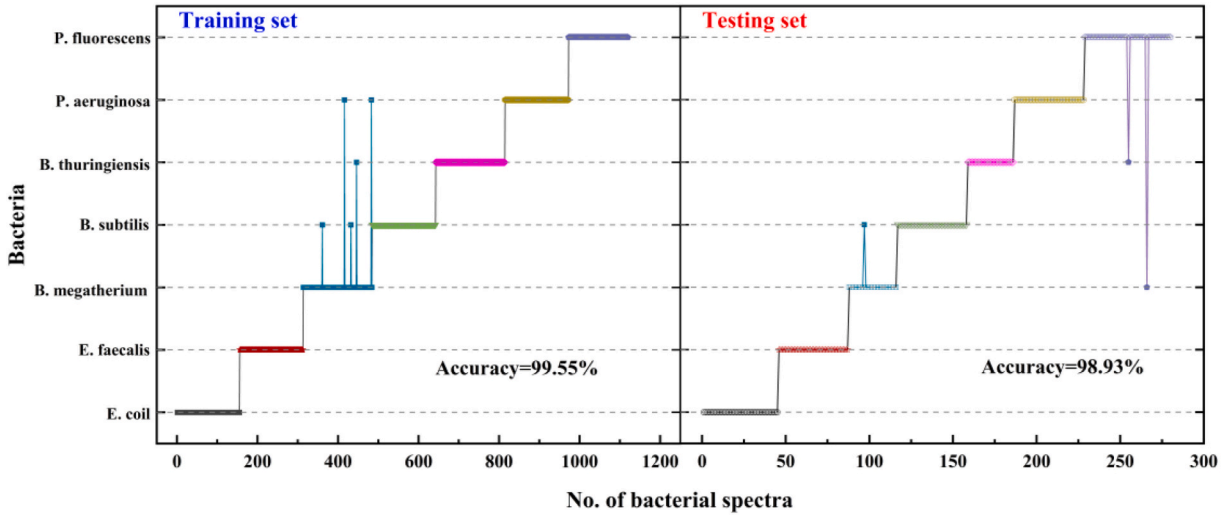

Fig. 5. Identification of seven types of bacteria using SVM with RBF kernel.

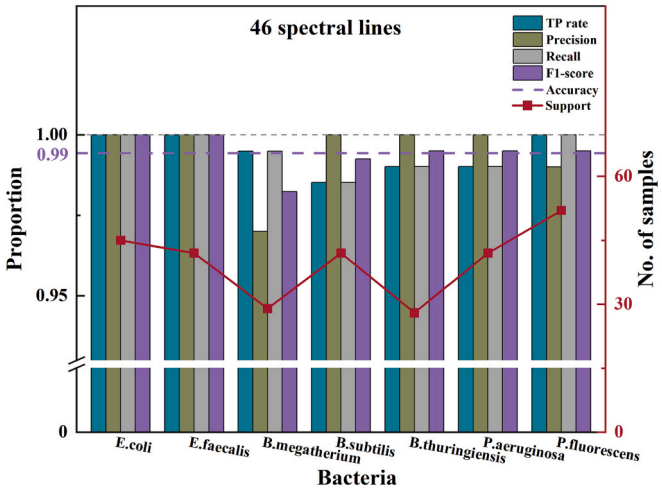

Fig. 6. Performance evaluation of classification model based on SVM with RBF kernel.

inactivation rate generally increases with an increase in laser repetition rate, with the maximum observed at 15 kHz and 20 kHz for *E. coli* and *B. subtilis*, correspondingly. However, both curves experience a decline at 5 kHz. It is speculated that the ions accumulated on the surface of the bacteria formed a protective film at this stage, and the classical coulomb repulsion generated by it prevents the entry of other ions, leading to a decrease in the inactivation rate. At a repetition rate of 30 kHz, the plasma life is shortened, reducing its effectiveness in stripping and damaging the bacteria, there was consequently a decrease in the inactivation rate.

To investigate the impact of laser defocusing amount on the inactivation rate, the experiment used a beam quality analyzer to measure the spot diameter and determine the focus position. The defocusing amount was obtained by fitting Gauss function for 500 iterations. The relationship between defocusing amount, spot diameter and inactivation rate is shown in Fig. 12. Results show that when the spot diameter exceeds 3 mm, the energy density is insufficient to generate plasma, resulting in a low inactivation rate. At this point, the inactivation mechanism is primarily thermal ablation. In the range of defocusing amounts from −50 to 50 mm, the inactivation rate of bacteria was high. The damage of the substrate surface was also tested (see Fig. 13), the defocusing amount of

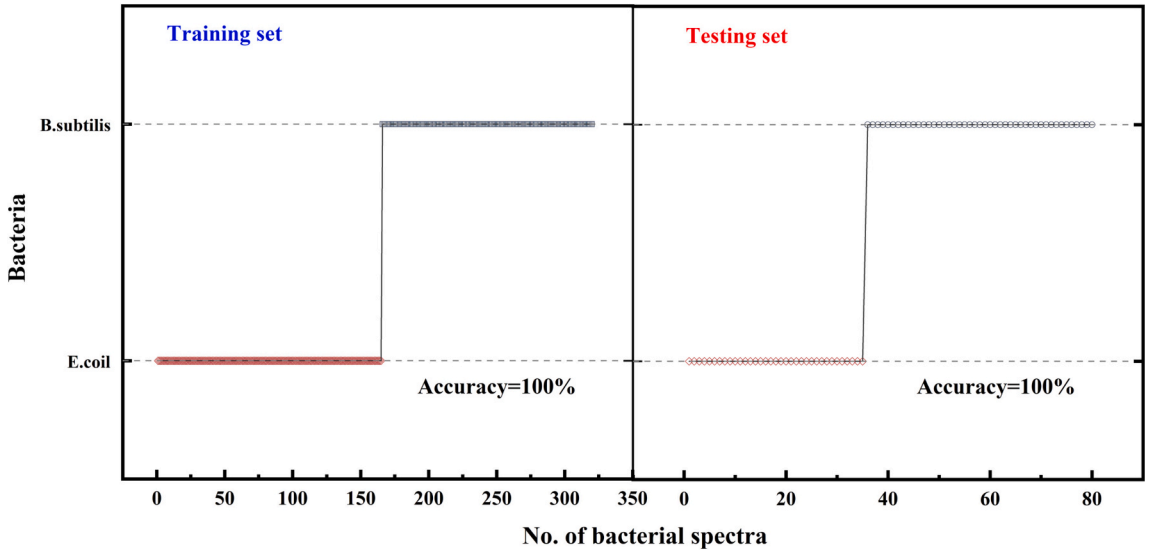

Fig. 7. Identification of two types of bacteria to be decontaminated using SVM with RBF kernel.

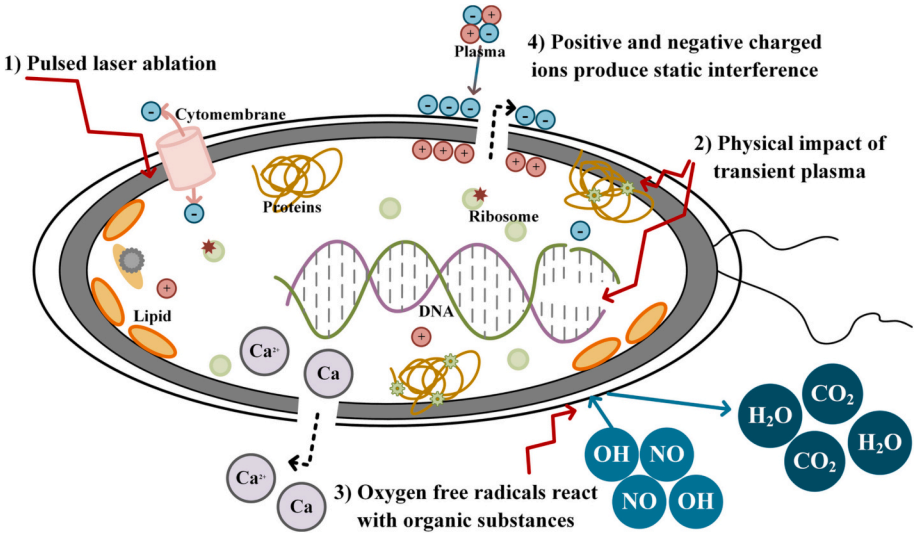

Fig. 8. Four mechanisms of LIBS induced low-temperature plasma to inactivate bacteria.

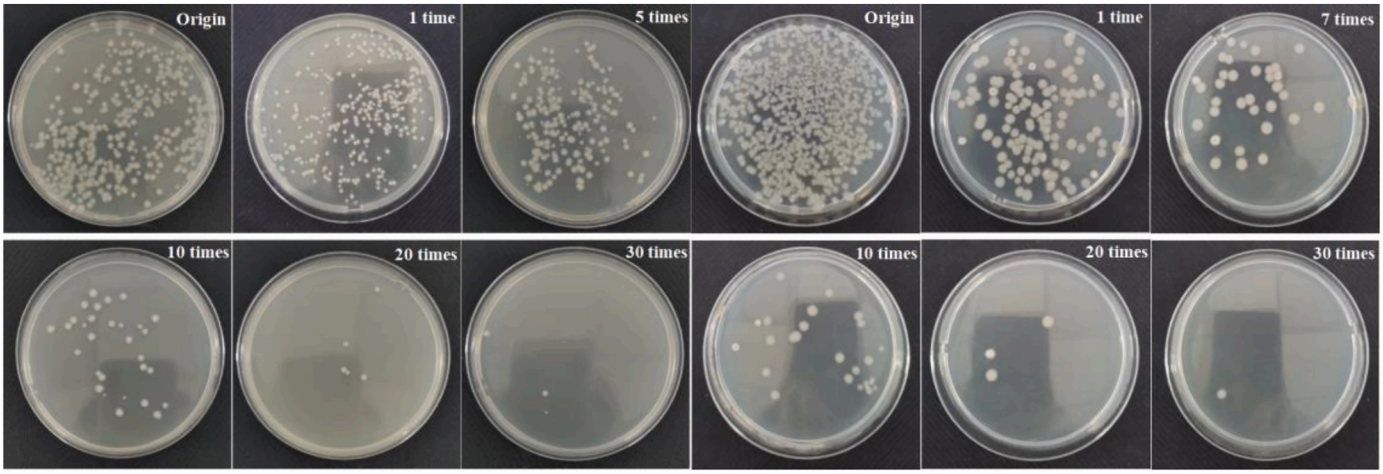

Fig. 9. Comparison of colony numbers of (a) *E. coli* and (b) *B. subtilis* on the culture medium under different laser number of scans.

−50 mm was chosen to achieve low-destructive decontamination, with a corresponding spot diameter of approximately 1 mm. With these optimal experimental parameters and rectangular scanning path with a spot catch-up rate of 98%, the decontamination area reached 14 mm × 14 mm within 10 min, corresponding to a decontamination rate of 25.6 mm^2/min. The inactivation rates of *E. coli* and *B. subtilis* were higher than 98%. Furthermore, it can be observed from the figure that the inactivation rate of thermal ablation is only about 20%, while that of plasma is almost 4 times higher, which also indicates that LIBS decontamination mainly relies on plasma rather than thermal ablation effects.

The above experimental results show that the optimal decontamination parameters of the setup for the two types of bacteria are a scanning speed of 150 mm/s, a number of scans of 10, a laser repetition rate of 15 kHz (for *E. coli*) or 20 kHz (for *B. subtilis*), and the corresponding single pulse energy of 680 μJ. In addition, under the same conditions, due to the slight differences in composition, size, shape, surface charge, and special structures between the two bacteria, LIBS slightly outperforms *E. coli* in terms of inactivation of *Bacillus subtilis*. This may also indicate that the two bacteria exhibit different sensitivities to plasma decontamination [25].

4. Conclusion

In this work, we carried out an experimental study on non-contact identification and decontamination of bacteria using LIBS. For bacterial identification, 46 characteristic spectral lines in the LIBS spectra were pretreated and dimensionality was reduced by PCA, and a classification model was established by SVM. The identification rate of the model for seven types of bacteria reached 98.93%. For bacterial decontamination, the experimental results showed that the inactivation rate was strongly dependent on laser pulse energy, laser repetition rate, scanning speed and number of scans, as well as the type of bacteria. The optimal laser decontamination parameters were determined as a laser defocusing amount of −50 mm, a scanning speed of 150 mm/s, a number of scans of 10, and a laser repetition rate of 15 kHz (for *E. coli*) or 20 kHz (for *B. subtilis*). Thus, the decontamination rate reached 25.6 mm^2/min, and the inactivation rates of *E. coli* and *B. subtilis* were >98%. This work provides a non-contact and all-optical means for the identification and decontamination of bacteria in unknown dangerous areas or on the surface of precision instruments in the military, medical and public health fields. In the future, we will try to establish quantitative analysis models for various bacteria, and further develop an adaptive decontamination technique that can automatically adjust parameters and plan scanning paths based on the concentration distribution of

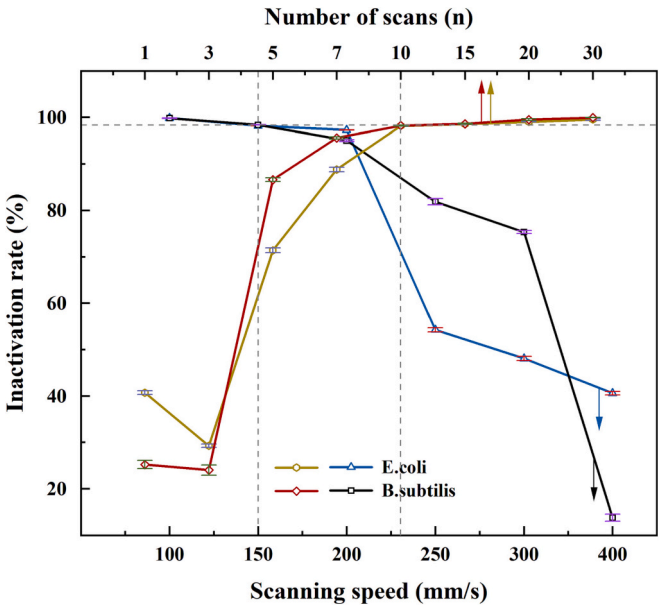

Fig. 10. Relationship between inactivation rate, scanning speed and number of scans.

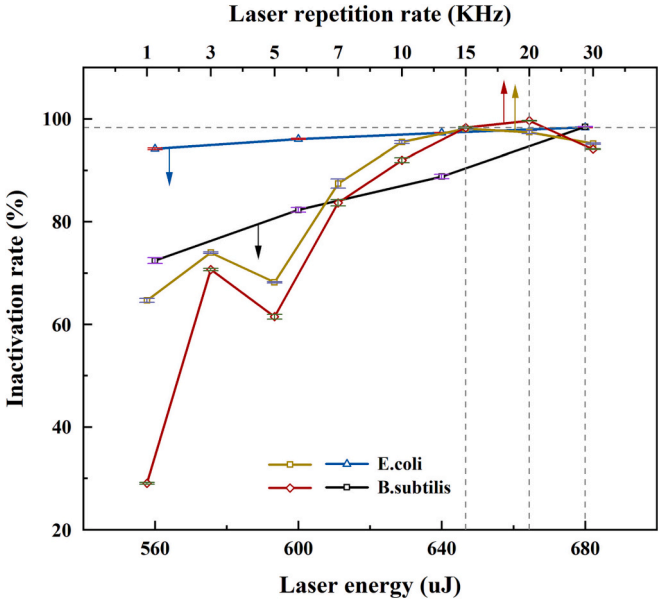

Fig. 11. Relationship between inactivation rate, laser energy and laser repetition rate.

bacteria on the surface.

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Author statement

Jiahui Liang is mainly responsible for the conceptualization, methodology, software and formal analysis of the experiment, and the writing

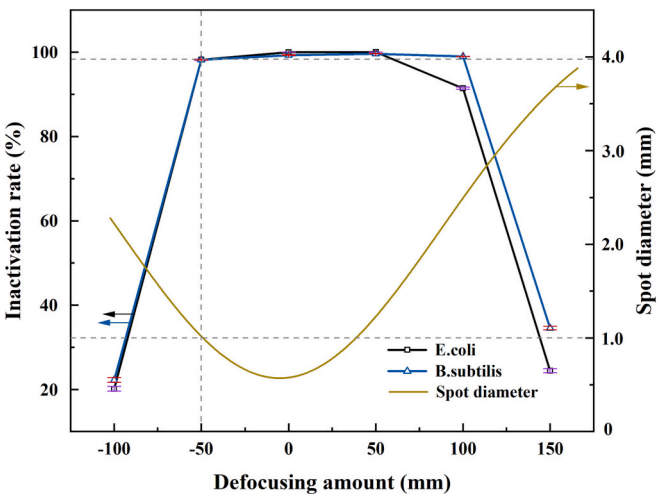

Fig. 12. Relationship between defocusing amount, spot diameter and inactivation rate.

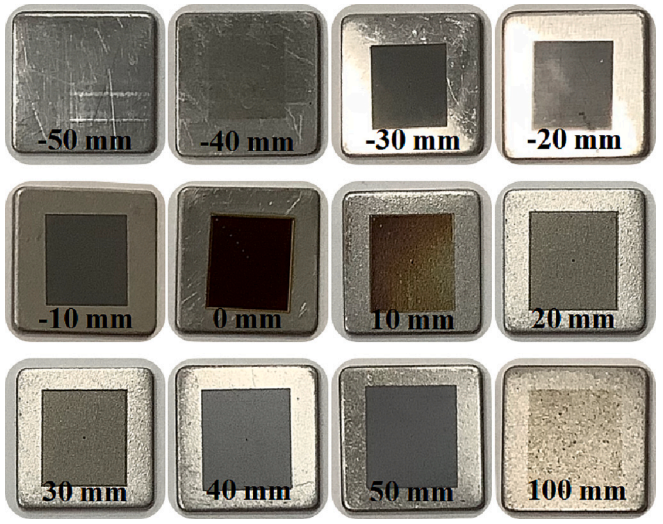

Fig. 13. Laser damage to substrate surface under different defocusing amounts.

of original draft. Yan Zhang and Lei Zhang are responsible for conceptualization and methodology, while Lei Zhang is also responsible for writing review and editing and project administration. Fei Chen is responsible for investigation, software and formal analysis, and Ziqi Mi is responsible for validation, data curation and supervision. Shuqing Wang, Xiaofei Ma, Gang Wang, Wanfei Zhang, Zhenrong Liu, Xuebin Luo, Zefu Ye and Zhujun Zhu are responsible for providing the necessary resources for the experiment. Wangbao Yin and Suotang Jia are responsible for project administration and funding acquisition, while Wangbao Yin is also responsible for writing review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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