Rapid detection of Escherichia coli and Salmonella typhimurium by surface-enhanced Raman scattering*

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In this paper, the surface-enhanced Raman scattering (SERS) is used as an analytical tool for the detection and identification of pathogenic bacteria of Escherichia coli (E. coli) and Salmonella typhimurium (S. typhimurium). Compared with normal Raman signal, the intensity of SERS signal is greatly enhanced. After processing all SERS data, the obvious differences between the SERS spectra of two species are determined. And applying the chemometric tools of principal component analysis and hierarchical cluster analysis (PCA-HCA), the SERS spectra of two species are distinguished more accurately. The results indicate that SERS analysis can provide a rapid and sensitive method for the detection of pathogenic bacteria.

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At present, the identification of pathogenic bacteria is always an ongoing task because many serious and even fatal medical conditions result from bacterial infection[1]. The food borne microorganism has become a serious threat to human health. With the continuous requirement for an effective method for the detection and characterization of microorganisms, surface-enhanced Raman scattering (SERS) has received much attention. It is emerging as a very powerful and sensitive biochemical detection method for providing information-rich spectra and microbial analysis[2]. In previous studies, many of non-SERS data have shown that whole-cell Raman vibrational fingerprints can serve as the basis for classification and identification of intact bacterial cells. But the normal Raman spectroscopy cannot guarantee that the reproducibility of experiment is good and the warp is small[1]. However, SERS is a surface-sensitive technique which enhances the Raman scattering by molecules adsorbed on rough metal surfaces or by SERS-active substrate, such as gold nanoparticles (AuNPs). AuNPs can greatly improve the strength of Raman signal and reduce the interference of background florescence[3]. In this paper, SERS spectra of Escherichia coli (E. coli) and Salmonella typhimurium (S. typhimurium) clearly indicate that these two fingerprinting regions, namely their characteristic peaks, are quite different. All of spectra are classified by principal component analysis (PCA) and hierarchical cluster analysis (HCA) for bacterial identification. The PCA-HCA treatment is used to highlight the enhanced bacterial species specificity[4]. With the convenience and maturity of the identification process, SERS can be used as a new analytical technique for portable diagnostic of bacterial pathogens, due to its high speed of analysis and high sensitivity[5].

The reagents used in the experiments include beef extract (Beijing Aoboxing Biotechnology Co., Ltd.), NaCl (Beijing Chemical Works), $K_2HPO_4{\cdot}3H_2O$ (Tianjin Fuchen Chemical Reagents Factory) and tryptone and bacterial agar (Beijing SeaskyBio Technology Co., Ltd.). Trisodium citrate ($Na_3C_6H_5O_7$) and chloroauric acid ($HAuCl_4{\cdot}4H_2O$) were purchased from Sigma-Aldrich.

Citrate-reduced gold (Au) colloidal solutions were set by a method based on Lee and Meisel's standard procedure[6]. Briefly, the preparation of AuNPs started by dissolving 240 mg $HAuCl_4$ in 500 mL ultrapure water (18 MΩ), and the aqueous solution was boiled with extensive stirring. Then 50 mL 1% sodium citrate was added dropwisely, and the solution was kept boiling for about 90 min. The complete citrate reduction finally resulted in a clear wine-red Au colloidal solution with a maximum absorption at approximately 520 nm, which corresponds to the average radius of colloidal Au particles about 16–20 nm.

In this paper, two species of E. coli and S. typhimurium used in the experiments were cultured in our laboratory. Bacterial cells were grown in nutrition broth medium at 37 °C (100 r/min), and the culture times are about 8 h for E. coli and 10 h for S. typhimurium[7]. The

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grown species were centrifuged at 7 500 r/min for 5 min, and washed three times with distilled water. The bacterial cells were resuspended in the distilled water, and used as SERS tested samples immediately. The optical density of the tested samples was about 0.6. Mix 0.1 mL bacterial suspension with 0.5 mL Au colloidal solutions in the sample bottle, and then let the mixture stand avoiding light for 1 min before the test. The non-SERS sample was 0.6 mL bacteria suspension without Au colloidal nanoparticles. The SERS spectrum of S. typhimurium was obtained from 15 times of detection, and the SERS spectrum of E. coli was obtained from 10 times of detection.

The main instrument of portable surface enhanced Raman spectrometer Ram Trace-200-NF is made by Ram Trace Optotrace technologies, Inc. All the spectra shown here were acquired by the Raman spectrometer with resolution of 2 cm^{-1}, and the power of Raman excitation light at 785 nm was 290 mW at the sample. Typically, the scanning spectrum range was 200–2 400 cm^{-1}, the integration time was set to be 10 s, and the integral was averaged for 2 times. Finally, put the sample bottle in detecting position and begin to test.

Before comparing SERS spectrum of E. coli with that of S. typhimurium, all spectra were required to normalize the intensity of the most intense band in each spectrum by Origin software (version 8.0). In addition, removing baseline can contribute to the assignment of SERS peaks. Based on the characteristics of two different species, the PCA analysis method can intuitively reflect the results, and thus confirming the SERS spectrum detection is a better way than that for non-SERS spectrum. The principal factors (PCs) of spectra are extracted by analysis, and choose PC1 and PC2 as the abscissa and ordinate of cluster formation according to the bacterial cell type by SPSS 19.0 software. Utilize PCA to reduce the dimension of the dataset, and then group similar SERS spectrum via data regression by HCA.

The comparison of SERS and non-SERS spectra can be used to determine the effectiveness of SERS in distinguishing E. coli and S. typhimurium and investigate the differences in characteristic peaks of these two bacterial species. The normal Raman signal is so weak that it is difficult to detect when it is submerged in intense background fluorescence. Fig.1 shows five unprocessed SERS spectra and a non-SERS spectrum of E. coli and S. typhimurium. The spectra are overlapped in order to demonstrate the SERS detection reproducibility with AuNPs, and the unprocessed SERS spectra are separately taken from E. coli and S. typhimurium with each repeating five times. By comparison, it can be found that the non-SERS spectra of E. coli and S. typhimurium are extremely similar without more information of spectral features as shown in Fig.1. Instead, there are more spectral distinctions which are palpable in the corresponding SERS spectra and with good reproducibility. The spectra consistency shows that the high degree of reproducibility of SERS spectra is already achieved with AuNPs as shown in Fig.1. The Raman signal is apparently enhanced when AuNPs attach to the bacterial cell wall. It is well known that the components and architectures of the bacterial cell wall of E. coli and S. typhimurium are different, which underlies the difference of their SERS spectra[8]. Moreover, SERS detection is able to further identify that the fingerprint Raman region for E. coli is from 600 cm^{-1} to 1 800 cm^{-1}, and that for S. typhimurium is from 600 cm^{-1} to 1 600 cm^{-1}. All results show that such structural differences can be clearly visible by SERS detection.

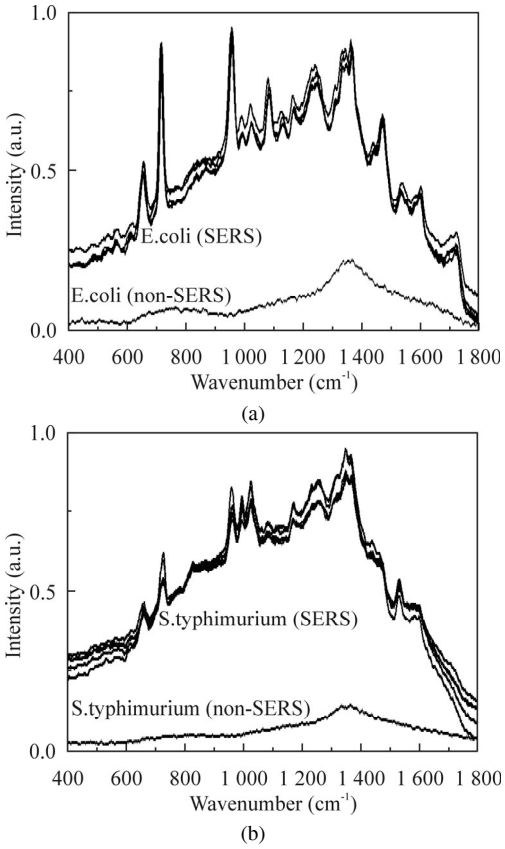

Fig.1 Five unprocessed SERS spectra and a non-SERS spectrum of (a) E. coli and (b) S. typhimurium

The second derivative is one of the effective approaches to identify the exact positions of characteristic peaks which can reflect more obvious features[9]. Every negative peak in the second-derivative spectra precisely corresponds to a peak or a shoulder peak in original spectra. The second-derivatives of non-SERS spectra of E. coli and S. typhimurium have a slight difference, and it is not obvious enough to distinguish both of them as shown in Fig.2(a) and (c), because the characteristic peaks do not stand out from other miscellaneous peaks. But a more distinct species-specific vibrational spectral signature can be seen in the SERS spectra shown in Fig.2(b) and (d) than the corresponding non-SERS spectra. The second-derivatives of SERS spectra can be used as a reference to identify the characteristic peaks clearly in the follow-up experiment. The results show that SERS spectral technology can enhance the original dim signal,

and it is more promising than ordinary Raman scattering[10].

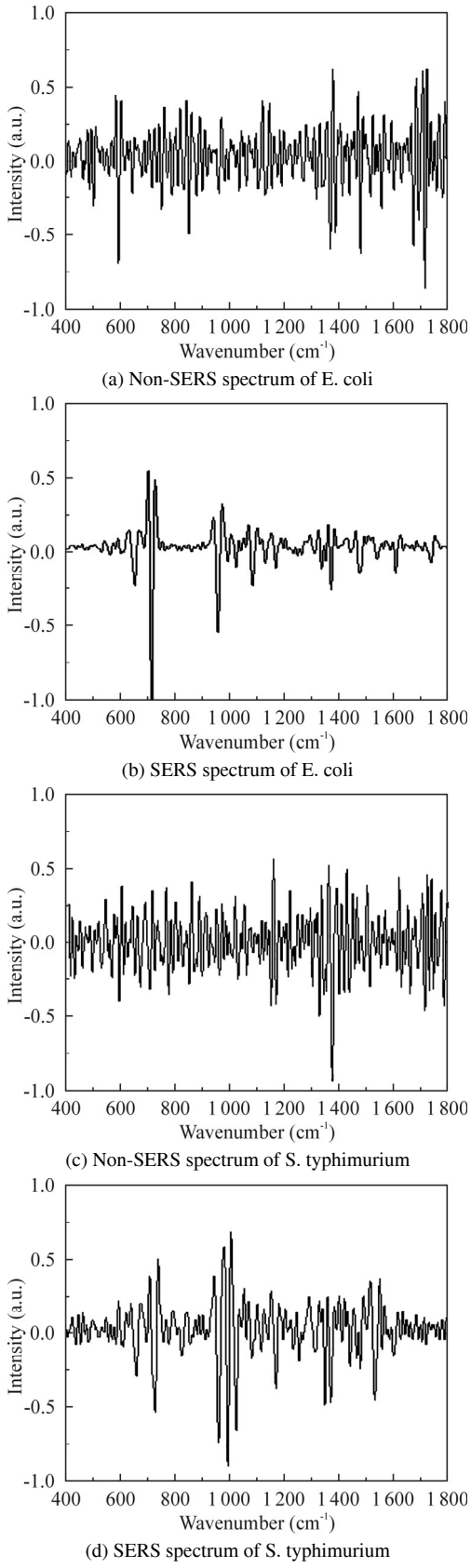

Fig.2 Second-derivatives of non-SERS and SERS spectra of E. coli and S. typhimurium

E. coli and S. typhimurium belong to Gram-positive bacteria, but there are strict differences for these two species in the peak position and relative strength. To better distinguish two species, the measured SERS spectra are pre-processed by baseline correction. The SERS spectra of E. coli and S. typhimurium and the positions of peaks are shown in Fig.3, and the differences are obviously presented.

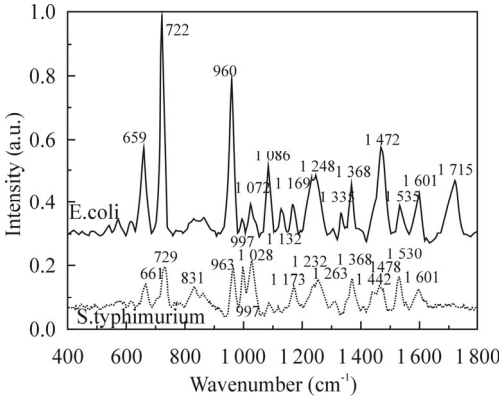

Fig.3 Comparison of SERS spectra of E. coli and S. typhimurium

In general, most Raman spectra analyses focus on the qualitative analysis. The main aims of peak identification are to classify different kinds of bacteria and to describe the differences in cell surface structures. At present, the surface structures of E. coli and S. typhimurium are not very clear, so the assignments of SERS peaks need to extrapolate by the existing data shown in Tab.1. The protein bands at 1 442 cm^{-1}, 1 472 cm^{-1} and 1 478 cm^{-1} belong to C-H_2 deformation of the protein molecules. And the bands associated with protein molecule can be found at 1 232 cm^{-1} (amide III)[11]. The results show the differences in structure and composition of proteins in both species. Moreover, the bands at 722 cm^{-1} and 729 cm^{-1} in E. coli and S. typhimurium are the deformational vibrations of adenine, and these bands are the typical spectral characteristics of DNA. The different bands are not interpreted in detail as they are not very conclusive, but they might be affected by cell lysates.

The differences generated by the data statistics analysis are the useful abstraction for SERS spectrum identification. The PCA reduced data sets are used as the inputs to HCA procedures. The PCA result shown in Fig.4(a) presents that the SERS spectra of E. coli and S. typhimurium are separately concentrated in two different locations. Then, the HCA result apparently shows that the same species will gather together due to high similarity by corresponding dendrogram shown in Fig.4(b). The dendrogram shows that the distance between the samples in the same species is smaller than that between the samples from different species, although there is a fine distinction between the samples in the same species[12]. The repeated experiments prove that the results of PCA-HCA analysis well coincide with the SERS spectrum measurement.

Tab.1 Tentative assignment of biochemical bands identified by Raman spectra with peaks shown in Fig.3

Peak position (cm^{-1})		
E. coli	S. typhimurium	Peak assignment
659	661	Guanine, (C-S)
722		Adenine
	729	Adenine, trans conformation of (C-S) or tryptophan
	831	O-P-O stretching or tyrosine
960	963	δ (C=C) or tyrosine
997	997	Phenylalanine, or glucose
1 027	1 028	A ring stretching, or (C-H) deformation
1 086		Phenylalanine
1 132		Not specified
1 169	1 173	12-Methyltetradecanoic acid or 15-Methylpalmitic acid or acetoacetate
	1 232	Amide III
1 248		($C-H_2$) stretching
	1 263	Cytosine, adenine(DNA), amide III random coil
1 335		($C-H_2$) deformation , or tryptophan
1 368	1 368	Not specified
	1 442	($C-H_2$) deformation in plane
1 472		($C-H_2$) (protein)
	1 478	($C-H_2$) (protein)
1 535	1 530	Adenine, cytosine, guanine
1 601	1 601	Tyrosine, (C-N) stretching vibration
1 715		(C=O)

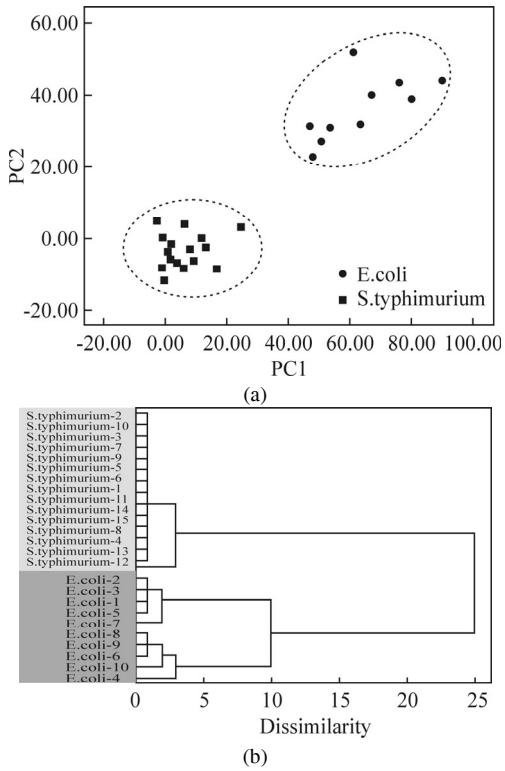

Fig.4 (a) PCA for Raman spectra of E. coli and S. typhimurium and (b) a dendrogram generated by HCA

In this paper, SERS is used in detection and identification of pathogenic microorganisms, such as E. coli and S. typhimurium. SERS can get more accurate results and is easier to operate than the ordinary Raman scattering[13]. It is validated by the PCA and HCA analysis that the method has higher accuracy, better repeatability and practicality. The main significance of the experiment is that it provides a systematic way to discern the difference between the two species, and can be applicable to other tests. The results show that SERS has become a highly sensitive research tool for the characterization of microorganisms. But the further investigation is needed to determine the effectiveness of using AuNPs to reproducibly identify the pathogenic bacteria in food inspection. Additionally, the effects of different growth phases on SERS spectrum are still a concern.

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