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Discrimination of foodborne pathogenic bacteria using synchrotron FTIR microspectroscopy

Ya-Di Wang[1,2] · Xue-Ling Li[3,4] · Zhi-Xiao Liu[1,2] · Xing-Xing Zhang[1] · Jun Hu[1] · Jun-Hong Lü[1]

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Abstract Traditional Fourier transform infrared (FTIR) spectroscopy has been recognized as a valuable method to characterize and classify kinds of microorganisms. In this study, combined with multivariate statistical analysis, synchrotron radiation-based FTIR (SR-FTIR) microspectroscopy was applied to identify and discriminate ten foodborne bacterial strains. Our results show that the whole spectra (3000–900 cm^{-1}) and three subdivided spectral regions (3000–2800, 1800–1500 and 1200–900 cm^{-1}, representing lipids, proteins and polysaccharides, respectively) can be used to type bacteria. Either the whole spectra or the three subdivided spectra are good for discriminating the bacteria at levels of species and subspecies, but the whole spectra should be given preference at the genus level. The findings demonstrate that SR-FTIR microspectroscopy is a powerful tool to identify and classify foodborne pathogenic bacteria at the genus, species and subspecies level.

Keywords Synchrotron FTIR microspectroscopy · Foodborne pathogens · Bacterial discrimination · Subdivided spectral regions · Multivariate statistical analysis

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✉ Xue-Ling Li
xlli@scbit.org

✉ Jun-Hong Lü
lujunhong@sinap.ac.cn

[1] Division of Physical Biology and CAS Key Laboratory of Interfacial Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences (CAS), Shanghai 201800, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Shanghai Center for Bioinformation Technology, Shanghai Academy of Science and Technology, Shanghai 201203, China

[4] Center for Clinical and Translational Medicine, Shanghai Industrial Technology Institute, Shanghai 201203, China

1 Introduction

Fast discrimination and accurate identification of foodborne pathogens are essential for the management of food safety and quality, including tracing contaminants and troubleshooting problems such as spoilage [1]. Bacterial species have been identified by culturing methods, relying on culturing processes coupled to morphological, physiological and biochemical characterization; and/or by DNA-based methods, such as real-time PCR and DNA microarrays. All these conventional methods are labor-intensive and time-consuming [3, 4]. In order to control and minimize the microbiological hazard of food products, efficient techniques for bacteria identification in a rapid and unequivocal way have been continuously pursued.

As a sensitive, rapid and noninvasive technique, Fourier transform infrared (FTIR) spectroscopy has been widely applied to typing and classification of bacteria since it can identify functional groups in bacterial specimen based on their vibration modes at different infrared wave numbers

[2, 3]. When an infrared light passes through a sample, certain wavelengths absorbed lead to stretching, contracting or bending vibrations of functional groups, thus an IR spectrum is produced which contains a number of absorption bands [4]. In the mid-infrared region (4000–400 wavenumber in cm^{-1}), three main spectral regions (Fig. S1) are commonly used [5, 6]. The wavenumber range 3000–2800 cm^{-1} (the "lipid region") reflects the information most of membrane lipids and some side chains of amino acids, since this region is dominated by C–H symmetrical or asymmetrical stretching vibrations of $-CH_3$ and $>CH_2$ functional groups [3, 7, 8]. The region of 1800–1500 cm^{-1} (the "protein region") is dominated by proteins, with two intensive bands mainly representing C=O stretching vibration of amide I and N–H bending or C–N stretching vibrations of amide II band of proteins [9, 10]. The wavenumber range 1200–900 cm^{-1} (the "polysaccharide region") is dominated by polysaccharides in the cell wall and phosphate-containing compounds like nucleic acids, as stretching vibrations of C–O–C, C–O–P and PO_2^- groups [3, 4, 11]. Thus, each type of bacteria would possess a fingerprint infrared absorption spectrum according to their specific chemical compositions [12]. During recent years, traditional FTIR spectroscopy has been widely reported for identification, discrimination and classification of bacteria [2, 7, 8, 13, 14], but in most studies, the whole spectra rather than spectra of subdivided wavenumber ranges were used.

Compared with conventional FTIR spectroscopy with ~75 μm spatial resolution, synchrotron radiation-based FTIR (SR-FTIR) spectroscopy is of higher signal-to-noise (by 100- to 1000-fold), higher collimation and luminance which can reach diffraction limit with 10 μm or better [15–19], so that it even probes the heterogeneities in the bacterial population at single cell level. In this study, SR-FTIR microspectroscopy coupled with multivariate regression analysis method was applied to characterize bacteria. Both whole spectra (3000–900 cm^{-1}) and subdivided spectra of lipid, protein and polysaccharide regions were chosen to discriminate bacteria at levels of genus, species and subspecies.

Fig. 1 FTIR spectra (*left*) and PCA results (*right*) of six genus bacteria (*L. innocua*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Y. enterocolitica*, *V. parahaemolyticus* and *Staphylococcus epidermidis*) of whole spectral region (**a**), lipid (**b**), protein (**c**) and polysaccharide (**d**) regions ▶

2 Materials and methods

2.1 Bacterial strains

Ten bacterial strains (Table 1) were used including *Staphylococcus epidermidis*, *Listeria innocua*, *Salmonella* spp., *Shigella dysenteriae*, *Vibrio* spp. Most of the bacteria were foodborne pathogens except *S. epidermidis*.

2.2 Bacteria culture and collection

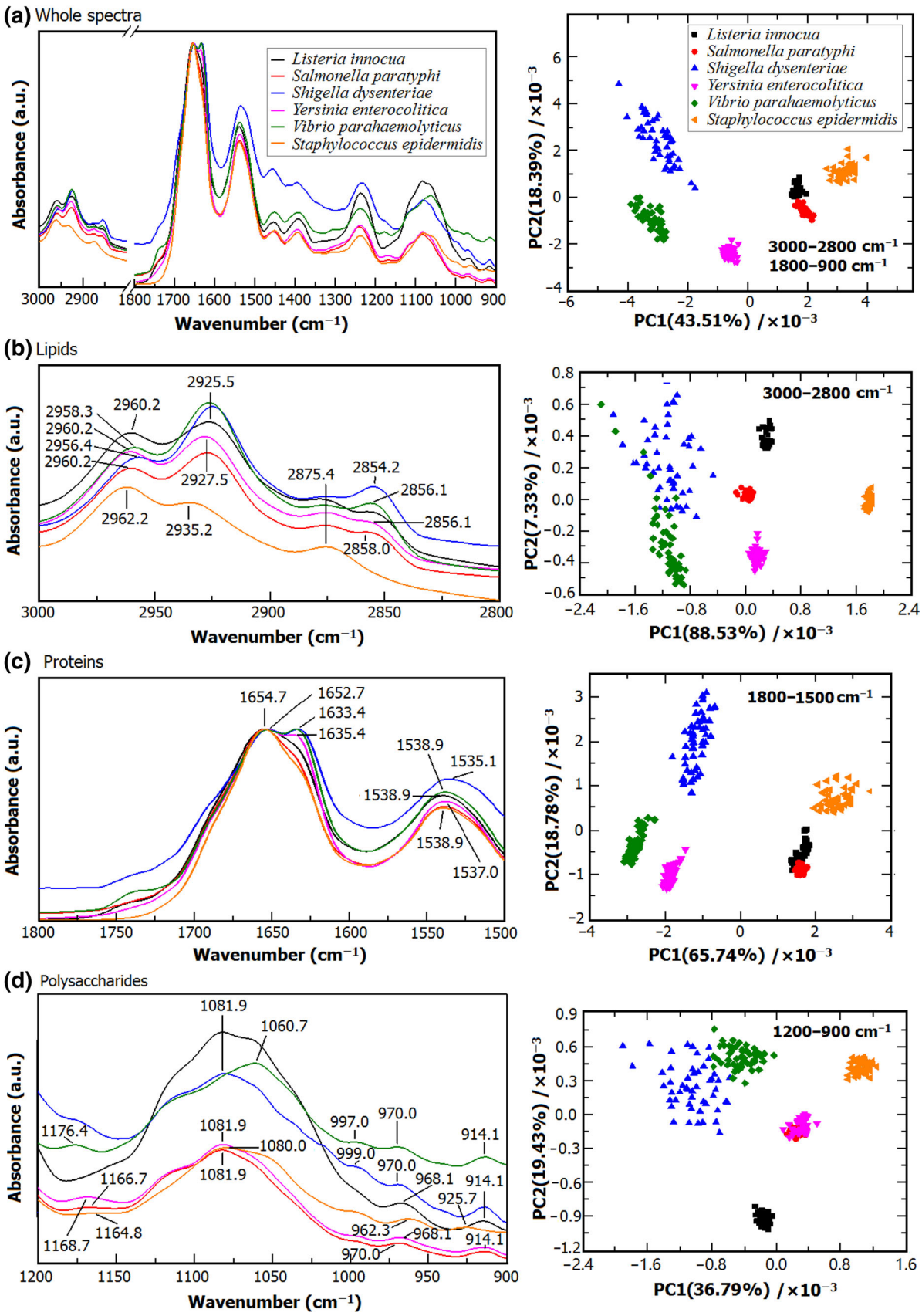
Bacterial strains were cultured in corresponding liquid culture medium overnight. For each species, 1 mL suspension (approximately 5×10^7 CFU/mL) was collected after centrifugation (8000 rpm, 5 min), the pellet was washed three times using Milli-Q water (18.2 MΩ cm^{-1}, Millipore, Bedford, MA, USA) and re-suspended in 50 μL absolute ethyl alcohol.

2.3 Synchrotron FTIR microspectroscopy

SR-FTIR microspectroscopy experiments were performed at the beamline BL01B1 of Shanghai Synchrotron Radiation Facility (SSRF). Before measurement, one drop of suspension was deposited on the BaF_2 window and air-dried at room temperature [7]. The absorption spectra were collected by Nicolet 6700 FTIR spectrometer with Continuum XL FTIR microscope equipped with 32× Schwarzschild objective (N.A. = 0.65). Transmission mode

Table 1 Strains used in this study

Genus	Strain	Culture medium and temperature (°C)
Staphylococcus	*S. epidermidis* (CGMCC 1.4260)	Nutrient agar, 37
Listeria	*L. innocua* (CICC 10417)	Brain-heart agar, 37
Salmonella	*S. enteritidis* (CICC 21482)	Nutrient agar, 37
	S. typhimurium (CICC 10420)	Nutrient agar, 37
	S. paratyphi (CICC 10437)	Nutrient agar, 37
Shigella	*S. dysenteriae* (CGMCC 1.1869)	Nutrient agar, 37
Yersinia	*Y. enterocolitica* (CICC 21669)	Nutrient agar, 25
Vibrio	*V. vulnificus* (CICC 10383)	Marine agar 2216, 30
	V. parahaemolyticus (CGMCC 1.1997)	Marine agar 2216, 30
	V. fluvialis (CGMCC 1.1609)	Marine agar 2216, 30


(a) Whole spectra
Absorbance (a.u.)
Wavenumber (cm⁻¹)
Listeria innocua
Salmonella paratyphi
Shigella dysenteriae
Yersinia enterocolitica
Vibrio parahaemolyticus
Staphylococcus epidermidis
PC2(18.39%) /×10⁻³
PC1(43.51%) /×10⁻³
3000–2800 cm⁻¹
1800–900 cm⁻¹
(b) Lipids
2925.5
2960.2
2958.3
2960.2
2956.4
2960.2
2927.5
2875.4
2854.2
2856.1
2856.1
2962.2
2935.2
2858.0
PC2(7.33%) /×10⁻³
PC1(88.53%) /×10⁻³
(c) Proteins
1654.7
1652.7
1633.4
1635.4
1538.9
1535.1
1538.9
1538.9
1537.0
PC2(18.78%) /×10⁻³
PC1(65.74%) /×10⁻³
1800–1500 cm⁻¹
(d) Polysaccharides
1081.9
1060.7
1176.4
1081.9
1080.0
997.0
970.0
999.0
970.0
914.1
1166.7
1081.9
968.1
914.1
925.7
1164.8
1168.7
962.3
968.1
914.1
970.0
PC2(19.43%) /×10⁻³
PC1(36.79%) /×10⁻³
1200–900 cm⁻¹

was chosen for sample testing, aperture was set 20 μm × 20 μm. Each specimen was measured at 50 different sites ($n = 50$) within the wavenumber 4000–650 cm^{-1}, with 64 co-added scans at 4 cm^{-1} resolution. Spectra were collected using OMNIC 9.2 (Thermo Fisher Scientific) followed by baseline correction, 15-point smoothing and normalization [5, 8]. Second derivative spectra were calculated using Savitsky–Golay method to improve resolution and minimize baseline variability.

2.4 Data analysis

Principal component analysis (PCA) makes it easy to distinguish the spectral differences by a data reduction method [3]. After essential information is extracted from the complex spectral data sets, and several uncorrelated variables (principal components, PCs) are listed in a descending order [20], the first two PCs are chosen and converted into a score plot. In our study, PCA was carried out on the second derivative spectra of 10 bacterial strains using MATLAB 8.3. The scatter plots were drawn using Origin 9.3.

3 Results and discussion

FTIR absorption spectra of six genus bacteria (*L. innocua*, *Salmonella paratyphi*, *S. dysenteriae*, *Y. enterocolitica*, *V. parahaemolyticus* and *S. epidermidis*) were acquired and analyzed. The average spectra of the 3000–2800 and 1800–900 cm^{-1} regions are shown in Fig. 1a. The spectral bands were too complex to distinguish. To find out the exact positions of all peaks and shoulder peaks in spectra, second derivative spectra of the six bacteria were calculated and PCA was performed. The results showed that PC1 and PC2 totally expressed 61.9% of the variation, so they were chosen to draw score plots. Scatter plots indicated that the six bacteria could be distinguished using the whole spectra, and therefore SR-FTIR microspectroscopy can identify and differentiate bacteria at the genus level.

To explore whether subdivided spectral regions can be used to differentiate bacteria, spectral peaks were labeled and PCA analysis was done. In the lipid region [21], most adsorption bands for the bacteria differed from each other, except a same absorption band at 2875 cm^{-1} (Fig. 1b). PCA results proved that the bacteria could be

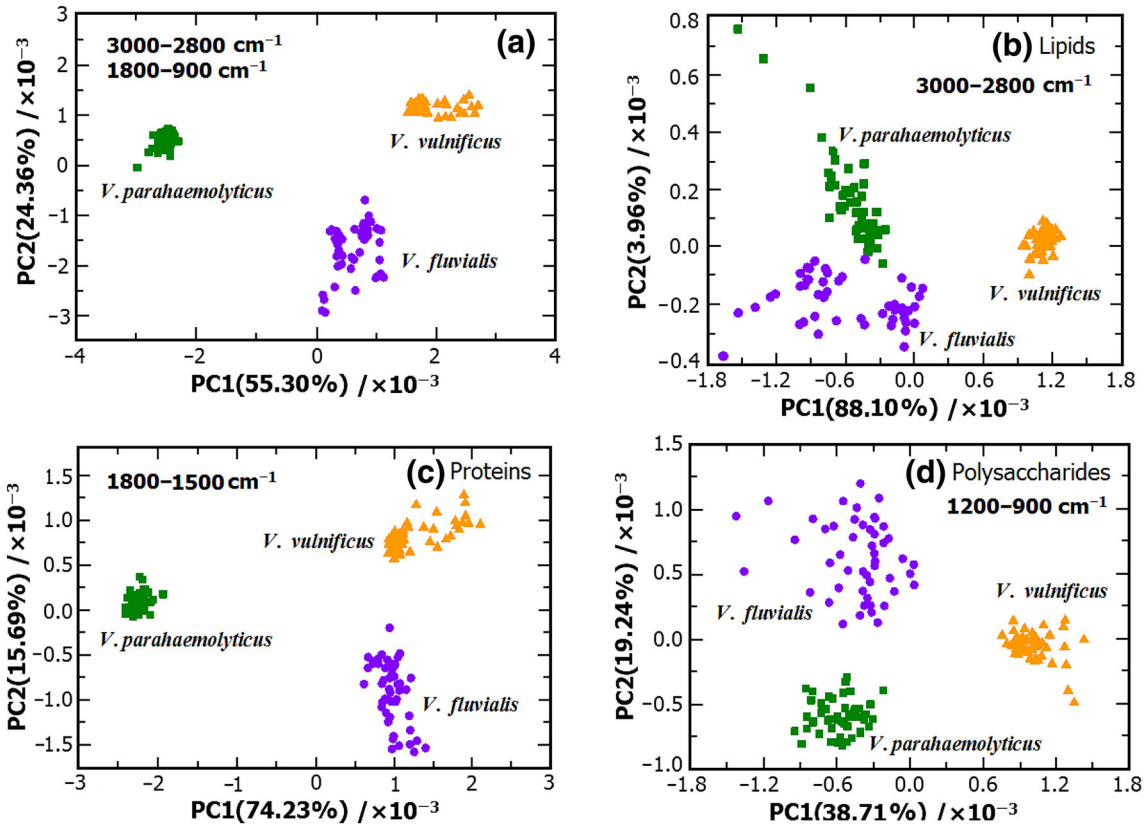


Fig. 2 PCA results of three different bacterial species from *Vibrio* (*V. parahaemolyticus*, *V. fluvialis* and *V. vulnificus*) of whole spectral region (**a**), lipid (**b**), protein (**c**) and polysaccharide (**d**) regions

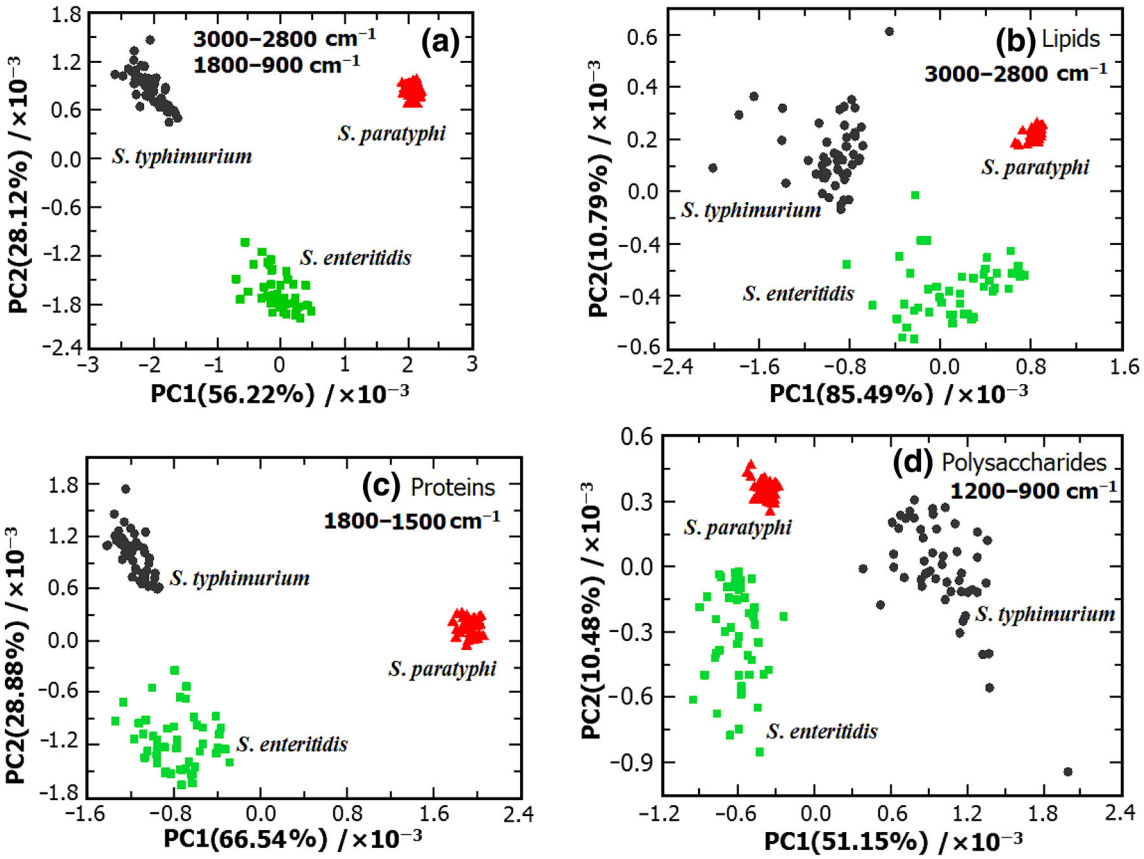


Fig. 3 PCA results of three different bacterial strains from *Salmonella enterica* subsp. (*S. enteritidis, S. typhimurium and S. paratyphi*) of whole spectral region (**a**), lipid (**b**), protein (**c**) and polysaccharide (**d**) regions

discriminated, though it was not very good between *S. dysenteriae* and *V. parahaemolyticus*. In the protein region [3], *L. innocua*, *S. paratyphi* and *S. epidermidis* had similar absorption spectra (Fig. 1c) and PCA results indicated that the protein spectra region could not be used to differentiate them. In the polysaccharide region (Fig. 1d), though the spectra had higher specificity, the PCA results could discriminate the bacteria except *S. paratyphi* and *Y. enterocolitica*.

Therefore, whole spectra were better than three subdivided spectral regions to differentiate the bacteria at genus level.

FTIR spectra of the same bacterial species of *V. parahaemolyticus*, *V. fluvialis and V. vulnificus* were analyzed, too. The whole spectra and three subdivided spectra with their absorption peaks are shown in Fig. S2. The PCA results done with whole wavenumber range, and the lipid, protein and polysaccharide regions, are shown in Fig. 2. The results show that each bacteria had specific absorption bands. Score plots proved that the three bacterial species were noticeably segregated with distinct clustering using either whole spectra or spectra of three spectral regions, indicating that SR-FTIR microspectroscopy is a sensitive technique to detect and discriminate subtle differences of chemical components between bacterial species from the same genus, and whole spectra or lipid, protein and polysaccharide regions can be used to discriminate bacteria at species level.

We further checked whether SR-FTIR microspectroscopy was sensitive enough to discriminate bacteria at subspecies level, with three bacterial strains of *Salmonella enterica* sub species (*S. enteritidis*, *S. typhimurium* and *S. paratyphi*). As shown in Fig. S3, the whole spectra and even three subdivided regions of the three bacterial strains can be discriminated with disparate absorption bands. Similarly, PCA analysis of whole spectra and the three subdivided regions (Fig. 3) showed that the bacteria can be well differentiated. These indicate that SR-FTIR microspectroscopy can discriminate different components of lipids, proteins and polysaccharides among the bacteria subspecies using either whole spectra or subdivided spectra.

4 Conclusion

Our work first demonstrated that SR-FTIR microspectroscopy was powerful and sensitive enough to discriminate bacteria at the genus, species and subspecies level.

More importantly, we found that either whole spectra or spectra of three subdivided wavenumber regions can be used to discriminate bacteria at species and subspecies level, although whole spectra are better when used at the genus level.

Compared with traditional FTIR spectroscopy, SR-FTIR microspectroscopy is advantageous for bacterial identification. For example, sample preparation is simple and only several microliter bacterial suspension is needed; one sample can be measured within minutes; little differences in chemical compositions within a population of closely related organisms can be detected. It is believed that, due to its high sensitivity, fast speed and in-invasive measurements, SR-FTIR microspectroscopy will find the growing demand and proper application in the microbiology field.

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