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Synchrotron infrared spectral regions as signatures for foodborne bacterial typing

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Abstract Fourier-transform infrared (FTIR) spectroscopy has emerged as a viable alternative to biochemical and molecular biology techniques for bacterial typing with advantages such as short analysis time, low cost and laboratorial simplicity. In this study, synchrotron radiation-based FTIR (SR-FTIR) spectroscopy with higher spectral quality was successfully applied to type 16 foodborne pathogenic bacterial strains. Combined with principal component analysis (PCA) and hierarchical cluster analysis (HCA), we found that the specific spectral region 1300–1000 cm^{-1}, which reflects the information of phosphate compounds and polysaccharides, can be used as the signature region to cluster the strains into groups similar with genetic taxonomic method. These findings demonstrated that FTIR spectra combined with HCA have a great potential in quickly typing bacteria depending on their biochemical signatures.

Keywords Synchrotron radiation · FTIR · Spectral signature · Bacterial typing · PCA · HCA

1 Introduction

Rapid identification and typing microorganisms are an increasingly important task in food safety and epidemiological diagnosis [1]. Traditional bacteria classification methods, such as serological test, PCR, DNA-DNA hybridization, and DNA sequencing [2], are always time-consuming and need laborious culturing procedures. Moreover, these phenotype or gene-based methods have difficulty in discriminating closely related strains which have similar phenotypic or genotypic properties [1].

Fourier-transform infrared (FTIR) spectroscopy has emerged as a real alternative of molecular biology and biochemical methods to describe microorganisms dependent on chemical components [3–5]. Due to the advantages of short analysis time and low cost [6], since it was introduced to characterize microorganisms by Naumann and co-workers [7, 8], FTIR spectroscopy was successfully applied to discriminate bacteria at genus, species, and even subspecies level [9]. The commonly used infrared spectra (Figure S1, Supporting Information) include a lipid region, protein region, mixed region, and polysaccharide region (3000–2800, 1800–1500, 1500–1200, and 1200–900 cm^{-1}, respectively) [10]. Since infrared spectra contain complex chemical information, multivariate statistical analysis methods, such as principal component analysis (PCA) and

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hierarchical cluster analysis (HCA), are usually applied to explain their diversities and to further type FTIR spectra of bacteria [11–13].

The development of synchrotron source for infrared spectroscopy has greatly improved the accuracy and signal-to-noise ration of the measurements [14]. In the previous work, we have successfully applied synchrotron radiation-based FTIR (SR-FTIR) spectroscopy to discriminate 10 bacterial strains. Herein, we tried to further classify and type 16 foodborne bacteria from seven genera by the combination of SR-FTIR and chemometric methods. Whole spectra (3000–2800, 1800–900 cm^{-1}) and spectra of four divided regions (lipid region, protein region, mixed region, and polysaccharide region) were used, respectively. We found that the spectra region between 1300 and 1000 cm^{-1} can be recognized as the signature region to cluster the bacteria from the same taxonomic levels into one group. With high reproducibility and low sample amount, SR-FTIR spectroscopy shows great potential in fast identifying and typing microorganisms based on the specific infrared signature.

2 Experimental section

2.1 Bacterial strains

A total of 16 foodborne bacteria were used, and their culture conditions are listed in Table 1 (Supporting Information).

2.2 Sample preparation

Bacterial strains were incubated overnight and collected by centrifugation, respectively. After re-suspended in 50 µl ethyl alcohol, 5 µl sample was dropped on the BaF_2 window and air-dried at room temperature [9, 14, 15].

2.3 Synchrotron FTIR spectroscopy

Transmission mode was chosen, and aperture was 20 µm × 20 µm. Data collecting was monitored by OMNIC 9.2 (Thermo Fisher Scientific), and the collected spectra were further processed by baseline correcting, 15-point smoothing, and normalization [11, 16]. The Savitzky–Golay method was used to calculate the first derivative spectra.

2.4 Data analysis

PCA and HCA were carried out on first derivative spectra using SPSS Statistics 22.0 (IBM). For PCA, the first two PCs (PC1 and PC2) were used to draw scatter plots. For HCA, Ward's linkage algorithm and Euclidian distance measurements (or Pearson's correlation coefficient) were chosen [11], the top horizontal axis of a dendrogram depicted the distance coefficient values, and the actual distance was assigned 0–25 in proportion.

3 Results and Discussion

Spectra were recorded from 16 different bacterial isolates (Table S1, Supporting Information). A typical SR-FTIR spectrum of *Vibrio parahaemolyticus* is shown in Fig. S1 (Supporting Information). The recorded spectra of these 16 bacterial strains showed similar bands in the whole spectral region (3000–2800 and 1800–900 cm^{-1}), and little differences were observed (Fig. S2a, Supporting Information). To further resolve the spectral differences among these strains, the first derivative transformations were performed. As shown in Fig. S2b (Supporting Information), the first derivatives of reduced FTIR spectra displayed minor differences in the mixed region (1500–1200 cm^{-1}) and in the polysaccharide region (1200–900 cm^{-1}).

To find out the better discriminating results, the first derivative spectra of the 16 bacterial strains were used and multivariate analysis methods PCA and HCA were carried out on the whole spectral region and specific regions (such as lipid region, protein region, mixed region, and polysaccharide region), respectively.

We firstly tried to carry out PCA on whole spectra of 16 bacteria while performing HCA to explain their affinity–disaffinity. As shown in Fig. 1, it can be seen that all *Listeria* strains are well separated by principle component one and two. The dendrograms from HCA of the average spectra showed close clustering of these species, suggesting that *Listeria* strains have a similar biochemical profile and all of them are from the same order. Two *Staphylococcus* strains were also grouped together. However, both *Vibrio* strains and *Salmonella* strains were decentralized and could not cluster into their corresponding group. These results indicated that the whole spectra could be difficult to distinguish all the bacterial strains at distinct taxonomic levels.

Since the infrared spectra of the microorganism reflect the information of several classes of biomolecules, PCA and HCA were carried out on these specific spectral regions, respectively. For the lipid region (3000–2800 cm^{-1}) and protein region (1800–1500 cm^{-1}), most strains with the same genus did not cluster correctly and only *Listeria* strains were partly grouped together (Fig. S3 and Fig. S4, Supporting Information). These results indicated that either the lipid region or protein

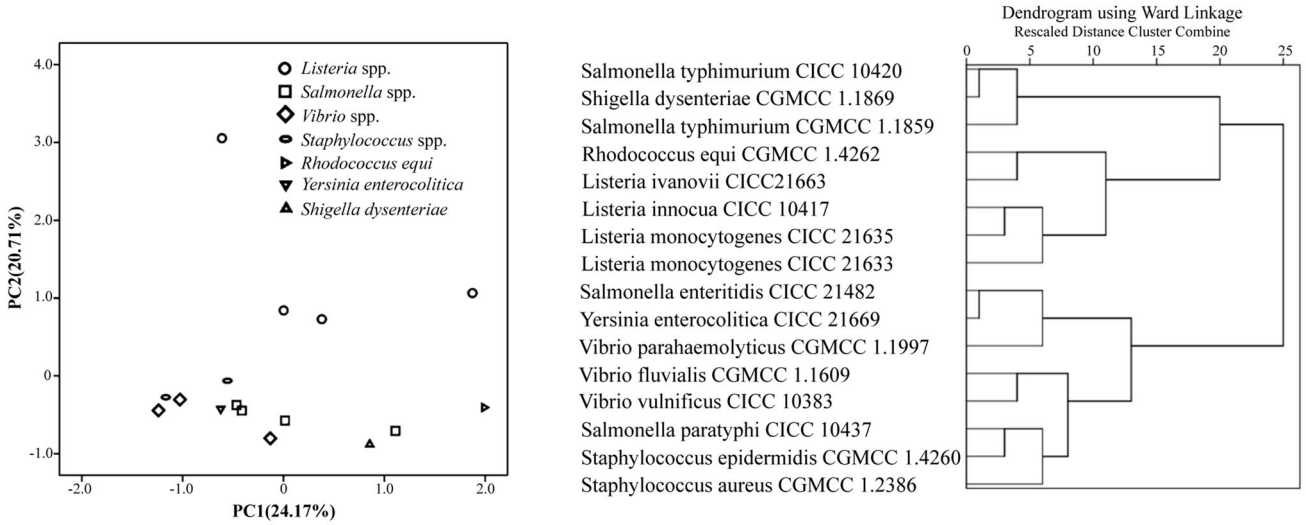

Fig. 1 Score plots from a PCA (first and second components, left) and HCA (right) of the whole infrared spectra of the 16 bacterial strains

region contained low specific information among these strains and could not be used to classify bacteria.

We further typed the bacteria based on the spectra of the mixed region 1500–1200 cm^{-1} (Fig. 2). PCA displayed that four *Listeria* strains and three *Vibrio* strains were significantly separated from other bacteria. HCA illustrated that *Listeria*, *Vibrio*, *Staphylococcus*, and *most Salmonella* (except *S. dysenteriae*) were formed as separate genus-specific clusters. Although not all strains were classified into their corresponding sub-clusters, this result was very close to the phylogenetic relatedness. Interestingly, similar results were obtained after PCA and HCA of the spectral region 1200–900 cm^{-1}, which mainly represent the information about nucleic acids and polysaccharides components (Fig. 3). For example, four *Listeria* strains were clearly differentiated from the others and three of four *Salmonella* species were classified into a same cluster. However, some strains, such as *Staphylococcus epidermidis*, *S. typhimurium* (CICC10420), and *Vibrio vulnificus*, were misclassified.

The above results suggested that the spectra information in the mixed region (1500–1200 cm^{-1}) and polysaccharide region (1200–900 cm^{-1}) had relatively good discriminating capability, which have been previously reported as the specific regions for characterizing these kinds of bacterial strains [17, 18]. To acquire the most appropriate spectra for typing bacteria within their corresponding taxonomy, the wavenumber, ranging from 1500 to 900 cm^{-1}, was carefully optimized and selected. Finally, the region 1300–1000 cm^{-1} was found of particular interest because

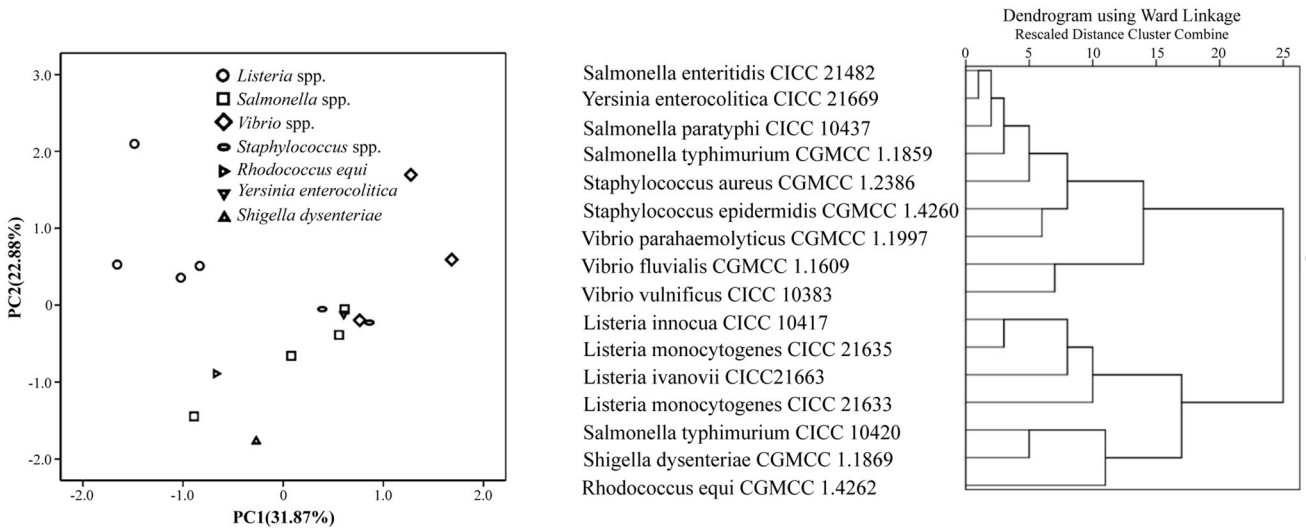

Fig. 2 PCA (left) and HCA (right) of the 16 bacterial strains based on the infrared spectra of the mixed region (1500–1200 cm^{-1})

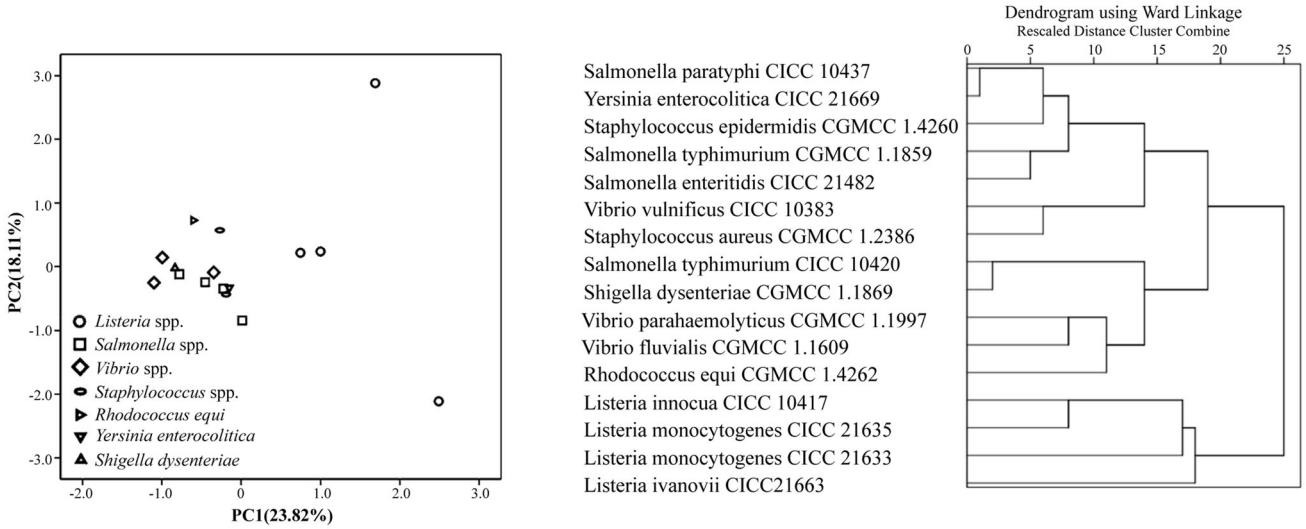

Fig. 3 PCA (left) and HCA (right) of the 16 bacterial strains based on the infrared spectra of the polysaccharide region (1200–900 cm^{-1})

of its proximity to the best clustering results (Fig. 4). This region mainly reflected the chemical information of P=O symmetric and asymmetric stretching vibrations of $> PO_2^-$ in nucleic acids or phospholipids and C–O–C, C–O vibrations in glycogen and carbohydrates [19]. The results showed that these bacteria were typed into three larger clusters, including four *Listeria* strains, three *Vibrio* strains, and two *Staphylococcus* strains which were clearly classified. Although the *Salmonella* species, *Yersinia enterocolitica* and *Shigella dysenteriae* were grouped together, the fact that all three of them belong to members of *Enterobacteriales* could explain the difficulty of discrimination, which was consistent with previous reports [12, 20].

4 Conclusion

We reported on the use of SR-FTIR spectroscopy to type 16 foodborne bacteria from seven genera. A whole spectral region and four subdivided spectral regions were analyzed, respectively. We found that the wavenumber range of 1300–1000 cm^{-1}, which mainly reflects the information of nucleic acids and polysaccharides, contained more specific information and could be referred to as the signature region. The spectral signature proceeded with PCA and HCA obtained a delineation among the bacterial classes, and cluster results matched with their phylogenetic relationship. These results proved that SR-FTIR spectroscopy with high spectral resolution has the greatest potential in fast identifying and typing microorganisms based on the specific infrared signature.

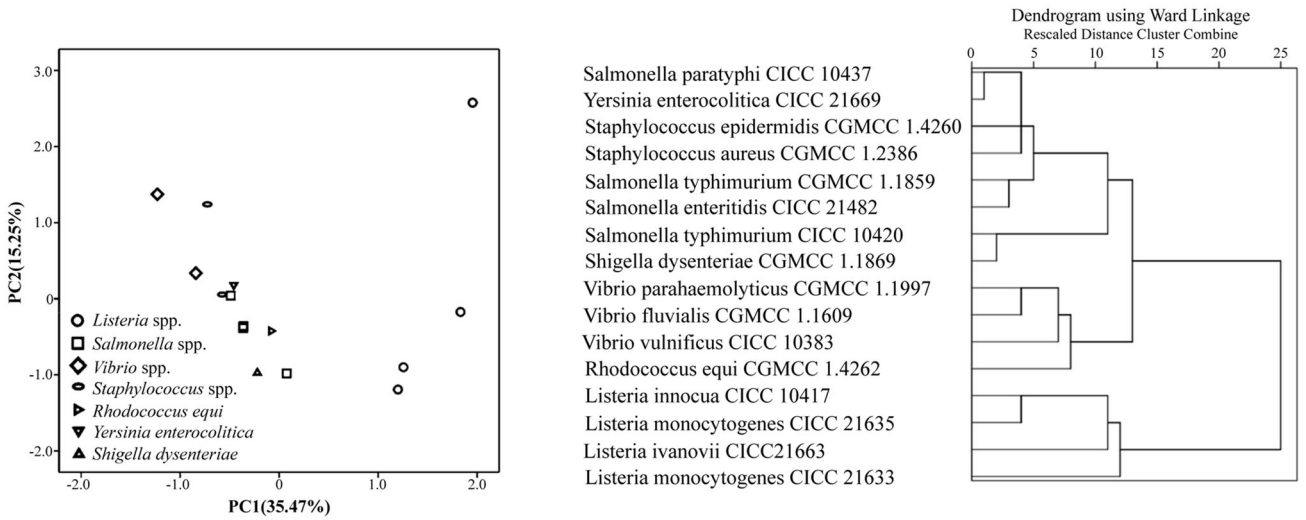

Fig. 4 PCA (left) and HCA (right) of 16 bacterial strains based on the infrared spectral region 1300–1000 cm^{-1}

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