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FTIR Analysis of *Burkholderia seminalis* from Apricot, Water and Rice Rhizosphere

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The present work compares isolates of *Burkholderia seminalis* from different sources with fourier transform infrared (FTIR) spectroscopy. Our results show that they present a typical *Burkholderia cepacia* complex sample spectrum, in which strong amide I and amide II bands, (at 1,656 and 1,540 cm^{-1}, respectively) were the dominant features, which were mainly due to proteins. There were significant differences in protein-to-lipid ratio, indicating significantly differences of membrane structure and dynamics of *B. seminalis* isolates from different sources.

Key Words: Comparative analysis, *Burkholderia seminalis*, Different sources, FTIR.

INTRODUCTION

Burkholderia seminalis, a member of *Burkholderia cepacia* complex (Bcc), is a new species recently proposed by Vanlaere *et al.*[1]. It was first recovered from sputum of a cystic fibrosis (CF) patient as an opportunistic pathogens[1], Then it was found to be widely distributed in natural environment such as soil, water and plant rhizosphere[1-4]. Recently, an causal pathogen of bacterial fruit rot of apricot in China has also been ascribed to *B. seminalis*[5,6]. These findings arouse our concern that cystic fibrosis patients may acquire *B. seminalis* by eating contaminated foods, or environmental strains may infect plants and fruits. Therefore, it is necessary to find out the difference of *B. seminalis* strains from different sources. Traditional methods used for the differentiation of various *Burkholderia cepacia* complex microorganisms are based on fatty acid composition, carbon source utilization, 16S rDNA sequencing and recA gene sequencing[5]. However, all of these methods can often be tedious and time consuming and occasionally not conclusive[3].

Recently, Fourier transform infrared (FTIR) spectroscopy has been successfully applied in bacterial identification and classification[7-12]. FTIR spectroscopy is an sensitively physico-chemical technique, which can detect very small alterations in bond lengths and angles in macromolecules and therefore unique molecular fingerprints can be easily distinguished from the absorption patterns of other molecules[13-16]. Moreover, FTIR spectroscopy allows the rapid analysis of small quantities of biomass, simultaneous characterization of different functional groups such as lipids, proteins, nucleic acids and polysaccharides in biological molecules and complex structures and without disturbing the systems and requires no consumables or reagents[14,16-18]. These findings have shown that it is possible to discriminate among various microorganisms at the genus, species and strain level.

The objective of this study was to compare FTIR spectra of bacteria from three different sources which were previously identified as *B. seminalis*.

EXPERIMENTAL

The nine strains of *B. seminalis* used in this study were isolated from diseased apricot, waters of West lake and rhizosphere of rice separately in our previous study[2,5,6,19].

Sample preparation: Bacterial cells were collected from overnight LB broth culture grown at 28 ºC by centrifugation at 10,000 rpm for 10 min. After removing the supernatants, the bacterial pellets were washed twice with double distilled water. After second wash in double distilled water, bacterial samples were dried under vacuum for 12 h and stored at 70 ºC until lyophilization. The samples for FTIR analysis were first grounded into fine particles using mortar and pestle. The 1 mg of each sample was then mixed with 100 mg potassium

bromide which extensively dried in microfuge tubes using a lyophiliser. These mixtures have been dried for an additional 2 h in the same microfuge tubes.

FTIR spectroscopy and data analysis: The FTIR spectroscopy data were analyzed as previously described by Garip *et al.*[7] with a small modification. Pellets were scanned at 4 cm^{-1} resolution with 100 scans in the spectral range of 4000-500 cm^{-1} at room temperature. The sample compartment in the FTIR spectrometer was continuously purged with dry air to prevent water vapour. Analysis of the spectral data was performed by using Grams 32 (Galactic Industries, Salem, NH, USA) software. The spectral range of 3500-800 cm^{-1} was analyzed. The band positions were measured according to the center of weight. The averages of the spectra belonging to the same experimental groups, baseline correction, normalisation and the band areas were obtained by using the same software program. The average spectra and normalisation process were applied only for visual representation of the differences, however for the determination of the spectral parameters and calculation of mean values and statistical analysis each baseline corrected original spectrum was taken into consideration.

Statistics: The software STATGRAPHICS Plus, version 4.0 (Copyright Manugistics Inc., Rockville, Md., USA) was used to perform the statistical analysis. Levels of significance ($p < 0.05$) of main treatments and their interactions were calculated by analysis of variance after testing for normality and variance homogeneity.

RESULTS AND DISCUSSION

The resulting infrared spectroscopy fingerprints of *B. seminalis* isolates showed the typical complex shape of bacterial cells spectra, corresponding to the building blocks present in biological samples (nucleic acids, proteins, carbohydrates and lipids).

The average spectra of all *B. seminalis* samples present a typical *Burkholderia cepacia* complex sample spectrum reported previously[20]: strong amide I and amide II bands, (respectively at 1656 and 1540 cm^{-1}) were the dominant features, which were mainly due to proteins[13,14]; the bands centered at 2962 and 2930 cm^{-1} correspond to the stretching mode of asymmetrical CH_3 and CH_2 vibrations mainly due to lipids[21]; the prominent peak centered at 2876 cm^{-1} was mainly due to proteins and with little contribution from lipids, carbohydrates and nucleic acids; the prominent peak centered at 2857 cm^{-1} was mainly due to lipids. The band at 1460 cm^{-1} correspond to the CH_2 bending mode of lipids[7,11]; the band at around 1383 cm^{-1} was mainly due to amino acid side chains and fatty acids. These bands around 1233 and 1060 cm^{-1} were due to nucleic acids and glycogen[7,15]; besides, a strong band at 1736 cm^{-1}, corresponding to the ester carbonyI stretch ν(C=O) was observed, this band corresponds to compounds of the poly-hydroxyalkanoates family (PHA) that are known to be produced by *Burkholderia* spp[22].

Fig. 1 shows the average FTIR spectra of three *B. seminalis* isolates: Bca0901, R456 and S9. Table-1 shows the band frequencies of main functional groups in these three strains. As seen from the Fig. 1 and Table-1, they showed similar FTIR spectra. This result indicates that it's difficult to differentiate them by FTIR. Nevertheless, it was found that the protein-to-lipid ratio was significantly different among these isolates (Fig. 2), which was calculated by taking the ratio of the area of the amide I band to the area of ester C=O stretching band, the area of the amide II band to the area of CH_3 asymmetric stretching band, CH_2 asymmetric stretching band and CH_2 symmetric stretching band. The ratio of protein-to-lipid in the membranes is an important factor affecting the membrane structure and dynamics[23], indicating significantly differences of membrane structure and dynamics of *B. seminalis* isolates from different sources. Therefore, result from this study may give a new strategy for the rapid bacterial identification and differentiation of *Burkholderia cepacia* complex isolates from different sources.

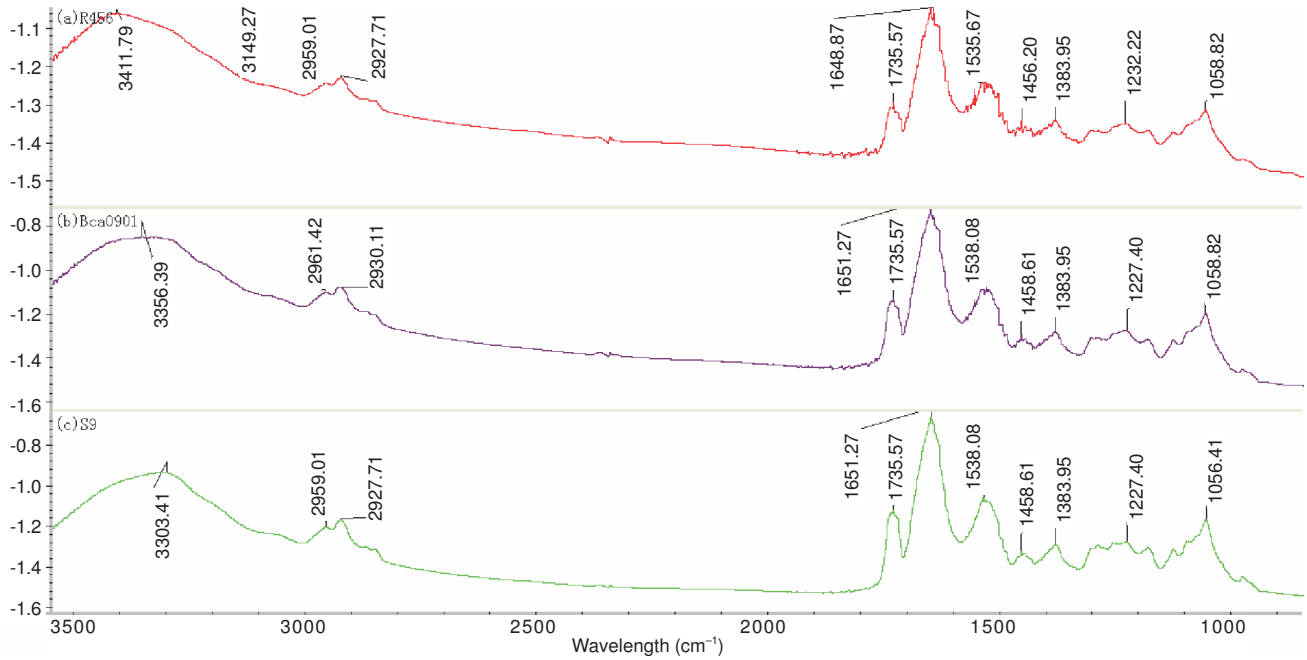

Fig. 1. Average spectra of three *B. seminalis* isolates in 3500-800 cm^{-1} region

TABLE-1
BAND FREQUENCIES OF VARIOUS FUNCTIONAL GROUPS IN THREE *B. seminalis* STRAINS OF DIFFERENT SOURCES

Functional groups[a]	Frequency (cm^{-1})[b]		
	Bca0901	R456	S9
CH_3 asymmetric stretching	2962.28 ± 0.00	2962.28 ± 0.00	2962.28 ± 0.00
CH_2 asymmetric stretching	2930.25 ± 0.00	2930.25 ± 0.00	2930.25 ± 0.00
CH_3 symmetric stretching	2875.80 ± 0.00	28750.8 ± 0.00	28750.8 ± 0.00
CH_2 symmetric stretching	2856.58 ± 0.00	2856.58 ± 0.00	2856.58 ± 0.00
Ester C=O stretching	1735.59 ± 0.00	1735.59 ± 0.00	1735.59 ± 0.00
Amide I	1655.51 ± 0.01	1655.51 ± 0.00	1655.52 ± 0.01
Amide II	1540.21 ± 0.00	1540.21 ± 0.00	1540.21 ± 0.00
CH_2 bending	1460.14 ± 0.00	1460.14 ± 0.00	1460.14 ± 0.00
COO^- symmetric stretching	1385.41 ± 1.85	1383.27 ± 0.00	1383.27 ± 0.00
PO_2^- asymmetric stretching	1233.82 ± 1.87	1232.74 ± 0.00	1232.74 ± 0.00
PO_2^- symmetric stretching	1059.78 ± 0.01	1059.78 ± 0.01	1059.78 ± 0.00

[a]Adapted from references[15,16,24]. [b]The experiment was performed trice and data from the repeated experiment were pooled.

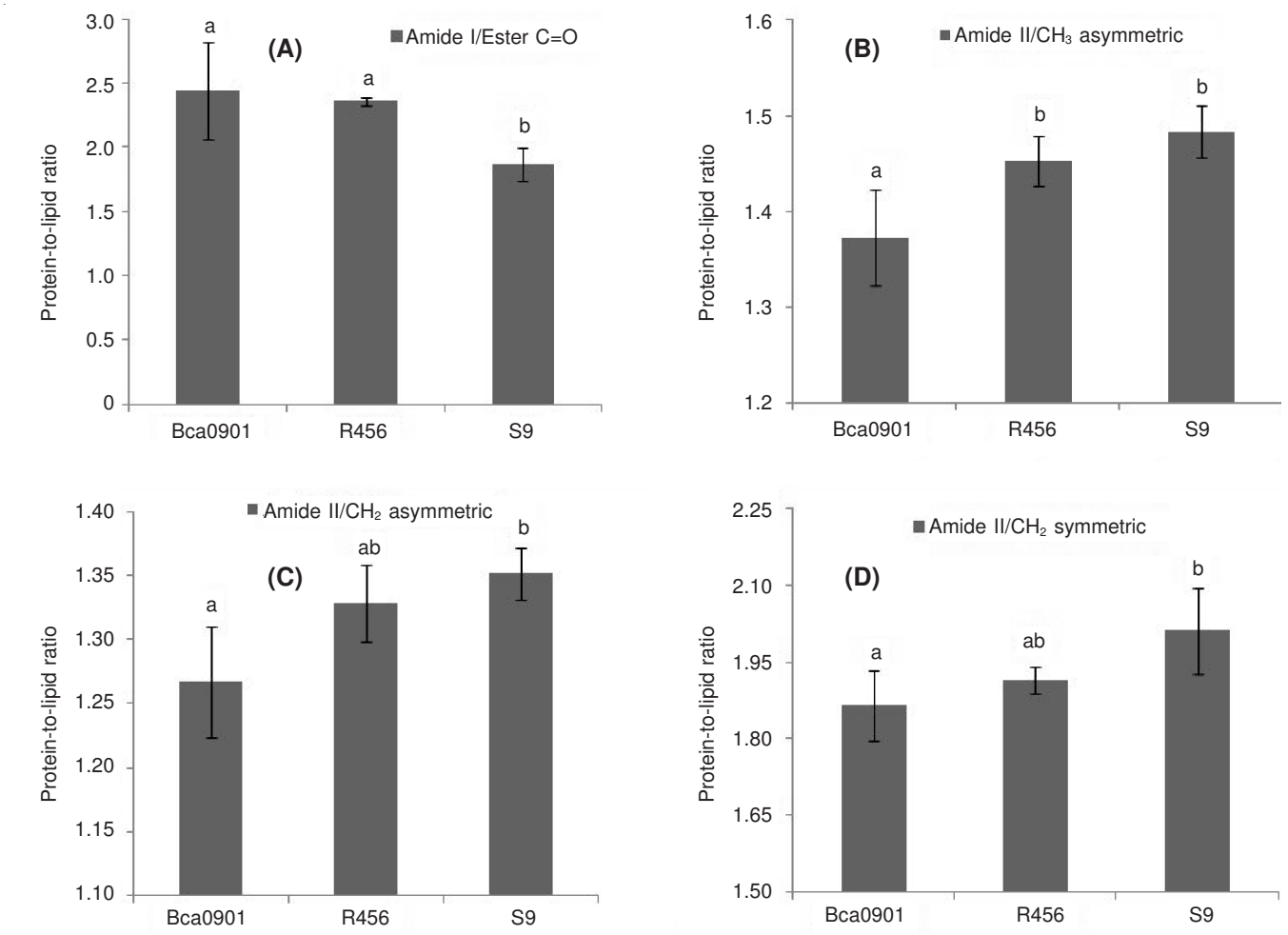

Fig. 2. Protein-to-lipid ratio of three *B. seminalis* isolates. The experiment was performed thrice and data from the repeated experiment were pooled. Means in a column followed by the same letter are not significantly different according to LSD test ($p = 0.05$)

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