Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 116 (2013) 478–484

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Identification and discrimination of bacteria using Fourier transform infrared spectroscopy

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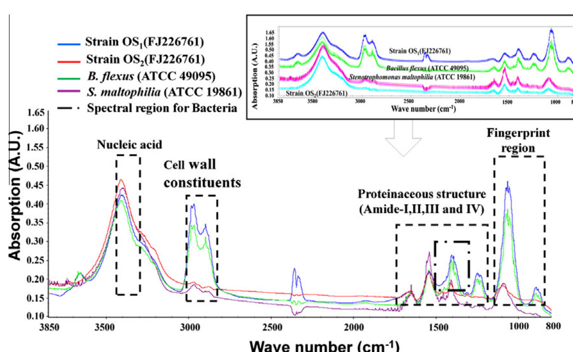
HIGHLIGHTS

- FTIR is applicable for bacterial classification and identification.
- Bacterial discrimination by proteins specific bands at 1339, 1382 and 1096 cm^{-1}.
- Cell wall constituent at 2987, 2971 and 2900 cm^{-1} found in both *B. flexus* and OS_1.
- Amide bands (I/II/III) exhibits OS_1 and *B. flexus* were closely related, except OS_2.
- Specific fingerprint at 1096 (OS_2), 1339 (OS_1) and 1382 cm^{-1} (*B. flexus*ATCC49095).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 December 2012
Received in revised form 17 June 2013
Accepted 22 July 2013
Available online 6 August 2013

Keywords:
FTIR
16S rRNA
B. flexus
S. maltophilia
Identification
Discrimination

ABSTRACT

Bacterial spectra were obtained in the wavenumber range of 4000–600 cm^{-1} using FTIR spectroscopy. FTIR spectral patterns were analyzed and matched with 16S-rRNA signatures of bacterial strains OS_1 and OS_2, isolated from oil sludge. Specific spectral bands obtained from OS_1 (FJ226761), reference strain*Bacillus flexus* (ATCC 49095), OS_2 (FJ215874) and reference strain *Stenotrophomonas maltophilia* (ATCC 19861) respectively, suggested that OS_1 and ATCC 49095 were closely related whereas OS_2 was different. The bands probably represent groups of proteins and lipids of specific bacteria. Separate peaks found in *B. flexus* were similar to those of OS_1. The *S. maltophilia* (ATCC 19861) and OS_2 exhibited a similar peak at 3272 cm^{-1}. Amide bands (I, II and III) exhibited that OS_1 and *B. flexus* were closely related, but were different from OS_2. In the fingerprint region, peak at 1096 cm^{-1} and 1360 cm^{-1} exhibited the specific fingerprints of OS_2 and reference strain *S. maltophilia* (ATCC 19861), respectively. The specific fingerprint signature was found at 1339 cm^{-1} for OS_1 and at 1382 cm^{-1} for *B. flexus* ATCC 49095, allowing these two strains of *B. flexus* to be differentiated. This spectral signature originated from phospholipid and RNA components of the cell. Principle components analysis (PCA) of spectral regions exhibited with distinct sample clusters between *Bacillus flexus* (ATCC 49095), *S. maltophilia* (ATCC 19861), OS_1 and OS_2 in amide and fingerprint region.

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Introduction

Detection and characterization of microorganisms by Fourier transform infrared spectroscopy (FTIR) technique promises to be

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http://dx.doi.org/10.1016/j.saa.2013.07.062

of great value because of the method's inherent sensitivity, non-destructive nature, small sample size, rapidity, simplicity, and the potential for complete computerization [1–11]. Spectroscopic techniques provide a wealth of specific qualitative and quantitative information about a given organism [9,12,13]. The infrared spectrum of any compound, including those found in living cells, is known to give a unique 'fingerprint' [14]. Fourier Transformation Infrared (FTIR) spectroscopy provides a non-destructive, fast, easy to use, and highly sensitive method for microbial analysis [9,15]. This technique permits non-destructive chemical characterization of living cells producing unique and reproducible biochemical spectra for different cell types [16–19]. Five spectral windows have been defined and are used for the differentiation of bacteria species [7,20,21]. Previous authors reported that the spectral region of 3000–2800 cm^{-1} was commonly assumed to be dominated by fatty acid related compounds; the region of 1700–1500 cm^{-1} by carbonyl residual of proteins (Amide I and II); the carboxylic groups of peptide (—C=O), at about 1650 cm^{-1} and —C—O— at 1500 cm^{-1}; free amino acids, and polysaccharides in the region of (1450–1400 cm^{-1}), and the region of 1250–1200 cm^{-1} by RNA/DNA (1245 cm^{-1} and 1080 cm^{-1} for $v_{as}PO_2^-$ and $v_{as}PO_2^-$) and phospholipids [22]. The region below 1500 cm^{-1} was referred as the fingerprint region (IUPAC), and contains information significant to strain-specific discrimination. Several reports have been published using FTIR spectroscopy as means of rapid identification of microorganisms [8,9,22–24], however there are insufficient reports on the identification of *Bacillus flexus* and *S. maltophilia* using the FTIR spectroscopy technique.

In this study, FTIR spectroscopy was used for detection of unique spectral parameters representing biochemical differences among different bacteria. We compare the identification of bacteria using FTIR techniques to 16S-rRNA gene sequence analysis.

Materials and methods

Isolation and cultivation of bacteria

Oil degrading bacteria were isolated from oil sludge of a sewage treatment plant (China Petroleum Refinery Company, Kaohsiung, Taiwan) using a dilution plate method in nutrient agar media (Sigma–Aldrich, Germany) under aerobic conditions at 30 °C [25]. The Isolated bacteria were grow in nutrient broth media (Peptone 5 g/L, beef extract 3 g/L, agar 15 g/L, and *Milli-Q* water, pH-7 at 25 °C). Cultivated bacteria was split into duplicate samples that were used for identification by FTIR spectroscopy and by 16S rRNA, respectively.

FTIR spectroscopy: sample preparation, spectral data collection and measurement of bacteria spectra

Samples (OS_1, OS_2, reference *B. flexus* (ATCC 49095) and *S. maltophilia* (ATCC 19861)) were prepared for the FTIR study as described by earlier workers [22,24]. Small amounts of bacterial colonies were suspended in 2 mL of saline solution and pelleted by centrifugation at 1000*g* for 2 min. The pellet was then re-suspended with 20 μL of saline solution and one drop of suspension was placed on a zinc selenide crystal (4 cm × 1 cm) and air dried for 5 min in a laminar flow hood at room temperature before being examined by FTIR spectroscopy. The FTIR spectra was recorded in transmission mode from individual cells at 4 cm^{-1} resolution with Happ-Genzel apodization, using a FTIR spectrometer (Thermo-Fisher-Nicolet (TFN), Magna-IR 860) fitted with an IR microscope system (TFN, Continuum) and a liquid–nitrogen-cooled mercury–cadmium–tellurite (MCT) detector, including KBr beamsplitter and a 32× magnification IR objective. The spectra were obtained in the wavenumber range of 4000–600 cm^{-1}. For each bacterial sample, the spectrum was taken as an average of nine different measurements at various sites of the sample (n = 9). The peak position, baseline corrections and smoothing were automatically performed by peak resolve (Thermo-Fisher-Nicolet, OMNIC 7.1). The FTIR spectra (4000–600 cm^{-1}) of bacteria were analyzed using Spectrum software (PerkinElmer V5.0) and KnowItAll software (Bio-Rad IR/NIR Edition) [26]. This software provided identification of the various functional groups and possible assignments of different bacteria.

Identification by 16S rRNA gene sequence analysis: DNA isolation, PCR (Polymerase Chain Reaction) amplification, nucleotide sequencing and accession numbers

Each strain of bacteria (OS_1, OS_2, reference *B. flexus* (ATCC 49095) and *S. maltophilia* (ATCC 19861)) was cultivated in nutrient broth for identification by 16S rRNA gene sequencing and the FTIR technique. Genomic DNA was extracted from bacteria using a DNA/RNA extraction kit (Viogene, Taipei, Taiwan). The 16S rRNA gene region of DNA was amplified by PCR using a pair of forward and reverse primers [27,28]. GenBank accession numbers were assigned from nucleotide sequences (≈99% identity) (OS_1: FJ226761; *B. flexus*: ATCC 49095; OS_2: FJ215874, and *S. maltophilia*: ATCC 19861) (*S. maltophilia* (FJ009381) is the synonym of *Pseudomonas beteli*) (http://www.ncbi.nlm.nih.gov).

Statistical analysis of FTIR spectral data

A significant level of difference between experimental bacteria was determined by factor analysis (n = 9), which was performed with the help of the PCA using STATISTICA 5.1. Also, this software was used to perform Hierarchical Cluster Analysis (HCA) on the FTIR region of the different bacteria.

Results and discussion

Identification of bacteria using two different techniques: FTIR spectroscopy and 16S-rRNA gene sequence

Different bacterial strains were investigated by FTIR spectroscopy to determine the specific spectroscopic biomarkers useful for identification and discrimination of selected bacterial species. The peak position, baseline corrections and smoothing were automatically performed by peak resolve for a profile of each peak from the same bacterial strain. FTIR spectra and possible assignments of each isolate (OS_1 and OS_2) were compared with *B. flexus* reference strain (ATCC 49095) and *S. maltophilia* (ATCC 19861) (Figs. 1–7; Table 1). The 16S-rRNA gene sequence of the OS_1 bacterial isolate aligned with that of the reference strain of *B. flexus* with ≈99% nucleotide similarity, suggesting that the OS_1 (FJ226761) isolate was closely related to *B. flexus*, whereas OS_2 (FJ215874) was closely related to *S. maltophilia* (ATCC 19861) (Fig. 2). During FTIR analysis, *B. flexus* (ATCC 49095) delivered a well differentiated spectra from OS_2 but provided an almost identical spectra to the OS_1 strain, with minimal differences in intensity of the individual bands (Figs. 1 and 3), nucleic acid and cell wall constituents (Figs. 1, 4 and 5), proteinaceous structure, and the fingerprint region (Figs. 6 and 7)

The bacterial spectra in the region of 1300–1500 cm^{-1} showed a unique absorption band at 1405 and 1313 cm^{-1}. The bands at 1394 and 1313 cm^{-1} probably represent absorption that is specific to *B. flexus* (Fig. 3). On the other hand, the band at 1394 cm^{-1} was observed in both OS_1 and *B. flexus* (ATCC 49095), indicating that OS_1 is very similar to *B. flexus.* The bands at 1094 cm^{-1} were specific for OS_2 and *S. maltophilia* (ATCC 19861). By contrast, the bands

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