Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 1105–1108
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

FTIR microscopy as a method for identification of bacterial and fungal infections

Vitaly Erukhimovitch*, Valentina Pavlov, Marina Talyshinsky, Yelena Souprun, Mahmoud Huleihel

The Institute for Applied Biosciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel

Received 18 April 2004; received in revised form 17 August 2004; accepted 17 August 2004
Available online 24 December 2004

Abstract

Fourier-transform infrared (FTIR) microscopy is considered to be a comprehensive and sensitive method for detection of molecular changes in cells. The advantage of FTIR microspectroscopy over conventional FTIR spectroscopy is that it facilitates inspection of restricted regions of the examined sample. In the present study, we examined the potential of FTIR microscopy as an easy, rapid and reliable technique for discrimination between bacteria and fungi both of which are involved in various human and other animal infections. In many cases, there is no easy and rapid technique for identifying the cause of such infections whether it is bacteria, fungi or both. Knowing such information in a rapid way could be highly important for effective therapy. Our results proved detectable and significant spectral differences between bacterial and fungal samples. Representative peaks of bacteria and fungi appeared clearly in the spectra of a mixture of bacteria and fungi. It seems that this technique could be used for rapid discrimination between bacterial and fungal infections and contaminations.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Bacteria; FTIR microscopy; Fungi; Spectral characteristics; Bacterial and fungal detection

1. Introduction

Both bacteria and fungi are responsible for large number of human and other animal diseases, part of them are life threatening. The time required for the identification of such pathogens plays an important role and, in some cases, a critical role in the course of treatment and can affect significantly the affectivity of such treatment [1]. Most commercially available identification systems in hospitals are based on the physiological and nutritional characteristics of microorganisms. Such identification systems are usually time consuming, between 1–5 days for bacteria and 1–2 weeks for fungi. It has been reported that a significant percentage of patients suffering from various infections are not initially receiving the correct therapy which might lead to additional severe complications and in some cases to death [2–4]. In addition to the risk that the chosen treatment is not appropriate and is not efficient, this treatment may lead to toxic side effects [5]. Early identification enables the clinician to precisely target a pathogen with the most effective drug. Between the techniques offering possibilities for rapid analysis, molecular biology methods are considered between the most rapid and sensitive method for identification of pathogens. Most tests are targeted at specific deoxyribonucleic acid (DNA) sequences allowing specific identification [6–8]. However, these molecular diagnostic techniques are highly expensive, with either false positive and false negative results [9–12].

The detection and identification of microorganisms by spectroscopic techniques promises to be of a great value, because of their sensitivity, rapidity, low expenses and simplicity. Furthermore, spectroscopic techniques provide a wealth of qualitative and quantitative information about a given sample. The infrared spectrum of any compound is known to give a unique ‘finger print’ [13]. Together with the large information already known about spectral peaks obtained

* Corresponding author. Tel.: +972 8 6479004; fax: +972 8 6472970.
E-mail address: evitaly@bgumail.bgu.ac.il (V. Erukhimovitch).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.08.010

from FTIR spectra of living cells [14], FTIR spectroscopy serves as an attractive technique for detection and identification of pathogens. This technique was used previously for the detection and characterization of cancer cells [15,16], cells infected with viruses [17], microorganisms [13,18,19] and for tracking pharmacological substances injected to animals [20]. Maquelin et al. [18] successfully used FTIR microspectroscopy and different mathematical models for the identification of various bacteria and yeast strains [18].

In the present study, FTIR microscopy was used for the detection of some spectral parameters representing biochemical differences between bacteria and fungi. Our results showed a representative specific spectral biomarkers for both bacteria and fungi which may be usable for future rapid identification of viral and bacterial pathogens.

2. Materials and methods

2.1. Bacteria and fungi

In the present study, we used three different strains from three different families of either bacteria (*Bacillus magaterium*, *Escherichia coli* and *Pseudomonas stutzeri*) or fungi (*Penicillium* sp., *Memnoniella* sp. and *Fusarium* sp.). All these bacterial and fungal strains were supplied by our coauthor Dr. Valentina Pavlov from the microbiology department in our Institute.

Bacterial strains were grown on Nutrient Agar (Difco) and fungal strains were grown on Potato Dextrose Agar (PDA) (Difco). Both bacteria and fungi were grown overnight at 37 °C.

2.2. Sample preparation

Since ordinary glass slides exhibit strong absorption in the wavelength range of interest to us, we used zinc selenide crystals, which are highly transparent to IR radiation. Small amounts from bacterial colonies or fungal were picked up with bacteriological loop, suspended in 100 μl of saline, pelleted by centrifugation at 1000 rpm for 2 min. Each pellet was suspended with 20 μl of saline and a drop of 1 μl of the obtained suspension was placed on a certain area on the zinc sellenide crystal, air dried for 15 min at room temperature (or for 5 min by air drying in a laminar flow) and examined by FTIR microscopy.

2.3. FTIR spectra measurement

FTIR measurements were performed in the transmission mode with a liquid-nitrogen-cooled MCT detector of the FTIR microscope (Bruker IRScope II) coupled to an FTIR spectrometer (BRUKER EQUINOX model 55/S, OPUS software). The spectra were obtained in the wave number range of 600–4000 cm^{-1}. Spectral resolution was set at 4 cm^{-1}. Baseline correction by the rubber band method and vector normalization were obtained for all the spectra by OPUS software. Peak positions were determined by means of a second derivation method by OPUS software. Since the samples to be analyzed were often heterogeneous, appropriate regions were chosen by FTIR microscopy, so as to eliminate different impurities (salts, medium residuals, etc.). The aperture used in this study was 100 μm, since this aperture gave the best signal/noise ratio. At lower apertures, the quality of the spectra was bad due to the high noise level. For each sample, the spectrum was taken as the average of five different measurements at various sites of the sample. Each experiment with each sample was repeated five times. It is important to mention that there were no significant differences in the spectra from various sites (S.D. did not exceed 0.005).

3. Results and discussion

3.1. FTIR spectra of different bacterial and fungal strains

Samples obtained from three different bacterial families and three different fungal families were examined by FTIR microscopy trying to find specific spectroscopic biomarkers for rapid identification and discrimination between bacterial and fungal infections. Developing specific biomarkers by FTIR microscopy could be highly important for future rapid and reliable detection and identification of these diseases. Quick and reliable identification of the disease might be critical, in many cases, for effective treatment.

Our results presented in Fig. 1A show the FTIR spectra of three strains from different bacterial families (*B. magaterium*, *E. coli* and *P. stutzeri*). It can be seen that despite the general similarity between the spectra of these different strains, there is a unique spectrum for each one with specific differences compared to the other strains. These results provide a preliminary indication for possible spectral parameters for identification of bacterial strains. These results are in agreement with previously published results that showed spectral unique ‘finger print’ for each of large number of bacterial strains [18].

The results presented in Fig. 1B showed the spectra of three strains from different fungal families (*Penicillium* sp., *Memnoniella* sp. and *Fusarium* sp.). It can be seen also, like bacteria, that there is a good similarity in the spectra of these different fungi. However, still specific parameters could be found for each of these fungi strains.

By a comparison between the obtained spectra of the bacteria and fungi, it is possible to point on several spectral peaks which could be considered as unique for bacteria or fungi. These peaks might be considered as a reliable biomarkers to be developed for future rapid discrimination between bacteria and fungi. This rapid discrimination could be highly significant in the case of clinical samples obtained from unidentified infections. For both bacterial and fungal spectra, the dominant bands at 1655 and 1546 cm^{-1} were attributed to protein

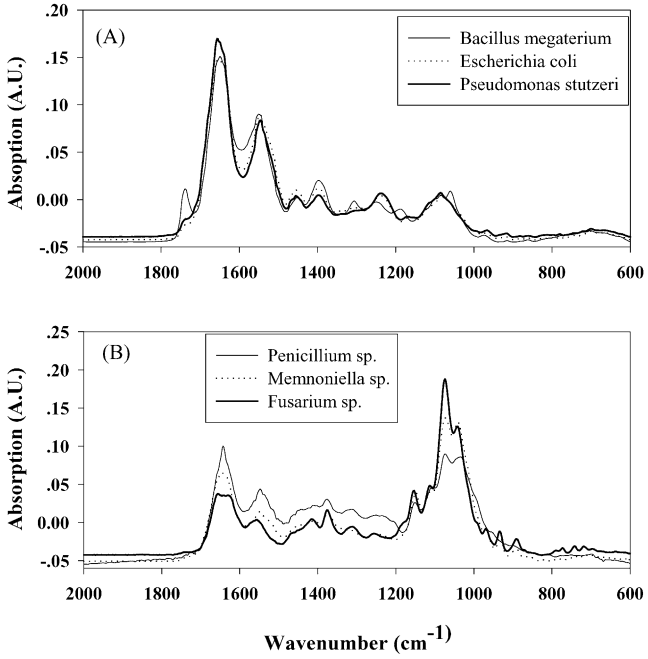

Fig. 1. FTIR spectra in the region of 600–2000 cm^{-1} of three different strains of bacteria (A) and (B) fungi from different families. Results are means of five different and separate experiments for each sample. The S.D. for these means was ≤0.001.

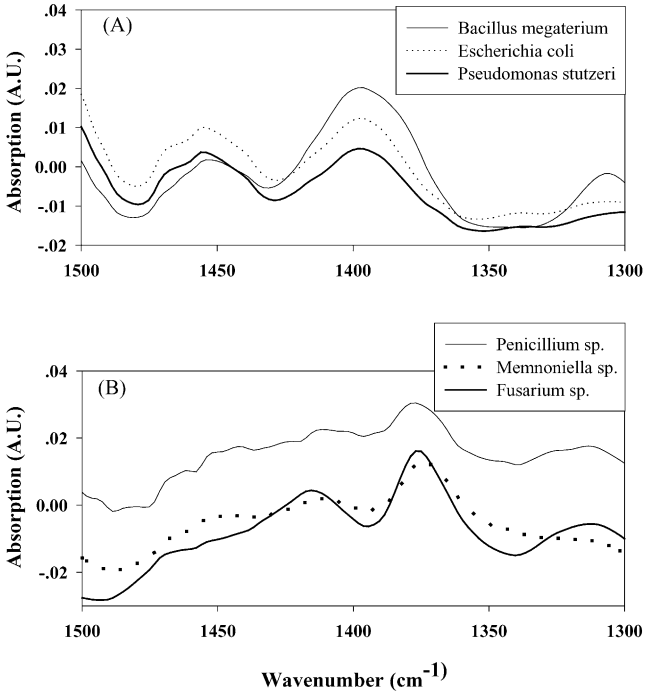

Fig. 2. FTIR spectra in the region of 1300–1500 cm^{-1} of the examined different strains of (A) bacteria and (B) fungi.

amide I and II bands [21]. The shoulder at about 1750 cm^{-1} was attributed to lipid C=O stretching vibrations [21]. The band at 1465 cm^{-1} was assigned to the CH_2 bending mode of the cell lipids. The band at 1450 cm^{-1} represents asymmetric CH_3 bending modes of end ethyl groups of proteins [22,23]. The band at 1396 cm^{-1} represents C=O symmetric stretching of COO^- [24] and assigned to lipids [22] and the band at 1377 cm^{-1} represents C–H bending mode of CH_2 [25]. From information obtained from previous studies [21], we assigned the remaining IR bands as follows: the peaks at 1237 and 1082 cm^{-1} were attributed to PO_2^- asymmetric and symmetric stretching vibrations and phospholipids. The peak at 1064 cm^{-1} resulted from the overlap of several bands, including absorption due to the vibration modes of CH_2OH and the C–O stretching vibration coupled to the C–O bending mode of cell carbohydrates [26].

3.2. Bacterial and fungal spectra at the 1300–1500 cm^{-1} region

It can be seen clearly that all examined bacterial samples show a clear and significant peak at 1396 cm^{-1}, which is completely missing in all fungi samples (Fig. 2A). On the other hand, all tested fungi samples have a unique peak at 1377 cm^{-1}, while this peak is completely missing in all tested bacteria samples (Fig. 2B). Both bands at 1377 and 1396 cm^{-1} probably represent groups of proteins and lipids, although each of them was attributed to different modes as detailed above. Furthermore, all tested bacterial samples show a sharp peak at 1450 cm^{-1}, whereas all fungal samples show only a moderate shoulder at this area (Fig. 2). This band, as mentioned above, was attributed to proteins [22]. These results demonstrate the ability of FTIR microspectroscopy for the detection of biochemical differences between bacteria and fungi.

It can be concluded that these three unique peaks may provide good biomarkers for an easy, rapid and inexpensive identification technique of bacterial and fungal infections. Certainly, the analysis of much more bacterial and fungal samples is required for more reliable and established conclusions. But, these results suggest a strong basis for a complementary study.

3.3. FTIR spectra of bacterial and fungal mixtures at the 1300–1500 cm^{-1} region

Based on the obtained unique spectral peaks for bacteria and fungi, the identification possibility of a bacterial and fungal mixture by this technique is of interest. In order to examine this point, similar aliquots of *B. magaterium* bacteria and each one of the examined fungi were suspended in 20 μl of saline. Both suspensions (the bacteria and one of the fungi strains) were then mixed well and 1 μl of the mixture was applied on the zinc sellenide crystal, dried and examined by FTIR microscopy. The results presented in Fig. 3A–C show clearly, in all the three examined different mixtures, a unique spectra for these mixtures which in fact demonstrate both specific peaks, the fungal peak at about 1377 cm^{-1} and the

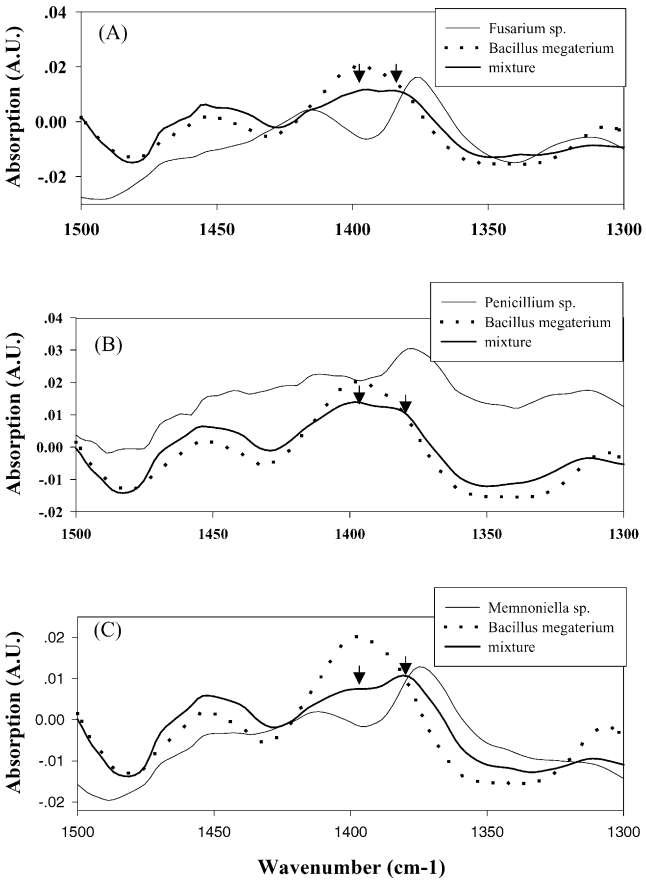

Fig. 3. Each part of this figure [A, B and C] represents FTIR spectra in the region of 1300–1500 cm^{-1} of the *Bacillus megaterium* bacteria, one of the examined fungi strains and the spectrum of a mixture of both the bacteria and fungi. The specific peaks, the fungal peak at about 1377 cm^{-1} and the bacterial peak at about 1400 cm^{-1}, are marked by arrows.

peak at about 1400 cm^{-1}. These results could mean that in case of infections caused by a combination of both bacteria and fungi, it might be possible to use these biomarkers for a rapid and easy detection and identification.

4. Conclusions

In the present study, we examined the potential of FTIR microscopy for easy and rapid discrimination between bacteria and fungi. Although the results obtained in this study could be considered only as preliminary results, but still the obtained interesting unique spectral peaks of bacteria and fungi may be considered as a promising baseline for a future study including large number of samples from different bacteria and fungi. Also, it seems to be that this technique might be valuable for rapid detection and identification of infections caused by pathogens which is highly important for successful therapy course. Furthermore, only a small amount of sample is required which can be easily obtained, and the final results could be obtained during very short time (approximately 1 h). It is therefore worthwhile to continue developing this technique as an efficient and reliable tool for the diagnosis and identification of bacterial and fungal pathogens.

References

[1] G.V. Doern, R. Vautour, M. Gaudet, B. Levy, J. Clin. Microbiol. 32 (1994) 1757–1762.
[2] E.H. Ibrahim, G. Sherman, S. Ward, V.J. Fraser, M.H. Kollef, Chest 118 (2000) 146–155.
[3] M.H. Kollef, Clin. Infect. Dis. 31 (2000) S131–S138.
[4] R.L. Wheeler, G.R. Bernard, N. Engl. J. Med. 340 (1999) 207–214.
[5] R.L. Thompson, A.J. Wright, Mayo Clin. Proc. 73 (1998) 995–1006.
[6] F.C. Tenover, M.B. Huang, J.K. Rasheed, D.H. Persing, J. Clin. Microbiol. 32 (1994) 2729–2737.
[7] S. Nikkari, D.A. Relman, Curr. Opin. Rheumatol. 11 (1999) 11–16.
[8] N.C. Clark, O. Olsvik, J.M. Swenson, C.A. Spiegel, F.C. Tenover, Antimicrob. Agents Chemother. 43 (1999) 157–160.
[9] M. Vaneechoutte, J. Van Eldere, J. Med. Microbiol. 46 (1997) 188–194.
[10] G.T. Noordhoek, J.D. Embden, A.H. Kolk, J. Clin. Microbiol. 34 (1996) 3522–3525.
[11] M. Ieven, H. Goosens, Clin. Microbiol. Rev. 10 (1997) 242–256.
[12] D.N. Fredricks, D.A. Relman, J. Clin. Microbiol. 36 (1998) 2810–2816.
[13] D. Naumann, D. Helm, H. Labischinski, Nature 351 (1991) 81–82.
[14] M. Diem, S. Boydstom-White, L. Chiriboga, Appl. Spectrosc. 53 (1999) 148–161.
[15] M. Huleihel, V. Erukhimovitch, M. Talyshinsky, M. Karpasas, Appl. Spectrosc. 56 (2002) 640–645.
[16] V. Erukhimovitch, M. Talyshinsky, Y. Souprun, M. Huleihel, Photochem. Photobiol. 76 (2002) 446–451.
[17] A. Salman, V. Erukhimovitch, M. Talyshinsky, M. Huleihil, M. Huleihel, Biopolymers 67 (2002) 406–412.
[18] K. Maquelin, C. Kirschner, L.P. Choo-Smith, N.A. Ngo-Thi, V. Vreewijk, M. Stammler, H.P. Endtz, H.A. Bruining, D. Naumann, G.J. Puppels, J. Clin. Microbiol. 41 (2003) 324–329.
[19] L. Mariey, J.P. Signolle, C. Amiel, J. Travert, Vib. Spectrosc. 26 (2001) 151–159.
[20] V. Crupi, D. Majolino, M.R. Mondello, P. Migliardo, V. Venuti, J. Pharm. Biomed. Anal. 29 (2002) 1149–1152.
[21] R.K. Dukor, Handbook of Vibrational Spectroscopy, Wiley, Chichester, UK, 2001, pp. 3335–3360.
[22] P. Wong, S. Goldstein, R. Grekin, A. Godwin, C. Pivik, B. Riga, Cancer Res. 53 (1993) 762–765.
[23] B. Stuart, Biological Applications of Infrared spectroscopy, Wiley, Chichester, UK, 1997.
[24] K. Maquelin, L.P. Choo-Smith, C. Kirschner, N.A. Ngo-Thi, D. Naumann, G.J. Puppels, Handbook of Vibrational Spectroscopy, Wiley, Chichester, UK, 2001, pp. 3308–3334.
[25] K. Brandenburg, U. Seydel, Handbook of Vibrational Spectroscopy, Wiley, Chichester, UK, 2001, pp. 3481–3507.
[26] D. Yang, D. Castro, I. EI-Sayed, M. EI-Sayed, R. Saxon, Y. Nancy, SPIE 2389 (1995) 543–550.