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The use of FTIR microscopy for the evaluation of anti-bacterial agents activity

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ABSTRACT

FTIR spectroscopy has been used by chemists as a powerful tool to characterize inorganic and organic compounds. In this study we examined the potential of FTIR microspectroscopy for early evaluation of the efficiency of anti-bacterial therapy. For this purpose, the effect of *caffeic acid phenethyl ester* (*CAPE*) and ampicillin on the development of bacterial infection in cell culture was examined. *CAPE* is one of the most active components of propolis which is a natural honeybee product with a potent anti-bacterial activity. Our results show early (2 h post-treatment), unique and significant spectral indicators for successful treatment with *CAPE* although some of these biomarkers showed different trends in Gram (−) compared with Gram (+) bacteria. For instance, the intensity of bands at 682 and 1316 cm^{-1} decreases in all examined Gram (−) bacterial strains while significantly increases in all examined Gram (+) bacterial strains. On the other hand, both Gram (+) and Gram (−) bacteria treated with ampicillin did not show any spectral differences compared with the control untreated bacteria. It seems that FTIR spectroscopy can be used as an effective tool for an early evaluation of the efficiency of the anti-bacterial effect of *CAPE* and probably other used drugs.

1. Introduction

Bacteria are considered as one of the major causes of serious and dangerous infections in human and animal. Although antibiotics are known as the most effective anti-bacterial drugs, bacteria can develop resistant mutants to the antibiotics used and, in fact, several bacterial mutants are known today to be resistant to all available antibiotics. Therefore, the search for both new anti-bacterial drugs and early evaluation techniques for the efficiency of the drugs used are highly essential and thus guarantee effective future treatment. In addition, death rates and costs associated with infectious diseases could be significantly reduced by employing rapid identification techniques and precise methods for the evaluation of the efficiency of the therapy used [1,2]. Most commercially available identification systems used in routine are based on physiological (morphology, growth temperature, etc.) and nutritional (media composition, sugar assimilation, enzymatic tests, etc.) characteristics [3]. However, these conventional tests are time consuming and are not always very specific.

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" [4]. This, together with the large information already known about spectral bands obtained from FTIR spectra of living cells, makes FTIR spectroscopy an attractive technique for the detection and identification of pathogens. FTIR spectroscopy was used successfully for the identification of different kinds of microorganisms [1,4,5], cancer cells, and cells infected with viruses [6–10]. It has also been used lately for the evaluation of the efficiency of antiviral drugs [11].

Propolis (PE), a natural product produced by honeybee and based on resins collected by bees from certain trees and plants, has been used for thousands of years in folk medicine for several purposes [12–14]. Flavanoids are the most important group of compounds of PE – in terms of amount and biochemical activity – which are thought to play a significant role in PE's bioactivities [12–14]. Among its various bioactivities, this product showed impressive antimicrobial activity mainly against Gram positive bacteria [15].

In the present study, we examined the potential of FTIR microscopy method for early evaluation of PE anti-bacterial activity against Gram positive [Gram (+)] and Gram negative [Gram (−)] bacteria.

2. Materials and methods

2.1. Bacteria

In the present study we used the following Gram (−) and Gram (+) bacteria:

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Gram (−) bacteria such as Escherichia (E.) coli, Serratia (S.) marcescens, Pseudomonas (P.) aeruginosa, Haemophilus (H.) influenza, Pseudomonas, Shegella, Salmonella (S.) enteridis, Neisseria and *Klebsiella*; *Gram (+) bacteria such as Staphylococcus (Staph.) aureus, Micrococcus, Streptococcus (S.) olysgalactiae, Streptococcus (S.) mitis, Bacillus (B.) subtilis, Bacillus cereus, Bacillus megaterium* and *Bacillus thuringiensis*.

All these bacterial strains were supplied by our co-author Dr. Valentina Pavlov from the microbiology department of our institute. All bacteria used were grown on Nutrient Agar (Difco) at 37 °C.

2.2. Amount of bacteria measurement

The amounts of bacteria were evaluated both by two methods:

(a) Examining their optical density (OD) by a spectrophotometer at wave length 620 nm. This method gives an evaluation of the live and dead bacteria.
(b) Counting the number of the obtained colonies by plating raising dilutions of each bacterium on agar plates for 24 h at 37 °C . This method gives the number of live bacteria only.

2.3. Caffeic acid phenethyl ester (CAPE)

This product was purchased as a powder from Sigma–Aldrich Corporation, USA. A stock solution of this product was prepared by dissolving it in dimethyl sulfoxide (DMSO) and then making the appropriate concentrations for examining its activity by dilution with bacterial growth medium (LB medium).

2.4. Bacterial DNA synthesis measurement

Bacterial cells (10^5) were grown in LB medium containing 10 μCi of ^{32}P orthophosphate, and at different times of post-labeling the DNA was extracted as previously described [16]. 500 μl of DNA in TE buffer were pipetted into counting vials, mixed with 10 ml scintillation fluid Quicksafe A, Zinsser Analytic and counted in a Packard liquid scintillation analyser.

2.5. Sample preparation

Since ordinary glass slides exhibit strong absorption in the wavelength range of interest, we used zinc–selenide crystals, which are highly transparent to IR radiation. The amount of the examined bacteria was determined by measuring its optical density with a spectrophotometer. Similar amounts of the examined bacteria were pelleted from a medium containing bacteria by centrifugation at 1000 rpm for 2 min. Each pellet was washed twice with H_2O and resuspended with 20 μl of H_2O. A drop of 1 μl of the obtained suspension was placed on a certain area of the zinc–selenide 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.

Table 1
Effect of *CAPE* treatment on the growth of various Gram (−) bacteria.

Bacteria strain	*CAPE* concentration (μM)					
	0		50		500	
	Bacteria amount		Bacteria amount		Bacteria amount	
	(OD)	(No/ml)	(OD)	(No/ml)	(OD)	(No/ml)
E. coli	1.401	8.1×10^8	1.010	1×10^8	0.731	4.1×10^6
P. aeruginosa	1.202	5.5×10^7	0.840	3.1×10^7	0.651	8.1×10^6
S. marcescens	1.301	4.6×10^7	0.950	2.0×10^7	0.761	6.2×10^5
H. influenza	1.110	3.5×10^6	0.711	5.1×10^5	0.630	0.5×10^5
Psedomonas	1.502	6.5×10^7	1.250	3.2×10^7	0.841	2.2×10^6
Shegella	1.150	6.6×10^6	0.851	2.1×10^6	0.701	3.1×10^5
S. enteridis	1.451	6.6×10^8	1.212	1.2×10^8	0.830	4.1×10^7
Neisseria	1.130	5.5×10^6	0.850	0.9×10^6	0.741	6.2×10^5
Klebsiella	1.371	6.6×10^7	0.822	1.3×10^7	0.701	7.1×10^5

Equal amounts (10^3 bacteria/ml) of Gram (−) bacteria were grown in 2 ml LB medium in the presence or absence of different concentrations of *CAPE* at 37 °C for 24 h. At the end of the incubation period, the amounts of bacteria were evaluated both by examining their optical density (OD)by spectrophotometer and by plating raising dilutions of each bacteria on agar plates for 24 h at 37 °C and counting the number of the obtained colonies. Results are means ± SD ($n = 5$); the SD for these means was negligible.

Table 2
Effect of *CAPE* treatment on the growth of various Gram (+) bacteria.

Bacteria strain	*CAPE* concentration (μM)					
	0		50		500	
	Bacteria amount		Bacteria amount		Bacteria amount	
	(OD)	(No/ml)	(OD)	(No/ml)	(OD)	(No/ml)
Staph. aureus	1.001	1.5×10^8	0.071	0	0.009	0
Micrococcus	0.901	6.4×10^7	0.180	1.2×10^2	0.010	0
S. olysgalactiae	0.411	5.1×10^8	0.121	1.0×10^2	0.011	10
S. mitis	1.302	3.1×10^8	0.071	0	0.003	0
Staph.	1.510	8.4×10^8	0.280	0.9×10^2	0.010	0
B. subtilis	1.150	2.5×10^8	0.092	1.7×10^2	0.013	8
B. cereus	1.011	7.5×10^7	0.053	0	0.008	0
B. megaterium	1.203	1.4×10^8	0.181	1.3×10^2	0.001	0
B. thuringiensis	1.505	7.0×10^8	0.210	3.2×10^2	0.021	20

Equal amounts (10^3 bacteria/ml) of Gram (+) bacteria were grown in 2 ml LB medium in the presence or absence of different concentrations of *CAPE* at 37 °C for 24 h. The amounts of bacteria were evaluated both by examining their OD and by plating on agar plates as described in Table 1. Results are means ± SD ($n = 5$), the SD for these means was negligible.