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Review

Fourier transform infrared spectroscopy as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria

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ABSTRACT

Vibrational spectroscopy techniques have shown capacity to provide non-destructive, rapid, relevant information on microbial systematics, useful for classification and identification. Infrared spectroscopy enables the biochemical signatures from microbiological structures to be extracted and analyzed, in conjunction with advanced chemometrics. In addition, a number of recent studies have shown that Fourier Transform Infrared (FT-IR) spectroscopy can help understand the molecular basis of events such as the adaptive tolerance responses expressed by bacteria when exposed to stress conditions in the environment (e.g. those that cells confront in food and during food processing). The current review gives an overview of the published experimental techniques, data-processing algorithms and approaches used in FT-IR spectroscopy to assess the mechanisms of bacterial inactivation by food processing technologies and antimicrobial compounds, to monitor the spore and membrane properties of foodborne pathogens in changing environments, to detect stress-injured microorganisms in food-related environments, to assess dynamic changes in bacterial populations, and to study bacterial tolerance responses.

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1. Introduction: technical and methodological aspects of FT-IR spectroscopy in food microbiology research

Infrared spectroscopy is a technique based on the use of infrared (IR) radiation which changes the vibrational behavior of molecules by delivering energy quanta and changing their vibrational and rotational modes. IR radiation, which can be obtained from the thermal emission of an appropriate source, excites certain molecular groups and the vibration resulting from the excited state only occurs at fixed wavelengths. This results in energy absorption at frequencies corresponding to the molecular mode of vibration of the corresponding molecule or chemical group. IR radiation commonly embraces the electromagnetic radiation whose frequency is between 14,300 and 20 cm^{-1}, but the most useful vibrational frequencies of

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most molecules correspond to the mid IR spectrum (between 4000 and 400 cm^{-1}). The far- and near-IR ranges are not usually employed as only overtones (secondary vibrations) and combination vibrations are registered in those regions, being difficult to study and interpret from an analytical viewpoint. Within the mid IR spectrum, five spectral windows are commonly used because of their properties (Fig. 1): 1) the window between 3000 and 2800 cm^{-1} (w_1), influenced by functional groups of membrane fatty acids and by some amino acid side-chain vibrations, since here the C – H stretching vibrations of – CH3 and >CH2 functional groups dominate; 2) the window between 1800 and 1500 cm^{-1} (w_2), affected by amide I and amide II groups belonging to proteins and peptides. These yield very intense peaks and provide global information on protein structure. Bands near 1740 cm^{-1} are due to >C═O stretching vibrations of the ester functional groups in lipids. Also absorptions of nucleic acids occur in this range due to >C═O, >C═N, and >C═C< stretching of the DNA or RNA heterocyclic base structures; 3) the window between 1500 and 1200 cm^{-1} (w_3), mixed region influenced by proteins, fatty acids and phosphate-carrying compounds due to the $>CH_2$ and $-CH_3$ bending modes; 4) the window between 1200 and 900 cm^{-1} (w_4), due to the symmetric stretching vibration of PO_2^- groups found in nucleic acids and to C – O – C and C – O – P stretching, which reveals the occurrence of carbohydrates and polysaccharides in the cell wall but also the influence of nucleic acids; 5) finally, the window between 900 and 700 cm^{-1} (w_5), which is called the true fingerprint region and holds very specific, weak spectral patterns from aromatic ring vibrations of aromatic aminoacids (tyrosine, tryptophan, phenylalanine) and nucleotides (Table 1).

A complex, fingerprint-like resonance absorption band is obtained when a sample of a substance is radiated with a continuous spectrum of IR light, and the intensity of absorption bands stems from scanning before and after passing of the IR beam through the substance. The frequencies and absorbance intensities of the IR bands are composed of unique broad and complex contours instead of isolated peaks and can be used in the identification, characterization and quantification of the sample. When a sample comprising bacterial cells is radiated, the IR spectrum reflects its global chemical composition, and it is able to afford information on taxonomic differences because of their chemical background, or to detect chemical changes undergone due to stressful environments.

Several circumstances have contributed to the successful development of Fourier Transform Infrared (FT-IR) spectroscopy. Currently IR spectra are obtained by using FT-IR spectroscopes which use a Michelson interferometer, technically superior to conventional monochromators. Besides, the Fast Fourier Transform algorithm is able to compute the discrete Fourier transform (a mathematical procedure able to transform a function from the time domain to the frequency domain) and its inverse, so the raw signal is converted into a recognizable absorbance spectrum. Also, the use of chemometric tools, which allows for the extraction of qualitative and quantitative information from the spectra, and the versatility of the spectroscopic technique, which permits the analysis of samples in different conditions (liquid, suspended, powered or dehydrated) are responsible for the expansion in scientific and technical applications as well as in the number of articles published in scientific journals (Table 2). To obtain reproducible data, a standardized experimental protocol in relation to media preparation, incubation time and temperature, cell harvesting conditions, sample preparation and FT-IR measurement should be followed.

Table 1
Tentative assignment of some bands frequently found in microbial IR spectra (peak frequencies have been obtained from the second derivative spectra). Adapted from Naumann (2000).

Frequency (cm^{-1})	Assignment
2959–2852	CH, CH_2, CH_3 in fatty acids
1655–1637	Amide I bands, of α-helical structures and β-pleated sheet structures
1548	Amide II band
1515	"Tyrosine" band
1468	C – H deformation of $>CH_2$
1310–1240	Amide III band components of proteins
1250–1220, 1084–1088	P═O stretching of PO_2^- phosphodiesters
1200–900	C – O – C, C – O of ring vibrations of de carbohydrates
720	C – H rocking of $>CH_2$
900–700	"Fingerprint" region

Several spectroscopic methods have been widely used as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria (Fig. 2). In FT-IR transmission sampling techniques the sample is placed into the path of the IR beam and scanned. Solid samples made of lyophilized, purified cultures can be finely ground with potassium bromide (a matrix transparent in the mid-IR region) and mechanically pressed to form a hard, translucent pellet. Liquid samples with a cell suspension can be deposited on a zinc selenide window and stove-dried before measuring. In Attenuated Total Reflectance (ATR) the sample is placed onto an optically dense crystal of relatively higher refractive index, needing little or no sample preparation. The IR beam reflects from the internal surface of the crystal and creates an evanescent wave, which extends beyond the surface of the crystal and projects into the sample in close contact with the ATR crystal. Some of the energy of the evanescent wave is absorbed by the sample and the reflected radiation is passed to the detector in the IR spectrometer as it exits the crystal. Diffuse reflectance FT-IR projects the IR beam into the sample where it is reflected, scattered and transmitted through the sample material. The part of the IR light that is diffusely scattered within a sample, and

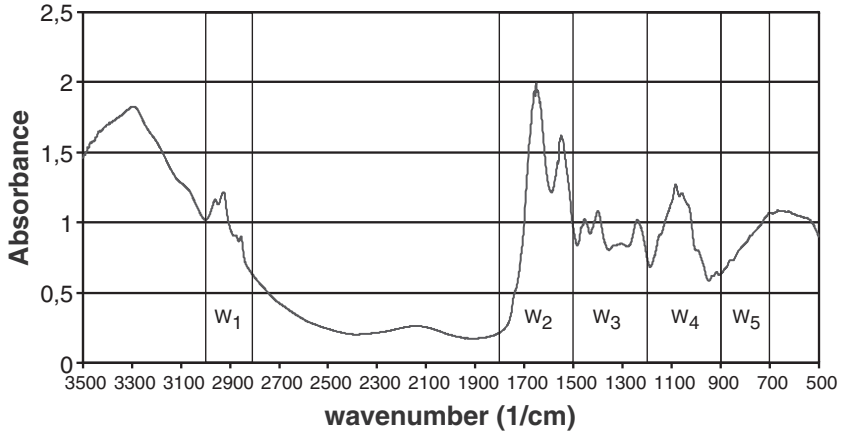

Fig. 1. Representative FT-IR spectrum (3500 to 500 cm^{-1}) from a bacterial cells sample.