WEBINAR SUMMARY

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Pharma Intelligence

Microbial Characterization, Identification and Typing Using FTIR Spectroscopy

Dr. Ashraf Ismail, Associate Professor, McGill University
Dr. Suja Sukumaran, Product Manager, Thermo Fisher Scientific
Andrew Warmington, Manufacturing Editor, Citeline (Moderator)

KEY TAKEAWAYS

- FTIR spectroscopy for biological sciences differs from its application in chemistry.
- Developing a spectral database for microbial identification requires standardization.
- Spectral acquisition methods offer distinct benefits and challenges.
- Retention of sample moisture is critical to microbial spectrum acquisition using ATR.

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OVERVIEW

Advances in FTIR technology for microbiological and molecular biochemistry research have facilitated routine implementation of FTIR spectroscopy for both microbial identification and strain typing. ATR-FTIR and transflection-FTIR measurements allow for rapid and reagent-free identification of microbes, among other advantages.

Researchers at McGill University leveraged the power of FTIR spectroscopy to reduce identification time of prevalent infectious yeasts and bacteria in hospitals, without sacrificing accuracy.

CONTEXT

Dr. Ismail explained how spectral databases of microorganism fingerprints are created using FTIR spectroscopy and gave examples of its application in hospital settings.

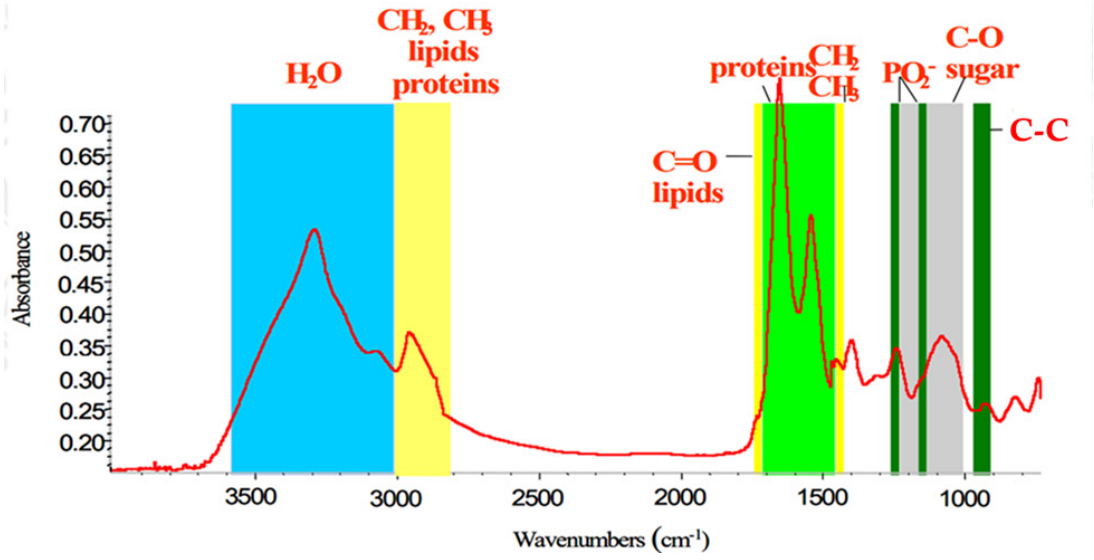
KEY TAKEAWAYS

FTIR spectroscopy for biological sciences differs from its application in chemistry.

In the past century, since its inception, infrared spectroscopy has been used for characterization of chemical substances and has found applications in many scientific domains and industry. The infrared spectrum of a chemical compound is its most characteristic physical property, and thus serves as its molecular "fingerprint," populating spectral databases for compound identification and product authentication.

Commercially available spectral databases containing many thousands of spectra are currently used by the chemical and pharma sectors, and IR spectroscopy is an important tool in forensic and material sciences and in atmospheric monitoring of greenhouse gases. However, extending its application to microbial identification faces challenges associated with biological variability. In particular, enormous spectral information is provided by contributions from all molecules that comprise a microbial cell, and it is the ensemble of overlapping spectral profiles of each biochemical constituent that forms the "fingerprint" of a microorganism. Therefore, IR spectroscopy is considered a "whole-organism" fingerprinting method.

Figure 1: Whole-organism fingerprinting using IR spectroscopy



Developing a spectral database for microbial identification requires standardization.

While there are many methods available to microbiologists for microbial identification, including whole-genome sequencing, FTIR spectral fingerprinting is low-cost, portable, and amenable to automation for high-throughput labs. Like most phenotypic methods, FTIR spectroscopy requires isolation of a pure colony. From a sample analysis perspective, there are no reagents and virtually no sample preparation, with a fast turnaround time of ~30 seconds per sample.

The classical method of pure colony isolation prior to undertaking sample identification often entails the use of selective culture media to suppress growth of certain microorganisms in order to screen for other species. Variations between growth media in their nutrient profile can profoundly affect the biochemical profile of the microorganism and, consequently, the infrared spectral fingerprint. Changes in incubation time and temperature can also impact the infrared spectral profile. Accordingly, consistent composition of the culture media, incubation time and temperature and selecting a well-isolated colony are essential for employing FTIR spectroscopy in microbial identification and strain typing.

A truly representative spectral database requires a sufficient number of well-characterized isolates for each species, cultured under standardized conditions prior to spectral acquisition. This enables the creation of a model that encompasses inherent variability in the biochemical composition of each species. All spectra must be recorded under exactly the same conditions regardless of elapsed time interval. To achieve full standardization and reproducibility, the FTIR spectrometer must be manufactured in a way to ensure all future spectrometers of the same model produce identical spectra for a given microbial isolate.

> The cardinal rule for the differentiation among different species by FTIR spectroscopy requires that inter-species spectral variability is greater than intra-species spectral variability.
>
> *Dr. Ashraf Ismail, McGill University*

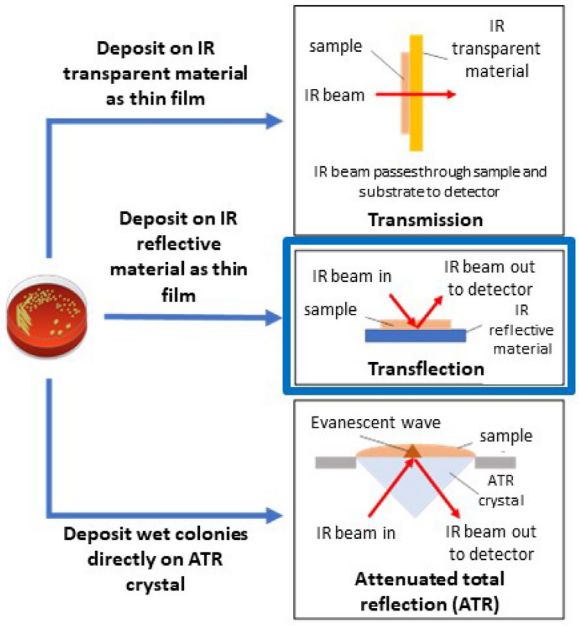
Spectral acquisition methods offer distinct benefits and challenges.

Over the past three decades, transmission-based measurements have been the technique of choice for acquiring FTIR spectral fingerprints of microorganisms. **Transmission-based FTIR spectroscopy** entails collecting a sample from a culture plate and suspending it in water, depositing the suspension onto an infrared-transparent plate or window, evaporating the water to form a uniform film, and recording the infrared spectrum by passing the infrared beam through the sample to reach the detector.

In recent years, **transflection FTIR spectroscopy** has had a prominent role in spectral profiling of human tissue and cells. When employed to acquire spectra of microorganisms, transflection measurements require that a microbial film be deposited on an infrared- reflective material so that the infrared beam passing through the microbial film is reflected back and passes through the sample a second time to reach the detector.

Attenuated total reflectance (ATR) FTIR spectroscopy has increasingly been used to acquire spectra of microorganisms. In ATR-FTIR spectroscopy, the sample is deposited on the surface of an internal reflection element (ATR crystal) where it interacts with the evanescent wave formed by total *internal* reflection of the infrared beam within the internal reflection element. This interaction results in attenuated total reflectance of the infrared beam reaching the detector. The ratio of the attenuated signal to that acquired in the absence of the sample yields the FTIR spectrum of the sample.

Figure 2: Three different methods of spectral acquisition



Retention of sample moisture is critical to microbial spectrum acquisition using ATR.

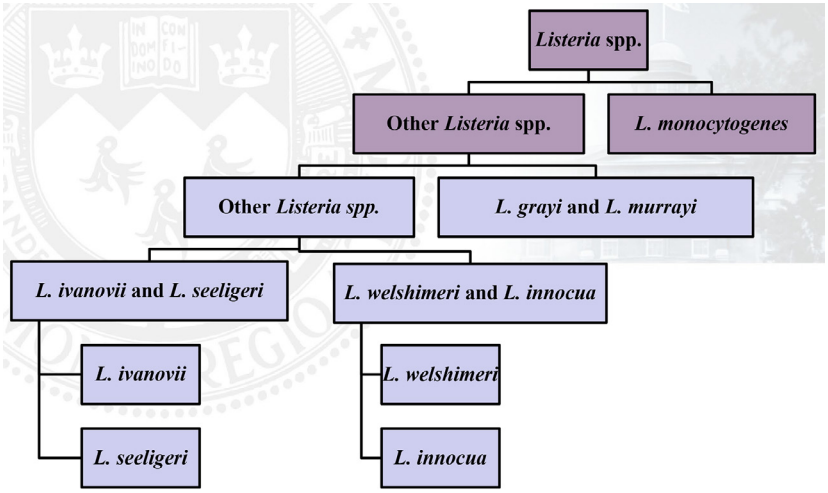
One of the attractions of ATR-based methods is that the sample can never be too thick. (Although, if there is little biomass, absorbance can be too low for spectral analysis.) Once a microbial colony is deposited onto the ATR surface, it begins to dry. As the water evaporates, the absorption bands of the microorganisms become clearly visible in the ATR-FTIR spectrum. However, changes in water content in the colony can cause both band shifts and pronounced changes in relative band intensities, particularly in spectral regions where biomolecules have absorptions that are sensitive to hydrogen bonding. To compound the problem, microbial colonies from different genera dry at different rates, which would effectively limit the viability of employing ATR-FTIR spectroscopy for database construction. To limit the evaporation rate and maintain consistent moisture content of the sample over time, place a piece of moist agar in a cap and suspend it close to the colony without allowing it to touch the surface.

Spectral pre-processing routines can employ computational methods that involve calculation of the first or second derivative of the spectrum, followed by vector normalization over the spectral range, to effectively compensate for variability in biomass. By ensuring consistent moisture content and sufficient biomass along with a good signal to noise ratio for each spectrum, a high degree of spectral reproducibility is achieved for database construction.

Identification of an unknown microbial isolate is achieved by comparing its FTIR spectrum against those in a reference spectral database. Usually, this comparison relies on multivariate statistical analysis techniques, which are employed to generate classification models (classifiers) from spectra in the database. A multitier database structure allows pairwise classification models to be developed independently from each other. Each classification model is optimized by selection of spectral features that achieve the discrimination between the paired genera or species.

In the algorithm implemented for the identification process, classifiers can be interrogated through increasingly specific levels, the process terminating if a classifier fails to return a result within the confidence limits set in the algorithm to safeguard against misidentifications.

Figure 3: Pairwise classification at species level



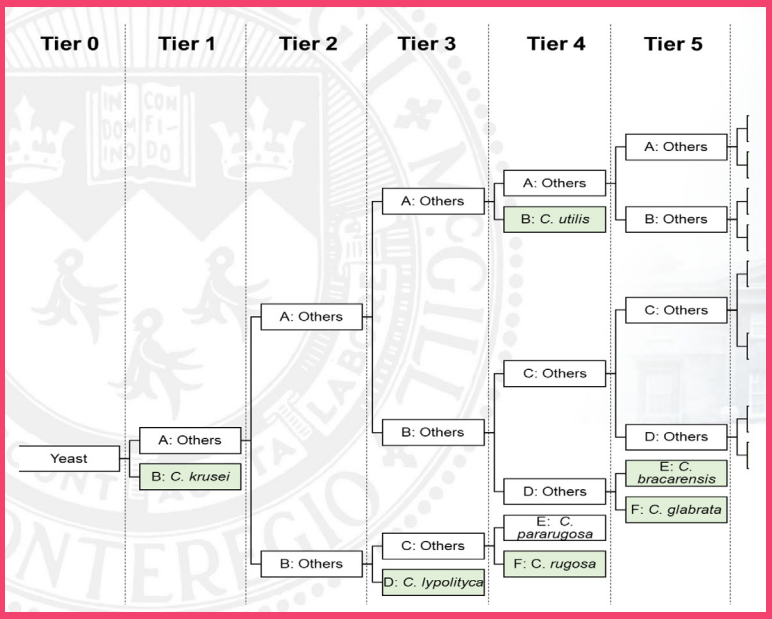
Case Study: Using ATR-FTIR spectroscopy to identify yeast strains on site at hospitals

The concept of a multitier database structure was employed by the McGill team in building a spectral database for species identification of clinically relevant yeasts using ATR-FTIR spectroscopy. The team achieved a 99.7% correct classification of yeast isolates collected across five months.

Based on this external validation, the team turned their attention to the use of ATR-FTIR spectroscopy on site at hospitals. Spectral acquisition took only about a minute, and the multivariate analysis was conducted in near-real time.

Of the 534 samples analyzed, 525 (98.3%) were correctly identified and 1.7% produced no identification. There were no instances of misidentification.

Given the success of this work, the team carried out an evaluation study on identification and strain typing of vancomycin-resistant *Enterococcus faecium* (VR-*E. faecium*), one of the most prevalent causes of hospital-acquired infections. The current gold standard in VRE surveillance and investigations of VRE outbreaks is pulsed-field gel electrophoresis (PFGE), which requires highly trained personnel, expensive reagents, and 3-7 days to complete. The study undertaken by the team explored real -time strain typing of VR-*E. faecium* isolates using transflection FTIR spectroscopy as a tool for hospital infection control. A supervised classification of the spectra of VRE isolates yielded complete separation of two outbreak strains. Ultimately, real-time strain typing by an unsupervised FTIR process correctly assigned 18 out of 19 isolates to one of two outbreaks, and an additional 18 out of 19 isolates were correctly identified as strains not related to either of the outbreaks.



BIOGRAPHIES

Dr. Ashraf Ismail

Associate Professor, McGill University

Dr. Ashraf Ismail is Associate Professor in the Department of Food Science and Agricultural Chemistry at McGill University. He obtained his B.Sc. (Biochemistry) and Ph.D. (Chemistry) degrees from McGill University and did postdoctoral studies at École Nationale Supérieure de Chimie de Paris. He has over 25 years of experience in the study of biomolecules and biological systems by FTIR spectroscopy and co-founded the McGill IR Group with Professor Fred van de Voort with the aim of advancing the application of FTIR spectroscopy in food science research and in food analysis. Over the past decade, a major component of his research program has been directed toward the development of rapid FTIR-based methods for microbial identification and typing that are of potential applicability in food or clinical microbiology.

Dr. Suja Sukumaran

Product Manager, Thermo Fisher Scientific

Dr. Suja Sukumaran is a Product Manager at Thermo Fisher Scientific. She received her PhD in Biophysics from Johann Wolfgang Goethe University, Germany, as part of the international Max Planck Research School. She has expertise and extensive experience in molecular spectroscopy, visible and fluorescence imaging, protein, and lipid biochemistry. Lastly, her current research interests are AI for protein folding, microplastics, and recycling.