

Application News

Fourier Transform Infrared Spectrophotometer (FTIR)

Using FTIR to Detect and Differentiate Microbial Contamination at the Species Level during the Production of Microbial Products

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User Benefits

- ◆ This technique can be used to detect and differentiate microbial contamination at the species level from a bacterium of the same genus during the production of microbial products.
- ◆ The diffuse reflectance accessory and microfocus plate enable efficient multi-sample measurements.
- ◆ An analysis time of around 30 seconds per sample enables the rapid completion of analysis tasks.

■ Introduction

The production of microbial products involves the following five steps (Fig. 1): thawing stock cells (activation), pre-culture (in a small volume of medium), expansion culture (in a large-scale culture), harvesting, and downstream processing to create the final product. However, microbial contamination of the production process has a critical effect on product quality and safety, so it must be detected quickly and accurately.

Fourier transform infrared spectrophotometry (FTIR), an analytical technique for characterizing the molecular structure of analyte substances, has attracted attention due to its effectiveness, particularly in identifying microorganisms. Infrared spectra recorded by FTIR analysis of a microorganism contain information about the lipids, polysaccharides, proteins, carbohydrates, and other molecular components of that microorganism,¹⁾ which can be used to monitor microorganisms being cultured under a fixed set of conditions (the same culture medium, temperature, and time).

This Application News describes combining the IRXross FTIR system (Fig. 2) with a DRS-8000 diffuse reflectance accessory (a custom model^{*1}) to detect and differentiate microbial contamination at the species level from a bacterium of the same genus during the production of microbial products. A previous Application News reported on using FTIR to detect contamination by two different types of bacteria. However, this Application News attempts the more difficult task of detecting and differentiating contamination at the species level from bacteria of the same genus. Differentiating between microorganisms of the same genus is comparatively difficult since the organisms are genetically extremely similar, as they share a large majority of the same DNA and have very similar structural cellular components.

^{*1}: It is a custom product. Contact a Shimadzu sales representative/distributor for further details.

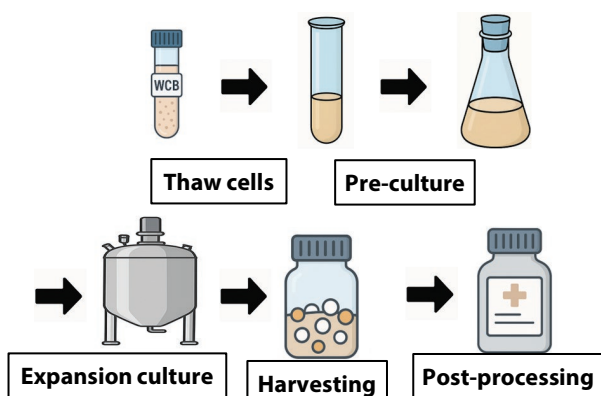


Fig. 1 Production Process for a Microbial Product



Fig. 2 IRXross™ FTIR

■ Microbial Culture

Experiments were performed using the gram-positive bacterium *Staphylococcus cohnii* (*S. cohnii*, strain: ATCC 29974), *Staphylococcus aureus* of the same genus (*S. aureus*, strain: ATCC 12600), and *Staphylococcus epidermidis*, also of the same genus (*S. epidermidis*, strain: ATCC 14990). Cultures of *S. cohnii* were spiked with cultures of *S. aureus* and *S. epidermidis* to create starting microbial concentrations between $1.0 \times 10^{-2}\%$ and $1.0 \times 10^{-7}\%$ (the percentages of *S. aureus* or *S. epidermidis*) and then co-cultured under identical aerobic conditions (37 °C, 24 hours).

■ Sample Preparation

Samples were prepared for analysis by the following process. Three spots were prepared and analyzed for each sample.

- (1) The entire culture solution was centrifuged in a centrifuge tube, and the supernatant was discarded.
- (2) The resulting precipitate was washed by adding physiological saline and centrifuging the mixture at room temperature.
- (3) After again discarding the supernatant, ultrapure water was added to the precipitate, and the mixture was agitated vigorously. Ethanol was then added to the mixture to a concentration of 70 % ethanol.
- (4) Zirconium beads were added and used to homogenize the mixture, creating a suspension.
- (5) Then 5 μ L of the dilute solution in (4) was instilled into a microfocus plate (64 well) and dried.

■ Analysis Conditions and Data Analysis

The conditions used for FTIR analysis are shown in Table 1. Analysis was performed by diffuse reflectance infrared Fourier transform spectroscopy (DRS), which offers the advantages of multi-sample analysis and ease of cleaning up samples. The FTIR system was also fully purged with nitrogen to reduce the presence of atmospheric water vapor and carbon dioxide during analysis.

Infrared spectra were recorded using automated DRS measurement software. As preprocessing for the infrared spectra, second derivative (Savitzky-Golay filter), selection of wavenumber range (1300 to 800 cm^{-1}), and unit vector normalization were performed. This processed data was then analyzed by principal component analysis (PCA) and cluster analysis (evaluated by averages).

Table 1 FTIR Analysis Conditions

Equipment:	IRXross, DRS-8000 diffuse reflectance accessory (custom model)
Resolution:	4 cm^{-1}
Number of Averaged Scans:	20
Apodization Function:	SqrTriangle
Detector:	DLATGS
Purge Gas:	Nitrogen

■ Detection of Contamination

The infrared spectrum of each culture solution is shown in Fig. 3. The same spectra processed with the second derivative are shown in Fig. 4 in the range of 1300 to 800 cm^{-1} .

The spectral profiles in Fig. 3 appear very similar, whereas the second derivative spectra in Fig. 4 reveal some notable differences between 1300 and 800 cm^{-1} . Fig. 5 (a) also shows the dendrogram obtained by performing a cluster analysis on the second derivative spectra. The dendrogram shows that *S. cohnii* cultures spiked with *S. epidermidis* at 10^{-2} to 10^{-3} %, *S. cohnii* cultures spiked with *S. aureus* at 10^{-6} to 10^{-7} %, and *S. cohnii* monocultures all formed a single cluster, with no contamination detected in this cluster. The dendrogram also shows that *S. aureus* monocultures and *S. cohnii* cultures spiked with *S. aureus* at 10^{-2} % form one cluster. *S. cohnii* cultures spiked with *S. aureus* at 10^{-3} and 10^{-4} % form another cluster, as do *S. cohnii* cultures spiked with *S. aureus* at 10^{-5} %. Four distinct groups were also revealed by principal component analysis (Fig. 5 (b)). The above results show that this technique may not be able to detect contamination by *S. epidermidis*, but it was able to detect contamination by *S. aureus* at starting concentrations above 10^{-5} %.

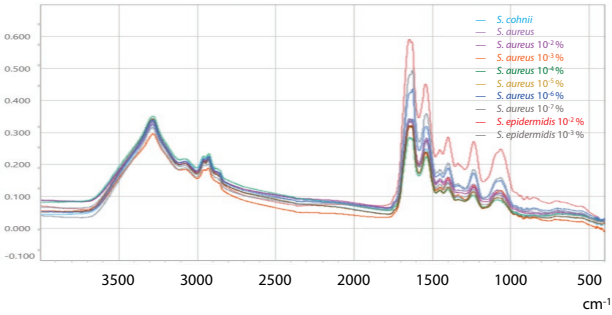


Fig. 3 Infrared Spectra

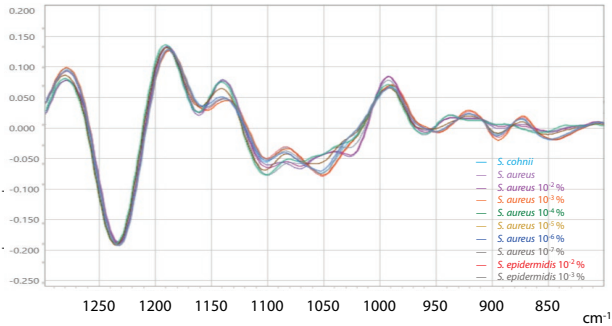


Fig. 4 Second Derivative Spectra

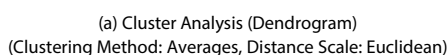


Fig. 5 Results of Analysis for Contamination

■ Bacterial Composition after Co-culture

The ratio of *S. cohnii* to *S. epidermidis* and *S. aureus* after co-culture was determined by real-time PCR using bacterial-specific primers for single-copy housekeeping genes. A calibration curve prepared based on Ct values obtained with known concentrations of control DNA was also used to determine the final ratios of the co-cultured bacteria from the Ct value for each culture. Although co-cultures were started at the same concentrations, *S. aureus* grew readily in co-culture with *S. cohnii*, whereas *S. epidermidis* had difficulty growing in co-culture with *S. cohnii* (Fig. 6). Notably, at starting concentrations above 10^{-6} %, the proportion of *S. aureus* was higher than *S. cohnii* at the end of each co-culture.



Fig. 6 Bacterial Composition Measured by Real-Time PCR

■ Conclusion

This Application News attempted the difficult task of detecting and differentiating contamination at the species level from a bacterium of the same genus. Specifically, diffuse reflectance infrared Fourier transform spectroscopy was used to detect contamination of an *S. cohnii* culture by two bacterial species of the same genus (*S. epidermidis* and *S. aureus*). The results showed that FTIR analysis can detect and differentiate contamination at the species level, but this depends on the growth of the contaminating microorganism.

As an Application News No. 01-00973-en, co-cultures were differentiated using cluster analysis and principal component analysis of derivative infrared spectra, and real-time PCR was used to determine which percentage compositions of bacteria could be differentiated by FTIR after co-culture. The technique described in this Application News can perform rapid monitoring of contamination at the species level from a bacterium of the same genus, and this has potential applications in the production of bacterial products.

This Application News is the result of joint research with JSR Corporation.

References

- 1) Lasch, P. Naumann, D. "Infrared Spectroscopy in Microbiology." Encyclopedia of Analytical Chemistry, 3: 1-32. Mar. 2015.

Related Applications

- ## 1. Using FTIR to Detect Microbial Contamination during the Production of Microbial Products

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01-01205-EN

First Edition: Aug. 2026



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