SHIMADZU
Excellence in Science

Application News

Fourier Transform Infrared Spectrophotometer (FTIR)

Using FTIR to Detect Microbial Contamination during the Production of Microbial Products

Shoko Iwasaki[1], Takahisa Araki[1], Masayoshi Ito[2], Hitoshi Sekine[2], Hidefumi Ishikawa[2], Kazuto Watanabe[2], Takayuki Arai[2]

1. Shimadzu Corporation, 2. JSR Corporation

User Benefits

- This technique can be used to detect microbial contamination of a production process for microbial products.
- The diffuse reflectance accessory and microfocus plate enable efficient multi-sample measurement.
- An analysis time of around 30 seconds per sample enables the rapid completion of analysis tasks.

■ Introduction

The production of microbial products can be summarized by the five steps in Fig. 1. First, stock microbial cells are thawed (activation), pre-cultured (in a small volume of medium), expansion cultured (in a large-scale culture), harvested, and then post-processed to create a final product that is suitable for commercial sale. However, microbial contamination of this 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).

In this Application News, the IRXross FTIR system (Fig. 2) was combined with a DRS-8000 diffuse reflectance accessory (a custom model*1) and used to detect microbial contamination during the production of microbial products.

*1: Custom product. Contact a Shimadzu sales representative/distributor for further details.

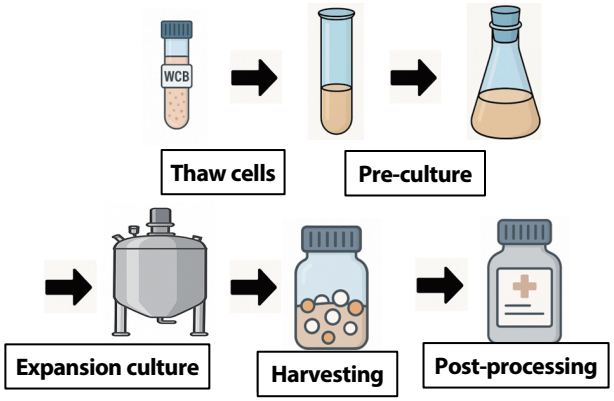


Fig. 1 Production Process for a Microbial Product

Fig. 2 IRXross™

■ Microbial Culture

Experiments were performed using the gram-positive bacteria *Staphylococcus cohnii* strain ATCC 29974 (*S. cohnii*) as the product microbe and *Escherichia coli* strain ATCC 700926 (*E. coli*) as the contaminating microbe. *E. coli* culture solution was added to *S. cohnii* culture solution at concentration ratios between 1 % and 1.0×10^{-6} % (percentage of *E. coli*) and co-cultured under 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 µL 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 the 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 on analysis.

Infrared spectra were recorded using automated DRS measurement software (Fig. 3). 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 and DRS-8000 diffuse reflectance accessory (a custom model)
Resolution:	4 cm^{-1}
Number of Averaged Scans:	20
Apodization Function:	SqrTriangle
Detector:	DLATGS
Purge:	Nitrogen

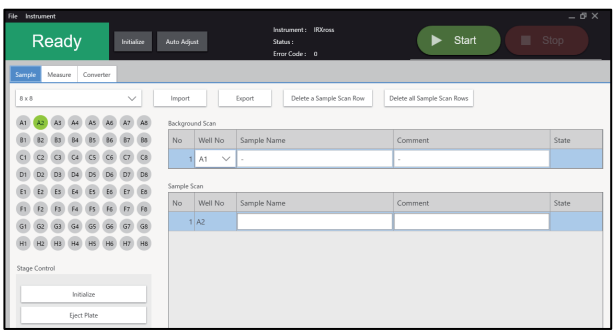


Fig. 3 Automated DRS Measurement Software

■ Detection of Contamination

The infrared spectrum of each culture solution is shown in Fig. 4. The same spectra processed with the second derivative are shown in Fig. 5 in the range of 1300 to 800 cm^{-1}.

The infrared spectral profiles in Fig. 4 appear very similar, whereas the second derivative spectra in Fig. 5 reveal some notable differences. Fig. 6 (a) shows the dendrogram obtained by performing a cluster analysis on the second derivative spectra. The dendrogram shows five clusters based on the starting *E. coli* concentration: *E. coli* monoculture, *S. cohnii* spiked with *E. coli* at 1 to 10^{-1} %, *S. cohnii* spiked with *E. coli* at 10^{-2} %, *S. cohnii* spiked with *E. coli* at 10^{-3} to 10^{-4} %, and *S. cohnii* spiked with *E. coli* at 10^{-5} to 10^{-6} %. Five groups are also revealed by principal component analysis (Fig. 6 (b)). The above results show that this technique may not be capable of detecting the lower starting concentrations of *E. coli* (10^{-5} to 10^{-6} %), but it was able to detect *E. coli* contamination at starting concentrations of 10^{-4} % and above.

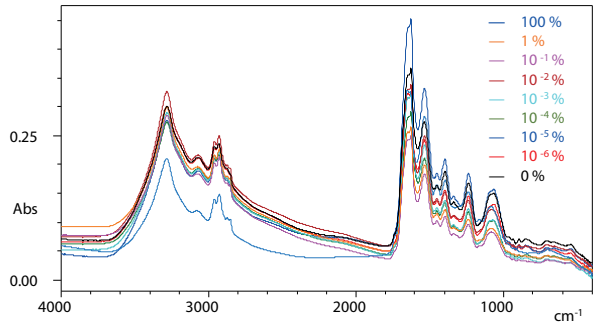


Fig. 4 Infrared Spectra

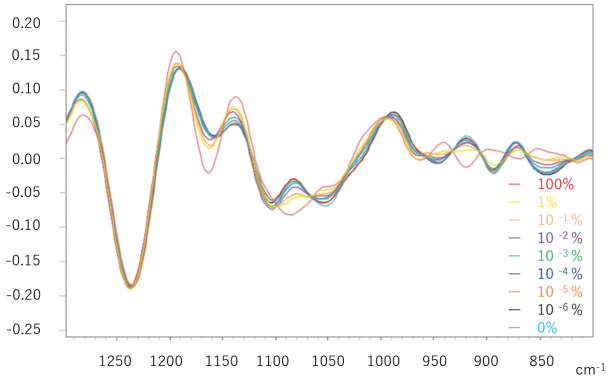
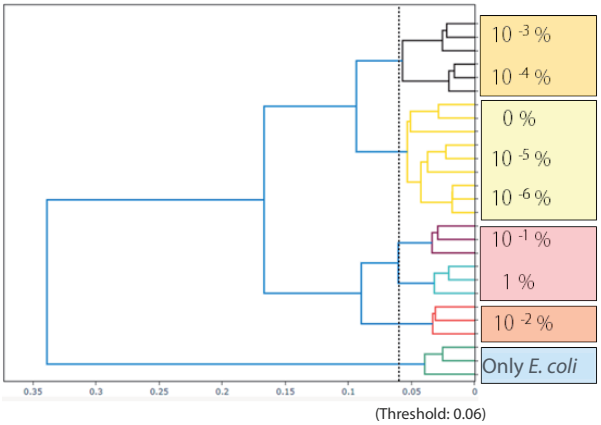
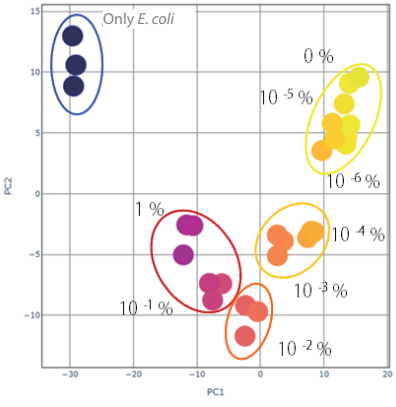


Fig. 5 Second Derivative Spectra



(a) Cluster Analysis (Dendrogram)
(Clustering Method: Averages, Distance Scale: Euclidean)



(b) Principal Component Analysis (Score Plot)

Fig. 6 Results of Data Analysis

■ Bacterial Composition after Co-culture

The ratio of *S. cohnii* to *E. coli* in the samples after co-culture for 24 hours was determined by real-time PCR using bacterial-specific primers of single copy housekeeping genes. The final ratios of the two bacteria were determined with a calibration curve prepared from Ct values using known concentrations of control DNA. At starting concentrations of 10^{-2} % and above, the quicker-growing *E. coli* overtook *S. cohnii* to account for a higher proportion of the final composition (Fig. 7). Also, given that separate clusters formed at over 10^{-4} %, Fig. 7 shows that FTIR can detect contamination at *E. coli* concentrations of 15 to 27 %.

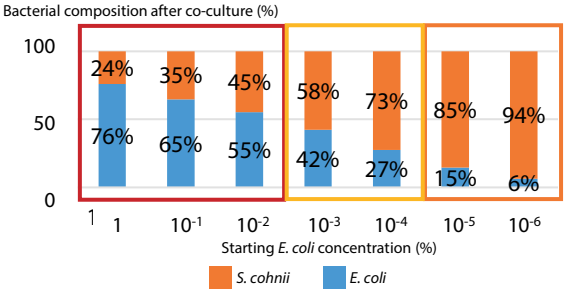


Fig. 7 Bacterial Composition Measured by Real-Time PCR

■ Conclusion

Diffuse reflectance infrared Fourier transform spectroscopy was used to detect contamination of an *S. cohnii* culture by a different bacterium (*E. coli*).

All samples were successfully and efficiently analyzed using a fully nitrogen-purged FTIR system combined with a DRS-8000 diffuse reflectance accessory. Furthermore, cluster analysis and principal component analysis of the second derivative spectra (Savitzky-Golay filter) identified *E. coli* contamination that was not apparent from a simple comparison of infrared spectra. Real-time PCR analysis was also used to show the range of proportions of contaminating and non-contaminating bacteria that can be identified with this FTIR technique. The technology described in this Application News can perform rapid contamination monitoring, which potentially has practical applications in the production of bacterial products.

<Acknowledgments>
This Application News is the result of joint research with JSR Corporation. We are sincerely grateful for the cooperation of all those who were involved.

<References>

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

<Related Application News Articles>

1) Identification Analysis of Lactobacillus Species/Strains Using IRXross
Application News No. 01-00567-EN

IRXross is a trademark of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

01-00973-EN First Edition: Sep. 2025

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
See http://www.shimadzu.com/about/trademarks/index.html for details.
Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.
The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2025

› Please fill out the survey

Related Products Some products may be updated to newer models.

› IRXross
Fourier Transform Infrared Spectrophotometer

Related Solutions

- › Life Science
- › Microorganisms
- › Pharmaceutical and Biopharmaceutical
- › Biopharmaceutical
- › Food and Beverages
- › Food Contamination

- › Price Inquiry
- › Product Inquiry
- › Technical Service / Support Inquiry
- › Other Inquiry