

## Application News

Fourier Transform Infrared Spectrometer (FTIR)

### Using an FTIR Spectrometer to Analyze How a Bacterial Culture is Affected by Differences between Culture Medium Ingredients (Peptones and Yeast Extracts)

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#### User Benefits

- ◆ It evaluates the impact of culture medium ingredients (peptones, yeast extract, etc.) from different manufacturers and of different types on the metabolism and composition of cultured bacteria
- ◆ It uses principal component analysis (PCA) and cluster analysis to differentiate between normal cultures and irregular cultures that are affected by using different culture medium ingredients.
- ◆ FTIR can be used for quality control of culture medium ingredients used in the production of microbial-based products and for equivalence testing when making changes to culture medium ingredients.

#### ■ Introduction

When microorganisms are used to produce materials or manufacture microbial products, the composition of the culture medium and the ingredients used in it have a major impact on the growth and metabolism of the microorganisms being cultured. Lot-to-lot and manufacturer-to-manufacturer variability in culture medium ingredients is a particular concern for naturally sourced ingredients, such as peptones and yeast extract, because variations in these ingredients can have unanticipated effects on the quality and yield of a final product. Therefore, ongoing monitoring of the impact of different culture ingredients on a microbial culture is an essential part of a stable manufacturing process (Fig. 1).

Fourier transform infrared (FTIR) spectrometry, an analytical technique used to characterize the molecular structure of analyte substances, has garnered attention for its effectiveness in identifying microorganisms. The infrared spectrum of a microorganism recorded by FTIR contains information on its lipids, polysaccharides, proteins, carbohydrates, and other molecular components,<sup>1)</sup> and it can be used to monitor microbial cultures performed under a fixed set of conditions (using the same culture medium, temperature, and time).

This Application News describes a technique that combines the IRXross™ FTIR spectrometer (Fig. 2) with a DRS-8000 diffuse reflectance accessory (custom model\*<sup>1</sup>) for monitoring cultures of microbial-based products.

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

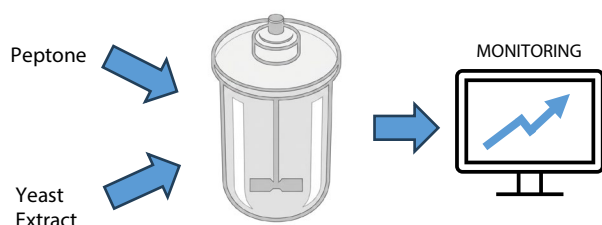


Fig. 1 Quality Monitoring of the Bioproduction Process for a Microbial Product



Fig. 2 IRXross™

#### ■ Microbial Culture

Experiments were performed using the Gram-positive bacterium *Staphylococcus cohnii* strain ATCC 29974 (*S. cohnii*).

Using the basal medium composition as a base, we prepared a commercially available PA medium and five in-house media (PB, PC, PD, PE, and PF: soybean-derived) prepared using different raw materials as peptone sources.

As for yeast extracts, we also prepared a commercially available YA medium and in-house prepared media using yeast extracts of different grades (YB\_STD and YB\_HQ), different lots (YD\_lot1 and YD\_lot2), and different manufacturers (YC and YE). Table 1 shows the ingredients used to prepare these culture media.

Each medium was used to culture *S. cohnii* under the same conditions (37 °C for 24 hours), and the resulting culture solution was used for analysis.

Table 1 List of Media

##### Peptones

| Name               | Medium                            |
|--------------------|-----------------------------------|
| PA                 | Commercial medium (soybean-based) |
| PB, PC, PD, PE, PF | In-house medium (soybean-based)   |

##### Yeast Extracts

| Name             | Sold By   | Medium                             |
|------------------|-----------|------------------------------------|
| YA               | Company A | Commercial medium                  |
| YB_STD, YB_HQ    |           | In-house medium (different grades) |
| YC               | Company C | In-house medium                    |
| YD_lot1, YD_lot2 | Company D | In-house medium (different lots)   |
| YE               | Company E | In-house medium                    |

#### ■ 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 diluted solution from (4) was dispensed into a 64-well microfocus plate and dried..

## ■ Analysis Conditions and Data Analysis

The conditions used for FTIR analysis are shown in Table 2. 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  $\text{cm}^{-1}$ ), and unit vector normalization were performed. This processed data was then analyzed by PCA and cluster analysis (evaluated by averages).

Table 2 FTIR Analysis Conditions

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

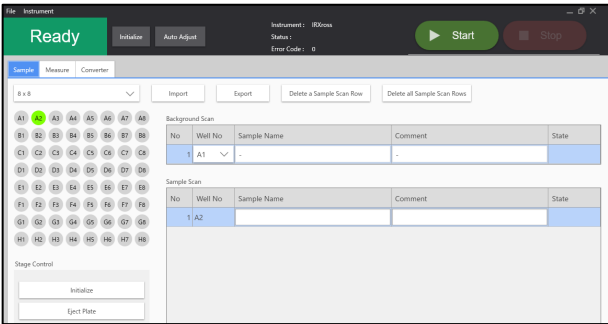


Fig. 3 Automated DRS Measurement Software

## ■ Differences between Peptones

Fig. 4 shows the infrared spectrum of each culture solution, and Fig. 5 shows their second derivative spectra in the range of 1300 to 800  $\text{cm}^{-1}$ . Each culture was performed three times ( $n = 3$ ), and the most representative spectrum recorded from the three cultures is shown in the figures.

The spectral profiles in Fig. 4 appear very similar, whereas the second derivative spectra in Fig. 5 reveal some notable differences at 1050  $\text{cm}^{-1}$  and 900  $\text{cm}^{-1}$ . Specifically, the spectral profiles recorded for the culture performed using a plant-based peptone (PF) and the commercially sourced peptone (PA) differ from the other spectral profiles. This indicates that using different culture medium ingredients had a distinct impact on the structural components and balance of metabolic products in the cultured bacteria.

A cluster analysis (dendrogram) and PCA were also used to better reveal the differences between the spectra. The results of these analyses are shown in Fig. 6 (a) and (b). The PCA and the dendrogram broadly separate the spectra into three groups.

The medium with commercially sourced peptone (PA) forms its own cluster, whereas the medium with PF is plotted far from the other groups and exhibits the most irregular behavior of all the culture media. Given that microorganism growth was poor in the PF media, the apparent major difference between the PF media spectra and the other media spectra probably reflects poor microbial growth in the PF media. All other culture media are closely grouped, indicating that the cultured *S. cohnii* are all in a similar state.

This study shows that FTIR spectrometers can be used to identify the effect that different culture medium ingredients have on microbial growth. Specifically, FTIR spectrometers can be used to clearly identify which ingredient has a distinct impact on microbial growth (in this case, PF).

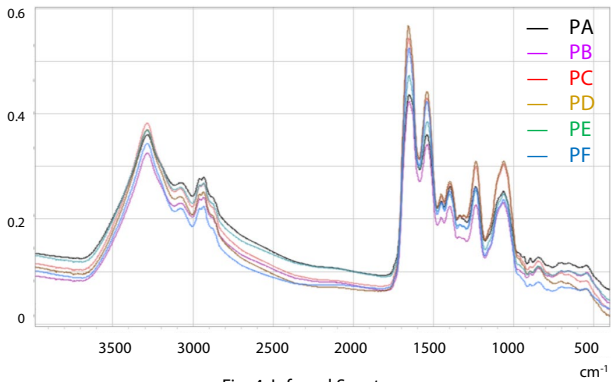


Fig. 4 Infrared Spectra

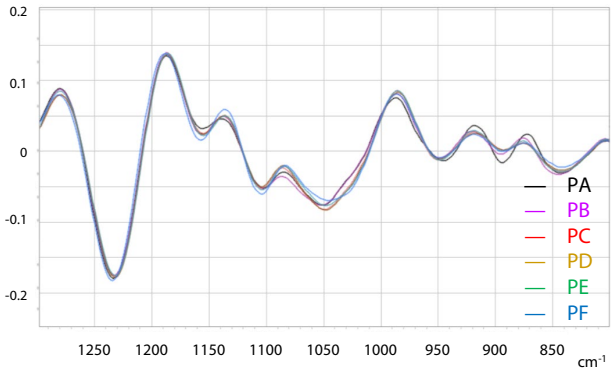
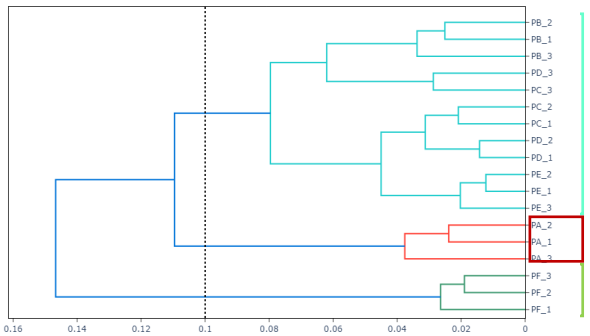
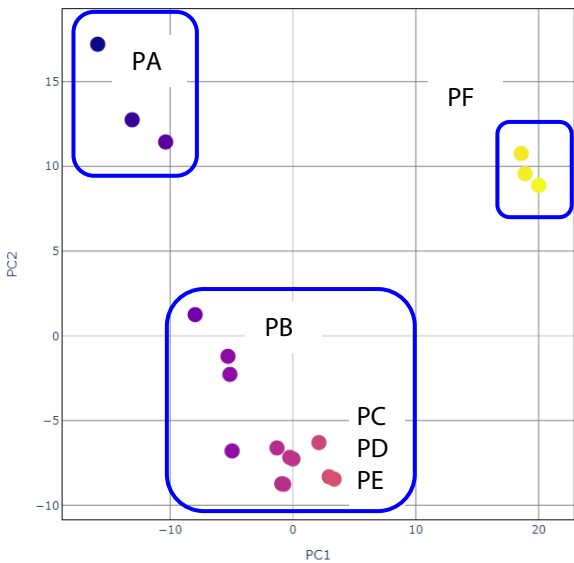


Fig. 5 Second Derivative Spectra



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



(b) Principal Component Analysis (Score Plot)

Fig. 6 Clustering of Different Peptones

## Differences between Yeast Extracts

Fig. 7 shows the infrared spectrum of each culture solution, and Fig. 8 shows their second derivative spectra in the range of 1300 to 800  $\text{cm}^{-1}$ . Each culture was performed three times ( $n = 3$ ), and the most representative spectrum recorded from the three cultures is shown in the figures.

The spectral profiles in Fig. 7 appear very similar, whereas the second derivative spectra in Fig. 8 reveal some notable differences at 1050  $\text{cm}^{-1}$  and 900  $\text{cm}^{-1}$ . Specifically, the spectral profiles recorded for the cultures performed using yeast extracts YD\_lot1, YD\_lot2, and YE differ from the other spectral profiles. This indicates that using different culture medium ingredients had a distinct impact on the structural components and balance of metabolic products in the cultured bacteria.

A cluster analysis (dendrogram) and PCA were used to better reveal the differences between the spectra. The results of these analyses are shown in Fig. 9 (a) and (b). The PCA and the dendrogram broadly separate the spectra into three groups.

Yeast extracts YD\_lot1 and YD\_lot2 form their own cluster, showing no apparent lot-to-lot difference. Yeast extract YE is plotted far from the other groups and exhibits the most irregular behavior of all cultures. Given that microorganism growth was poor in the YE media, the apparent major difference between the YE media spectra and the other media spectra probably reflects poor microbial growth in the YE media. All other culture media are relatively closely grouped, suggesting the *S. cohnii* cultured in these media are all in a similar state. However, a closer inspection reveals a difference between YB\_STD and YB\_HQ cultures, that is, *S. cohnii* cultures were affected by different grades of yeast extract.

This study shows that FTIR spectrometers can be used to identify the effect that different culture medium ingredients have on microbial growth.

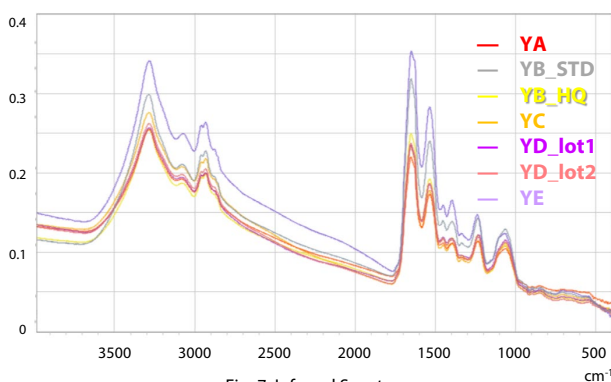


Fig. 7 Infrared Spectra

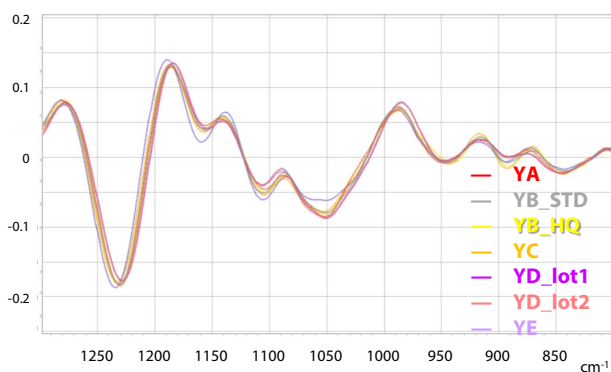
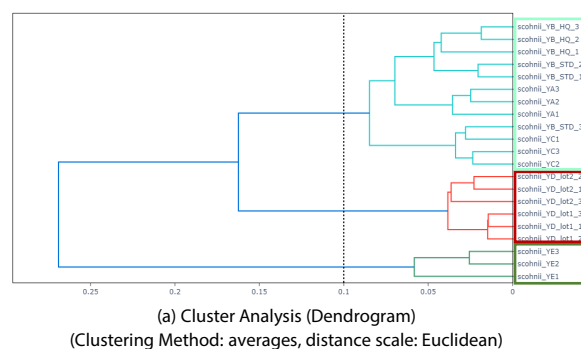
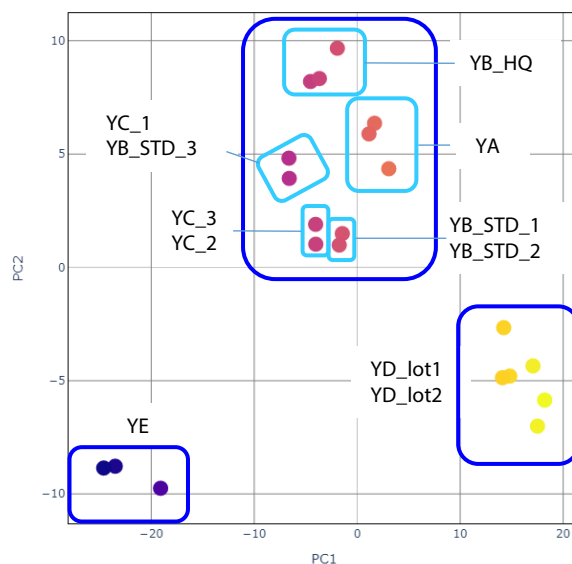


Fig. 8 Second Derivative Spectra



(a) Cluster Analysis (Dendrogram)  
(Clustering Method: averages, distance scale: Euclidean)



(b) Principal Component Analysis (Score Plot)

Fig. 9 Clustering of Different Yeast Extracts

## Conclusion

An FTIR spectrometer was used to monitor how *S. cohnii* cultures were affected by using different culture medium ingredients, specifically, peptones and yeast extracts.

The use of different culture medium ingredients resulted in differences between the recorded infrared spectra (especially the second derivative spectra) of cultured *S. cohnii*. Furthermore, principal component analysis and cluster analysis of the recorded spectra provided a clear grouping of the specific culture ingredients that resulted in poor microbial growth.

These findings show that an FTIR spectrometer can be an effective tool for acceptance testing of culture medium ingredients used to manufacture a microbial product for performing quality assessments when changes are made to a culture medium and for detecting abnormal changes in a culture-based process.

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 Application

1. Identification Analysis of Lactobacillus Species/Strains Using IRXross

[Application News No. 01-00567](#)

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01-01206-EN

First Edition: Sep. 2026



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➤ **IRXross**  
Fourier Transform Infrared  
Spectrophotometer

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➤ Pharmaceutical and  
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➤ Biopharmaceutical

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➤ Technical Service /  
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➤ Other Inquiry