

Application News

IRXross™ / IRTracer™-100 Fourier Transform Infrared Spectrophotometer

Identification Analysis of Lactobacillus Species/Strains Using IRXross

Shoko Iwasaki, Takahisa Araki and Risa Fuji

User Benefits

- ◆ Efficient measurement of multi-specimen is possible by using a diffuse reflectance measurement accessory and microfocus plate.
- ◆ Since the measurement time with this technique is about 30 seconds per specimen, analysis work can progress in a shorter time than with other techniques.
- ◆ This technique is also applicable to samples other than bacteria, and enables identification analysis by principal component analysis and cluster analysis.

■ Introduction

Identification analysis of bacteria is necessary in applied research in the clinical medical field and for quality control in food and drug manufacturing processes. As conventional techniques, the polymerase chain reaction (PCR) method and pulsed-field gel electrophoresis (PFGE) method are widely used, but these methods have various problems, including the complexity of sample preparation and the time required for measurements.

The Fourier transform infrared spectrophotometer (FTIR) is an analytical instrument that is applied to a wide range of target samples, and has the potential to become a new technique which solves the above-mentioned problems owing to its simple operation and fast measurement speed.

Since the infrared spectrum of bacteria acquired by FTIR contains information originating from lipids, polysaccharides, proteins, carbohydrates and other compounds⁽¹⁾, it is possible to identify the species and strains of bacteria cultured under the same conditions (culture medium, temperature, time).

This Application News article introduces an identification analysis of lactic acid bacteria (lactobacilli) using a combination of an IRXross Fourier transform infrared spectrophotometer (Fig. 1) and a diffuse reflectance measurement accessory^{*1}.

*1: Special order product. For details, please contact Shimadzu sales or an agent.



Fig. 1 Appearance of IRXross™

■ Culture and Sample Preparation

Table 1 shows the seven lactobacilli strains used in this experiment. These were all cultured under the same culture conditions (MRS culture medium, 30 °C, 24 hours).

Table 1 Lactobacilli Used in Experiment

Genus	Species	Subspecies	Strain
<i>Lactocaseibacillus</i>	<i>paracasei</i>	<i>paracasei</i>	NBRC 15889 ^T
		<i>tolerans</i>	NBRC 15906 ^T
	<i>rhamnosus</i>	-	NBRC 3425 ^T
			NBRC 12521
	<i>casei</i>	-	NBRC 15883 ^T
	<i>chiayiensis</i>	-	NBRC 112906 ^T
	<i>zeae</i>	-	JCM 11302 ^T

The superscript T following the strain number indicates that the strain is a type strain.

The sample preparation procedure is as follows. Three suspensions were prepared from one medium, and measurements were carried out three times for each strain.

- ① Add 50 μ L of pure water, 50 μ L of ethanol, and three 1 μ L inoculation loop portions (1 μ L loop \times 3) of lactobacillus cultured in an agar medium to a microtube and stir well.
- ② Add ϕ 1.0 mm zirconia beads (15 beads), and expose the useful substance in the cells by using a cell disintegration device (30 s \times 4 times).
- ③ After centrifuging, drip 2 μ L of the suspension onto a microfocus plate (64 wells) and dry.



Fig. 2 Microfocus Plate

■ Measurement Conditions and Analysis Technique

Table 2 shows the IRXross measurement conditions. In this experiment, the measurements were carried out while purging the entire measurement system with nitrogen to eliminate the effects of water vapor and CO₂ in the atmosphere.

Table 2 Measurement Conditions of IRXross

Instruments	: IRXross, diffuse reflectance measurement accessory (special order product)
Resolution	: 4 cm ⁻¹
Accumulation	: 20 times
Apodization function	: SqrTriangle
Detector	: DLATGS
Purge	: Nitrogen

In the data analysis, Aspen Unscrambler™ manufactured by Aspen Technology Inc. was used. Aspen Unscrambler is a multivariate analysis program that can carry out preprocessing, classification, and prediction of the spectra obtained with various types of analytical instruments.

The analysis of the infrared spectra acquired by LabSolutions™ IR was conducted by the following procedure.

- ① Export data in the txt format from LabSolutions IR.
- ② Copy and paste the txt data into Excel®, and create a table in which the wavelengths (cm⁻¹) are shown in column A and absorbance of each strain is shown in column B and the following columns. Then swap rows with columns.
- ③ As data preprocessing, select the 2nd derivative (Savitzky-Golay, number of smoothing points: 31, polynomial order: 2) and wavelength range (1300 to 800 cm⁻¹), and apply normalization (unit vector normalization) and centering (mean).

■ Identification Analysis

Fig. 3 shows the infrared spectra of the 7 lactobacillus strains, and Fig. 4 shows the 2nd derivative spectra for the wavelength range of 1300 to 800 cm^{-1} .

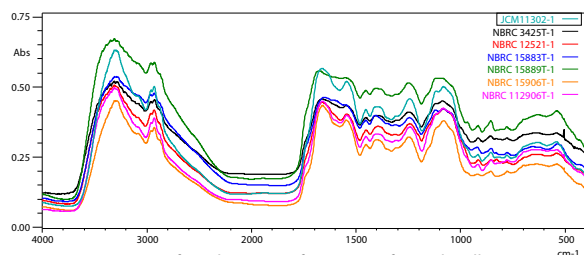


Fig. 3 Infrared Spectra of 7 Strains of Lactobacilli

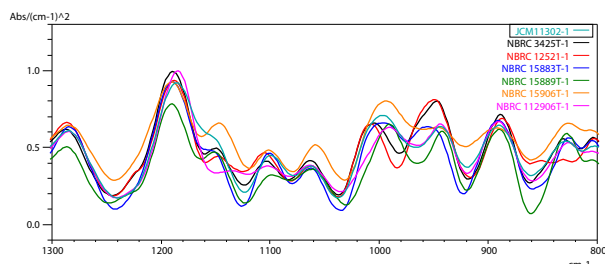
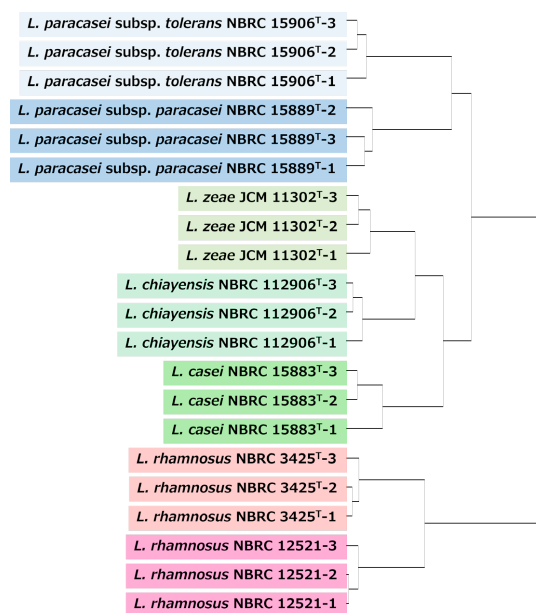
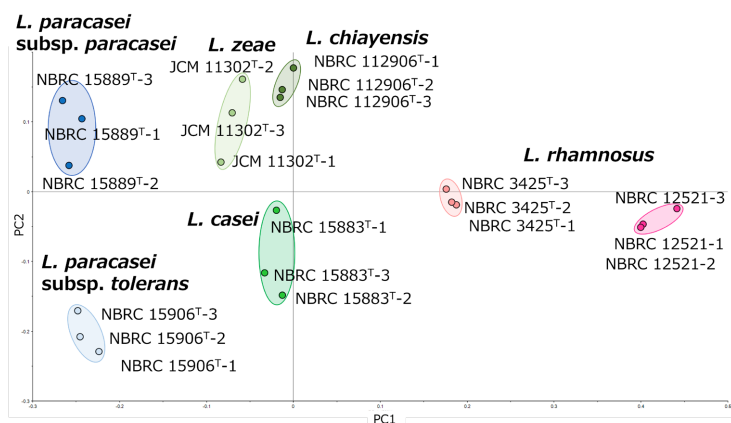


Fig. 4 2nd Derivative Spectra of Lactobacilli Strains

Since the infrared spectral shapes are extremely similar, as can be seen in Fig. 3, it is difficult to identify the strains simply by comparing the infrared spectra. In addition, variations in the peak intensity due to differences in the surface shapes of the dried samples could also be seen. Therefore, in this experiment, identification was carried by a principal component analysis and cluster analysis using the 2nd derivative spectra. Fig. 5 (a) and (b) show the results of the cluster analysis (dendrogram) and principal component analysis (score plots), respectively.



(a) Results of cluster analysis (dendrogram)
(Clustering method: Ward's method, distance measure: Squared Euclidean)



(b) Results of principal component analysis (score plots)

Fig. 5 Results of Identification Analysis

From Fig. 5 (a), the clusters were divided into *L. rhamnosus* and others, and were further divided in *L. paracasei* (two subspecies) and *L. zeae*, *L. chiayensis*, and *L. casei*. It was possible to distinguish the lactobacilli at the subspecies/strain level, and this taxonomic structure is in good agreement with the prior literature⁽²⁾. In Fig. 5 (b), similar strains are arranged at close distances, and it was found that identification by species and subspecies is possible.

■ Conclusion

Diffuse reflection measurements of seven strains of lactobacilli were carried out using an IRXross spectrophotometer and a diffuse reflectance measurement accessory. This technique enabled efficient measurement of all samples while maintaining purging of the entire system with nitrogen. Although the differences between strains could not be read by comparison of the infrared spectra, it was possible to identify the differences between all strains by a principal component analysis and cluster analysis using the derivative spectra.

<References>

- 1) Lasch, P, Naumann, D, "Infrared Spectroscopy in Microbiology." Encyclopedia of Analytical Chemistry, 3: 1-32. Mar. 2015.
- 2) Liu, DD, Gu, CT, "Proposal to reclassify *Lactobacillus zhaodongensis*, *Lactobacillus zeae*, *Lactobacillus argenteratensis* and *Lactobacillus buchneri* subsp. *silagei* as *Lactocaseibacillus zhaodongensis* comb. nov., *Lactocaseibacillus zeae* comb. nov., *Lactiplantibacillus argenteratensis* comb. nov. and *Lentilactobacillus buchneri* subsp. *silagei* comb. nov., respectively and *Apilactobacillus kosoi* as a later heterotypic synonym of *Apilactobacillus micheneri*." Int J Syst Evol Microbiol, 70: 6414-6417. Dec. 2020.

IRXross, IRTracer, and LabSolutions are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.
Excel is a registered trademark or trademark of the American company Microsoft Corporation in the United States and/or other countries.
Aspen Unscrambler is a trademark of Aspen Technology Inc.



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.

01-00567-EN

First Edition: Jul. 2023

➤ Please fill out the survey

Related Products

Some products may be updated to newer models.



➤ **IRXross**
Fourier Transform Infrared
Spectrophotometer

Related Solutions

➤ Life Science

➤ Food and Beverages

➤ Biopharmaceutical

➤ Microorganisms

➤ Price Inquiry

➤ Product Inquiry

➤ Technical Service /
Support Inquiry

➤ Other Inquiry