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IRXross™ / IRTracer™-100 Fourier Transform Infrared Spectrophotometer

Application News

Identification Analysis of Lactobacillus Species/Strains Using IRXross

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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
Lacticaseibacillus	*paracasei*	*paracasei*	NBRC 15889^T
		tolerans	NBRC 15906^T
	rhamnosus	-	NBRC 3425^T
			NBRC 12521
	casei	-	NBRC 15883^T
	chiayiensis	-	NBRC 112906^T
	zeae	-	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 µL of pure water, 50 µL of ethanol, and three 1 µL inoculation loop portions (1 µL loop × 3) of lactobacillus cultured in an agar medium to a microtube and stir well.

② Add φ1.0 mm zirconia beads (15 beads), and expose the useful substance in the cells by using a cell disintegration device (30 s × 4 times).

③ After centrifuging, drip 2 µL of the suspension onto a microfocus plate (64 wells) and dry.

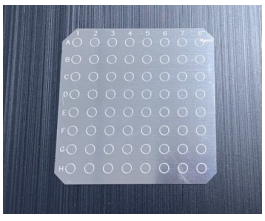


Fig. 2 Microfocus Plate

■ 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 CO_2 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}.

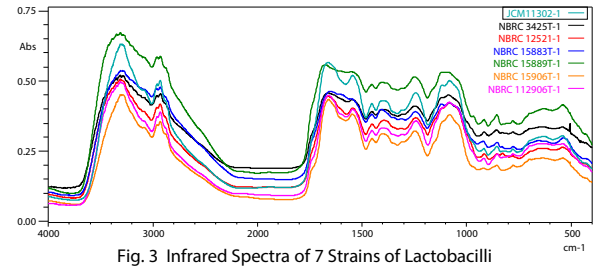


Fig. 3 Infrared Spectra of 7 Strains of Lactobacilli

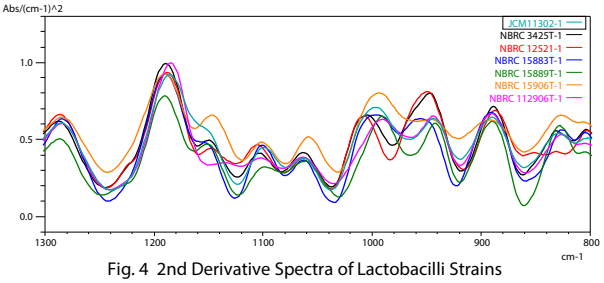


Fig. 4 2nd Derivative Spectra of Lactobacilli Strains

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.

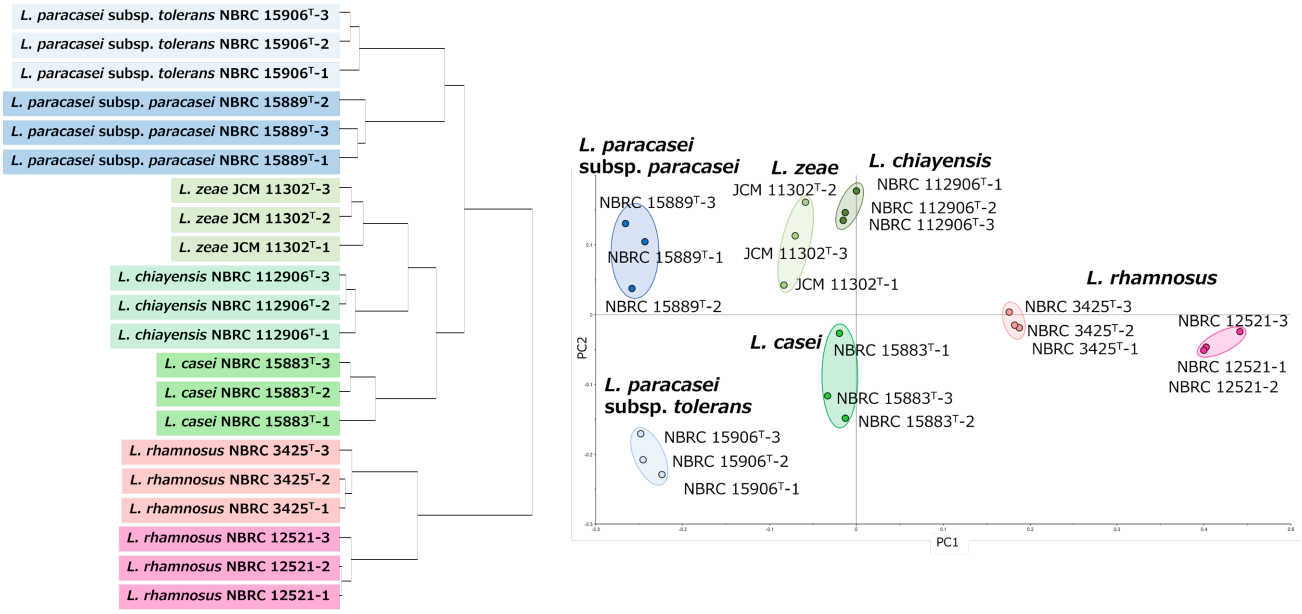
From Fig. 5 (a), the clusters were divided into *L. rhamnosus* and others, and were further divided in *L. paracasei* (two subspecies) and *L. zeae*, *L. chiayensis*, and *L. casei*. It was possible to distinguish the lactobacilli at the subspecies/strain level, and this taxonomic structure is in good 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 argentoratensis and Lactobacillus buchneri subsp. silagei as Lacticaseibacillus zhaodongensis comb. nov., Lacticaseibacillus zeae comb. nov., Lactiplantibacillus argentoratensis 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.



(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

01-00567-EN First Edition: Jul. 2023

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