Lait 80 (2000) 445–459

Original article

Potentiality of Fourier Transform Infrared Spectroscopy (FTIR) for discrimination and identification of dairy Lactic acid bacteria

Caroline AMIEL[a], Laurence MARIEY[a]*,
Marie-Christine CURK-DAUBIÉ[b], Patricia PICHON[a], Josette TRAVERT[a]

[a] Laboratoire de Physico-Chimie et Biotechnologies, IUT de Caen,
Université de Caen Basse Normandie, 14032 Caen Cedex, France
[b] ADRIA Normandie, boulevard du 13 juin 1944, BP 2, 14310 Villers Bocage, France

(Received 6 October 1999; accepted 15 February 2000)

Abstract — The potentiality of FTIR spectroscopy for the identification of lactic acid bacteria (LAB) used in the dairy industry was tested. For this purpose, spectra of different strains were recorded using standardized conditions. These strains were species of *Lactobacillus* (12 species, 3 subspecies), *Lactococcus* (4 species, 3 subspecies), *Leuconostoc* (3 species, 3 subspecies), *Weissella* (1 species) and *Streptococcus* (2 species) involved in the soft cheese industry. Spectral libraries were then established by statistical analysis. Reference strains were then tested on these libraries, and 100% of correct identifications were obtained at the genus and species level, 86% at the subspecies level. Well-identified strains were included in libraries. Wild isolates (n = 48) previously identified by biochemical tests and RAPD method were then tested. We obtained 100% at the genus level and 69% at the species level of correlation between FTIR and other methods. Finally, spectra of reference strains performed in other laboratories were tested with the same method, and the results tallied with official identification in 100% of the cases at the genus level and in 41% of the cases at the species level. These results concerning a few strains allow us to plan the development of a more complete database for rapid identification of LAB.

FTIR spectroscopy / lactic acid bacteria / classification / identification

Résumé — **Potentialités de la spectroscopie infrarouge à transformée de Fourier (IRTF) pour la discrimination et l'identification de bactéries lactiques d'intérêt laitier.** La discrimination et l'identification de souches de bactéries lactiques d'origine laitière ont été étudiées par spectroscopie IRTF. Les spectres de 28 souches de bactéries lactiques appartenant aux genres *Lactobacillus* (12 espèces, 3 sous-espèces), *Lactococcus* (4 espèces, 3 sous-espèces), *Leuconostoc* (3 espèces, 3 sous-espèces), *Weissella* (1 espèce) et *Streptococcus* (2 espèces) ont été enregistrés dans des conditions

* Correspondence and reprints
Tel.: (33) 2 31 56 71 19; fax: (33) 2 31 56 71 65; e-mail: l.mariey@iutcaen.unicaen.fr

standardisées. Ces spectres ont permis d'établir des bibliothèques spectrales de référence auxquelles ont été comparés de nouveaux spectres de souches de collection et de souches sauvages. L'identification des souches de collection (5 espèces, 7 sous-espèces) a été correcte dans 100 % des cas au niveau genre et espèce, et 86 % au niveau sous-espèce. Les souches correctement identifiées ont été introduites dans les bibliothèques. L'identification IRTF de 48 isolats a été corrélée à 100 % au niveau genre et à 69 % au niveau espèce avec les identifications biochimiques et les résultats de la RAPD (Random Amplified Polymorphic DNA) préalablement obtenus. Enfin, les spectres de 17 souches de collection enregistrés par d'autres équipes ont été testés sur nos bases de données. Leur identification a été correcte dans 100 % des cas au niveau genre et 41 % des cas au niveau espèce. La spectroscopie IRTF s'est donc avérée une méthode efficace pour la discrimination et l'identification des espèces étudiées. Les résultats obtenus nous permettent d'envisager le développement d'une base de données plus complète pour l'identification des bactéries lactiques.

spectroscopie IRTF / bactérie lactique / identification / discrimination

1. INTRODUCTION

The use of infrared spectroscopy to differentiate bacteria has been studied since the 1950's [26, 29]. Unfortunately, due to the weak performances of dispersive spectrometers, these kinds of studies ceased in the 1970's. For about ten years, the development of modern interferometric infrared spectroscopy, Fourier transform techniques and efficient low-cost computers have given a new impulse to this research field [12, 14, 21–23, 32, 33].

Bacteria spectra are usually recorded in the mid-infrared. They are specific to one bacterial strain and show the vibrational characteristics of all the cellular components: fatty acids, intracellular and membrane proteins, polysaccharides, nucleic acids. Statistical treatment of spectral data allows discrimination between different genera, species and even strains. That is why more and more research teams are interested in FTIR characterization of bacteria [3, 4, 8, 9, 13, 15, 18, 19, 28]. Lactic acid bacteria involved in the dairy industry are varied. It is important to be able to identify and characterize them with rapid, reliable and cheap methods, in the aim of controlling all the stages of fabrication.

Our research team has performed different studies to identify lactic acid bacteria and coryneform bacteria [1, 6, 31]. This paper describes the use of FTIR spectroscopy to discriminate some dairy lactic acid bacteria at different levels: genus, species and subspecies. Until now, only *Lactobacillus* involved in breweries have been studied by FTIR Curk et al. [3, 4]. We established libraries of species involved in the soft cheese industry: *Lactobacillus (Lb.), Lactococcus (Lc.), Leuconostoc (Ln.), Weissella (W.)* and *Streptococcus (Strep.)* [24]. This tool could be proposed for the characterization of such LAB for the rapid screening of strains isolated in these environments and products.

2. MATERIALS AND METHODS

To obtain reproducible data, we have set an experimental procedure relative to growth conditions, sample preparation, and spectra recording. Previous studies [3, 25, 34] have shown that spectra were especially sensitive to these parameters.

2.1. Strains and growth conditions

– Type strains come from international collections: ATCC, CNRZ, LMG, NCDO, NCFB, or CIP. Fourteen strains of *Lactobacillus*, six of *Lactococcus*, five of

Leuconostoc, one of *Weissella* and two of *Streptococcus* were studied, corresponding to usually used strains in the soft cheese industry (Tab. I), according to Dellaglio et al. [8], Larpent et al. [16], and Novel [24].

– Collection strains come from CNRZ collection: six strains of *Lactobacillus*, six of *Lactococcus*, four of *Leuconostoc*, and one of *Streptococcus* (Tab. II).

– Wild strains were isolated from "Pont l'Evêque" cheese by ADRIA Normandie and "Camembert" by LMA, University of Caen Basse-Normandie. They were identified by biochemical tests (API 50 CHL) as *Lactobacillus* (19 strains), *Lactococcus* (26 strains), and *Leuconostoc* (three strains). For these strains, we had also RAPD results.

– All the strains were grown for 24 ± 2 h on different liquid media, according to optimal growth conditions [27]: MRS [7], medium at 30 °C and 42 °C for mesophilic and thermophilic lactobacilli respectively, MRS medium at 25 °C for *Leuconostoc* and *Weissella* , M17 [30], medium at 30 °C for *Lactococcus*, M17 medium at 42 °C for *Streptococcus*.

Table I. Lactic acid bacteria genera differentiation by discriminant analysis.
Tableau I. Différenciation des genres de bactéries lactiques par analyse discriminante.

Strain		Distance to classes				
		Lactobacillus	*Lactococcus*	*Leuconostoc*	*Streptococcus*	*Weissella*
Lb. delbrueckii delbrueckii	**CNRZ 225T**	0.4	1.1	1.5	1.8	3.3
Lb. delbrueckii lactis	**CNRZ 207T**	0.6	1.3	1.7	2.2	3.5
Lb. delbrueckii bulgaricus	**CNRZ 208T**	0.4	0.9	1.2	1.9	3.7
Lb. helveticus	**CNRZ 223T**	0.4	0.8	1.4	1.3	3.0
Lb. plantarum	**ATCC 14917T**	0.6	1.3	1.8	2.3	3.5
Lb. paraplantarum	**CIP 104668T**	0.4	1.2	1.7	2.1	3.9
Lb. fermentum	**CNRZ 209T**	0.5	1.2	1.5	2.1	3.7
Lb. paracasei	**CNRZ 62T**	0.3	0.7	1.4	1.7	3.0
Lb. acidophilus	**CNRZ 204T**	0.4	0.9	1.4	1.6	3.9
Lb. rhamnosus	**CNRZ 212T**	0.7	1.3	1.6	2.1	3.6
Lb. casei	**CNRZ 313T**	0.5	1.0	1.9	2.0	4.4
Lb. zeae (ex rhamnosus)	**CIP 103253**	0.4	1.1	1.3	1.8	3.5
Lb. brevis	**CNRZ 215T**	0.4	0.7	1.1	1.4	3.6
Lb. pentosus	**LMG 10755T**	0.4	1.2	1.1	1.8	3.0
Lc. plantarum	**NCDO 1869T**	1.0	0.4	1.7	2.0	4.6
Lc. garvieae	**NCDO 2155T**	0.9	0.4	1.7	1.8	3.9
Lc. lactis cremoris	**CNRZ 105T**	0.7	0.2	1.6	1.2	4.7
Lc. lactis lactis	**CNRZ 142T**	0.5	0.1	1.5	1.5	4.4
Lc. lactis lactis var diacetylactis	**NCFB 176T**	0.6	0.1	1.9	1.7	4.8
Lc. raffinolactis	**NCFB 617T**	1.3	0.3	2.4	1.9	5.6
Ln. lactis	**NCDO 533T**	1.6	2.2	0.5	1.8	2.4
Ln. pseudomesenteroides	**CIP 103316T**	1.0	1.5	0.2	1.3	2.6
Ln. mesenteroides mesenteroides	**CIP 102305T**	1.6	2.3	0.4	1.8	2.5
Ln. mesenteroides dextranicum	**CIP 102423T**	1.4	1.8	0.3	1.6	2.4
Ln. mesenteroides cremoris	**CIP 103009T**	1.4	2.1	0.4	1.9	2.5
Strep. thermophilus	**NCDO 573T**	1.2	1.3	1.3	0.2	3.6
Strep. salivarius	**ATCC 7073T**	1.8	1.9	1.7	0.2	4.3
W. paramesenteroides	**CIP 102421T**	3.2	4.5	2.2	3.8	0.1

Table II. Identification of collection strains by FTIR.
Tableau II. Identification de souches de collection par IRTF.

Strain	Official name	FTIR identification		
		Genus	Species	Sub-species
Lb. delbrueckii bulgaricus	**CNRZ 10**	*Lactobacillus*	*delbrueckii*	*bulgaricus*
Lb. delbrueckii bulgaricus	**CNRZ 1004**	*Lactobacillus*	*delbrueckii*	*bulgaricus*
Lb. delbrueckii bulgaricus	**CNRZ 11**	*Lactobacillus*	*delbrueckii*	*bulgaricus*
Lb. delbrueckii lactis	**CNRZ 700**	*Lactobacillus*	*delbrueckii*	*bulgaricus*
Lb. helveticus	**CNRZ 34**	*Lactobacillus*	*helveticus*	
Lb. helveticus	**CNRZ 241**	*Lactobacillus*	*helveticus*	
Lc. lactis cremoris	**CNRZ 1454**	*Lactococcus*	*lactis*	*cremoris*
Lc. lactis cremoris	**CNRZ 1458**	*Lactococcus*	*lactis*	*cremoris*
Lc. lactis lactis var diacetylactis	**CNRZ 1453**	*Lactococcus*	*lactis*	*lactis var diacetylactis*
Lc. lactis lactis var diacetylactis	**CNRZ 1456**	*Lactococcus*	*lactis*	*lactis var diacetylactis*
Lc. lactis lactis	**CNRZ 1455**	*Lactococcus*	*lactis*	*lactis*
Lc. lactis lactis	**CNRZ 261**	*Lactococcus*	*lactis*	*lactis*
Ln. mesenteroides dextranicum	**CNRZ 1273**	*Leuconostoc*	*mesenteroides*	*dextranicum*
Ln. mesenteroides dextranicum	**CNRZ 1275**	*Leuconostoc*	*mesenteroides*	*dextranicum*
Ln. mesenteroides mesenteroides	**CNRZ 1274**	*Leuconostoc*	*mesenteroides*	*mesenteroides*
Ln. mesenteroides mesenteroides	**CNRZ 1284**	*Leuconostoc*	*mesenteroides*	*dextranicum*
Strep. thermophilus	**CNRZ 368**	*Streptococcus*	*thermophilus*	

2.2. Sample preparation

Samples were prepared using a standardized procedure:

– Absorbance of bacterial cultures was measured at 600 nm (UV) using a 1.6 cm length cell in a spectrophotometer (Spectronic 301, Milton Roy) to determine culture concentration for further dilutions.

– Culture medium was removed by centrifugation at 4 000 r.p.m. for 10 min.

– Cells were washed twice with 4 mL of saline solution (NaCl, 9 $g \cdot L^{-1}$), and centrifuged in the same previous conditions.

– The pellet was suspended in a defined volume of saline solution to obtain an adequate concentration for spectral measurements. The volume was calculated so that a culture, characterized by a $OD_{600} = 0.7$, is retrieved in 50 µL.

– 5 µL of the concentrated bacteria was deposited on a ZnSe (zinc selenide) window (13 mm (diameter) × 2 mm (thick)), then dried for an hour at 50 °C.

For each strain, two cultures were prepared in similar conditions to take into account cultural fluctuations [34]. Three samples were studied for each culture to verify repeatability of the method. When spectra obtained with these two cultures were not satisfactory (see below Sects. 2.3 and 2.5), a third culture was performed.

2.3. Spectroscopic measurements

Bacterial spectra were recorded between 4 000 and 700 cm^{-1} using a FTIR spectrometer (Nicolet 250, Nicolet Instrument, Thermo-Optek) equipped with a KBr beamsplitter and a DTGS detector. Sixty-four interferograms were averaged per spectrum at a resolution of 4 cm^{-1}. A background spectrum was recorded with the ZnSe window free of any sample. Each spectrum

results from the ratio of sample spectrum and background spectrum and is registered in absorbance units. All spectra were submitted to a "quality test" (adapted from Helm et al. [13]). After this test, validated spectra were normalized to one absorbance unit using the amide I spectral band located at about 1 640 cm^{-1}, and then included in the study.

The required time from sample preparation to spectrum is about 2 h.

2.4. Statistical methods

Two types of statistical methods were employed (one for discrimination, the other for identification), both applications of Omnic TQ Analyst Software 1.2 (Nicolet Instrument, Thermo-Optek): Discriminant Analysis and Search Standards Method.

– Discriminant Analysis (DAM) is a discrimination method. Spectra classes are predetermined (one class corresponding to one genus, species or subspecies). The chosen spectral region is specified. Bacteria spectra are introduced in their respective classes, and then the software calculates the Mahalanobis distances [17] between each spectrum and the center of its class. The distances between each spectrum and center of each other class are also calculated, and allow us to determine the proximity of classes. Besides, classes can be graphically represented by scatter plot diagrams whose axes are Mahalanobis distances to two classes. Several spectral regions are tested, chosen in accordance to Naumann studies [23], to obtain the most discriminating results. Classes are well discriminated if intra-class distances are clearly lower than inter-class distances.

– Search Standards Method (SSM) is a comparison method. Spectra of the database are introduced. A spectral region is chosen for the comparison of spectra. When a new spectrum is proposed, the software determines its similitude percentage with the spectra of the database. Preliminary studies show that when spectra obtained with bacteria from the same culture are compared, the result is generally superior to 99% similitude. With spectra obtained from different cultures of the same strain, more than 97% is commonly obtained. For the same species or subspecies, the result is more than 95%. So we can validate an identification only if the result of correlation with spectra of the database is over 95%.

2.5. Elaboration of reference libraries and strains identification

To establish genera library (LAB library), the following procedure was applied:

– Using SSM, spectra were selected only when the similitude percentage was more than 99% between the three spectra of one culture, and more than 97% between the nine spectra of one strain.

– A DAM was prepared, with five classes corresponding to the five genera. Spectra previously selected were introduced as standards in their respective classes.

For *Lactobacillus*, two options were chosen:

– According to Dicks et al. [9] and to Mori et al. [20], we classified the strain CIP 103253 = ATCC 15820 (*Lb. rhamnosus)* as type strain of *Lb. zeae*.

– We have introduced the type strain *Lb. paraplantarum* CIP 104668^T described by Curk et al. [5], to verify the discrimination of *plantarum*, *paraplantarum* and *pentosus* species.

To study the discrimination at the species and subspecies level, we had to make new libraries (with the same spectra) with one class corresponding to one species or one subspecies. Three species libraries were established: one for *Lactobacillus* (14 type strains corresponding to 12 species), one for *Lactococcus* (six type strains corresponding to four species), and one for both *Leuconostoc* and *Weissella* (six type strains

corresponding to four species). *Leuconostoc* and *Weissella* are grouped in the same library because *Weissella* was formerly known as *Leuconostoc* [2], and because *Leuconostoc* class is the nearest one from *Weissella* class in our library.

In the same way, three subspecies libraries were established: one for *Lactobacillus delbrueckii*, one for *Lactococcus lactis*, and one for *Leuconostoc mesenteroides*.

For each library, several spectral regions were tested, and the one giving the best discrimination was chosen. Table III summarizes data contained in the libraries.

To identify a strain, SSM is performed using LAB genera library. First, the best discriminating spectral region at the genus level is used. Then, according to the genus obtained, the best discriminating spectral region at the species level is set. And so on for the subspecies level.

3. RESULTS AND DISCUSSION

3.1. Type strains discrimination

The best discrimination between LAB genera was obtained using DAM with the following spectral region: 1 500–1 200 cm^{-1} + 1 200–900 cm^{-1} + 900–700 cm^{-1}. Indeed, according to several other teams [3, 11, 13], we found that discrimination was better with combined windows than with an entire one. Table I shows the average of the distances obtained between the spectra of each type strain and the five classes. Figure 1 shows partial results concerning distances to center of *Lactococcus* and *Streptococcus* classes. We can see in Table I that the average distances between the spectra of one class (i.e. one genus) and the center of this class is clearly inferior to distances to other classes. In spite of short distances, especially between *Lactobacillus* and *Lactococcus*, a good discrimination is obtained between genera. This is confirmed by Figure 1 where we can see four separate clusters corresponding to *Lactococcus, Lactobacillus, Leuconostoc* and *Streptococcus*. Graphic representation proposed by TQ Analyst software does not allow us to see the *Weissella* group which is at a Mahalanobis distance superior to three from *Streptococcus* and *Lactococcus* groups.

For species libraries, spectral regions were chosen as follows to obtain the best discrimination between species:

– 1 500–1 200 cm^{-1} + 1 200–900 cm^{-1} + 900–700 cm^{-1} for *Lactobacillus* library (Tab. IV).

– 1 200–900 cm^{-1} for *Lactococcus* library (Tab. V).

Table III. Data contained in libraries.

Tableau III. Données contenues dans les bibliothèques.

Library	Number of classes	Number of spectra/database	Spectral region
Genera	5 genera	213	1 500–1 200/1 200–900/900–700 cm^{-1}
Lactobacillus	12 species	82	1 500–1 200/1 200–900/900–700 cm^{-1}
Lactococcus	4 species	38	1 200–900 cm^{-1}
Leuconostoc + *Weissella*	4 species	31	1 200–900/900–700 cm^{-1}
Lb. delbrueckii	3 subspecies	19	1 500–1 200/1 200–900/900–700 cm^{-1}
Lc. lactis	3 subspecies	21	1 200–900/900–700 cm^{-1} first derivative
Ln. mesenteroides	3 subspecies	15	1 200–900/900–700 cm^{-1}

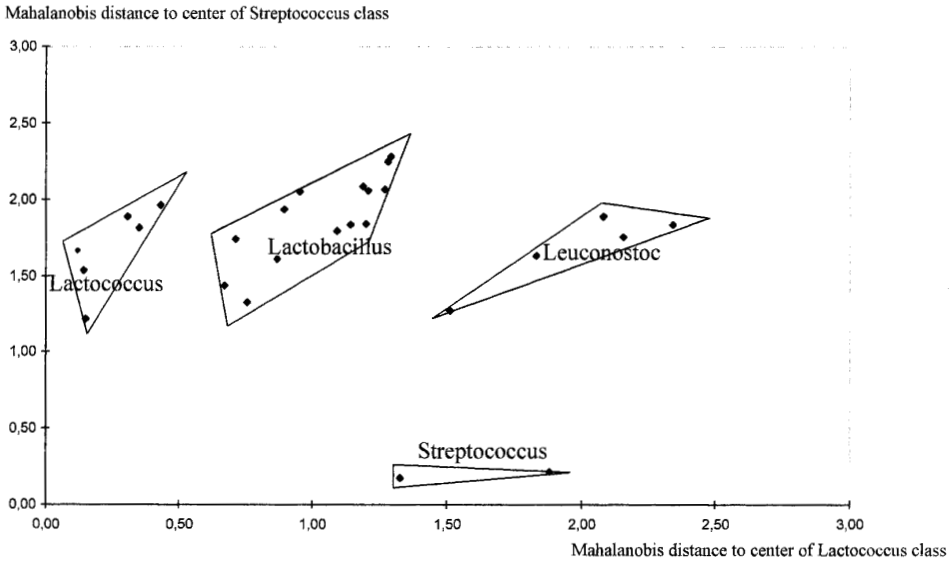

Figure 1. Discrimination of 4 classes of LAB using discriminant analysis.
Figure 1. Discrimination de 4 classes de bactéries lactiques par analyse.

– 1 200–900 cm^{-1} + 900–700 cm^{-1} for *Leuconostoc* and *Weissella* library (Tab. VI).

We can see in Tables IV, V and VI that distances inside one class representing one species vary between 0.1 to 0.9. Conversely, distances between classes are from 12 (between *Lc. garvieae* and *Lc. lactis*) to 315 (between *Lb. plantarum* and *Lb. rhamnosus*). We can conclude that species are well discriminated.

Moreover, we can notice that the two species *Lb. plantarum* and *Lb. paraplantarum* are well discriminated. Indeed, we can see in Figure 2 that spectra of these two species present differences, particularly in two regions: (i) the band at 1 740 cm^{-1} characterizing CO stretching vibration of the ester functional groups of the phospholipids, (ii) the region 1 200–900 cm^{-1} characterizing oligo and polysaccharides COC and COP stretching vibration, known to be selective at the species and strain levels [23].

For subspecies libraries, we can see the results in Tables VII, VIII and IX.

The type strains of *Lb. delbrueckii delbrueckii, Lb. delbrueckii bulgaricus* and *Lb. delbrueckii lactis* are separated on the combined windows: 1 500–1 200 cm^{-1} + 1 200–900 cm^{-1} + 900–700 cm^{-1}. We can notice that the subspecies *lactis* is as closed to *bulgaricus* as to *delbrueckii.*

The type strains of *Lc. lactis lactis, Lc. lactis cremoris* and *Lc. lactis lactis var diacetylactis* were discriminated on the combined windows 1 200–900 cm^{-1} + 900–700 cm^{-1}. In this case, the discrimination was improved by the use of first derivative spectra.

The type strains of *Ln. mesenteroides mesenteroides, Ln. mesenteroides dextranicum* and *Ln. mesenteroides cremoris* were discriminated on the combined windows 1 200–900 cm^{-1} + 900–700 cm^{-1}.

We obtain a good discrimination for the three subspecies libraries.

3.2. Validation with collection strains

The spectra of 17 collection strains were recorded in the same previous conditions

Table IV. *Lactobacillus* type strains calibration.
Tableau IV. Calibration des souches types de *Lactobacilles*.

		Distances to classes											
Name	Strain	*acidophilus*	*brevis*	*casei*	*delbrueckii*	*fermentum*	*helveticus*	*paracasei*	*paraplantarum*	*pentosus*	*plantarum*	*rhamnosus*	*zeae*
Lb. acidophilus	**CNRZ 204T**	**0.45**	104.35	181.20	69.01	87.92	29.54	57.45	168.48	44.93	256.79	259.52	128.95
Lb. brevis	**CNRZ 215T**	104.42	**0.52**	46.92	26.07	90.79	109.53	89.31	73.64	76.76	95.09	139.17	72.14
Lb. casei	**CNRZ 313T**	181.14	46.80	**0.40**	93.50	222.62	208.03	75.90	31.23	90.81	89.59	156.19	30.23
Lb. delb.bulgaricus	**CNRZ 208T**	68.83	25.86	93.67	**0.57**	85.65	67.17	73.68	128.57	77.20	148.88	159.31	91.17
Lb. delb. delbrueckii	**CNRZ 225T**	69.28	25.77	93.07	**0.55**	86.20	67.78	73.66	128.18	77.21	148.60	157.87	90.70
Lb. delb. lactis	**CNRZ 207T**	69.21	26.67	94.28	**0.61**	66.77	67.64	74.23	128.72	77.40	149.22	159.68	91.70
Lb. fermentum	**CNRZ 209T**	87.94	90.75	222.70	86.16	**0.48**	54.67	181.16	243.86	155.38	302.23	183.93	243.45
Lb. helveticus	**CNRZ 223T**	29.23	109.15	207.77	67.14	54.33	**0.15**	91.47	211.99	92.49	295.82	249.68	186.17
Lb. paracasei	**CNRZ 62T**	57.28	89.04	75.76	73.56	180.94	91.58	**0.26**	77.26	26.32	174.56	212.86	35.05
Lb. paraplantarum	**CIP104668T**	168.51	73.59	31.31	128.38	243.86	212.33	77.47	**0.48**	62.78	80.72	238.08	48.93
Lb. pentosus	**LMG 10755T**	44.83	76.59	90.76	77.05	155.25	92.69	26.41	62.65	**0.35**	124.48	254.63	47.48
Lb. plantarum	**ATCC 14917T**	256.74	94.96	89.59	148.72	302.15	296.07	174.69	80.63	124.52	**0.40**	315.63	93.32
Lb. rhamnosus	**CNRZ 212T**	259.77	39.34	156.49	159.04	184.15	250.23	213.30	238.30	254.98	315.93	**0.70**	206.58
Lb. zeae	**CIP 103253**	129.07	72.18	30.40	91.18	243.54	186.59	35.35	49.02	47.70	96.49	206.44	**0.56**

Table V. *Lactococcus* type strains calibration.
Tableau V. Calibration des souches types de Lactocoques.

Strain		Distances to class			
		garvieae	*plantarum*	*raffinolactis*	*lactis*
Lc. garvieae	**NCDO 2155T**	0.4	80.9	21.8	12.4
Lc. plantarum	**NCDO 1869T**	81.1	0.6	42.4	71.7
Lc. raffinolactis	**NCFB 617T**	22.0	42.4	0.5	19.2
Lc. lactis lactis var diacetylactis	**NCFB 176T**	12.3	71.5	19.0	0.3
Lc. lactis lactis	**CNRZ 142T**	12.8	71.1	19.0	0.7
Lc. lactis cremoris	**CNRZ 105T**	12.5	72.1	19.3	0.4

Table VI. *Leuconostoc* and *Weissella* type strains calibration.
Tableau VI. Calibration des Leuconostoc et des *Weissella*.

Strain		Distances to class			
		Ln. lactis	*Ln. mesenteroides*	*Ln. pseudomesenteroides*	*W. parameseteroides*
Ln. lactis	**NCDO 533**	0.5	51.8	76.8	38.6
Ln. mesenteroides	**CIP 103009T**	51.4	0.9	48.6	17.9
Ln. mesenteroides	**CIP 102423T**	52.3	0.7	49.0	17.6
Ln. mesenteroides	**CIP 102305T**	52.2	0.9	49.4	18.0
Ln. pseudomesenteroides	**CIP 103316T**	76.7	48.8	0.4	54.0
W. paramesenteroides	**CIP 1024241T**	38.4	17,3	53.9	0.3

and tested by SSM, using the spectral regions determined in Section 3.1. Results are presented in Table II. Collection strains are correctly identified in 100% of the cases at the genus and species levels and in 86% of the cases at the subspecies level according to official nomenclature.

The identification at the subspecies level is not optimal (86%). It would be certainly improved by the introduction of a greater variety of strains of the same subspecies in the database. It is a fact that information concerning subspecies is more difficult to point out, and perhaps more efficient statistical treatments [10] could give better results.

All the well identified strains were then included in previous libraries for the following studies, to increase the representativity of each species, and so to improve the following results.

3.3. Identification of wild LAB

Forty eight wild isolates of *Lactobacillus, Lactococcus,* and *Leuconostoc* were tested in our libraries. Comparative results are presented in Table X. FTIR identification of wild strains tallies with previous identification (RAPD or biochemical methods) in 100% of the cases at the genus level.

For species level, a few discrepancies were observed:

– One strain identified to *Lb. casei* by biochemical tests and RAPD has been identified to zeae by FTIR.

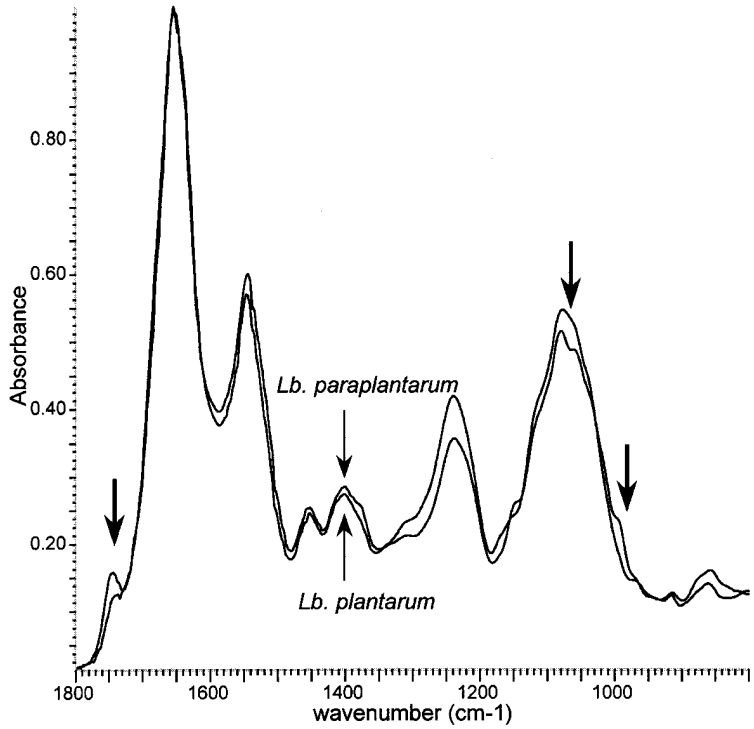

Figure 2. FTIR spectra of *Lb. plantarum* and *Lb. paraplantarum*.
Figure 2. Spectres IRTF de *Lb. plantarum* and *Lb. paraplantarum*.

Table VII. *Lb. delbruekckii* subspecies calibration.
Tableau VII. Calibration des sous-espèces *Lb. delbrueckii*.

Strain		Distances to class		
		bulgaricus	*delbrueckii*	*lactis*
Lb. delbrueckii bulgaricus	**CNRZ 208T**	**0.66**	25.73	14.73
Lb. delbrueckii delbrueckii	**CNRZ 225T**	25.55	**0.47**	12.64
Lb. delbrueckii lactis	**CNRZ 207T**	14.61	12.71	**0.54**

– One strain identified to *Lb. acidophilus* by RAPD tests has been identified by FTIR to another thermophilic species: *Lb. delbrueckii bulgaricus*.

– Four strains identified to *Lb. plantarum* by RAPD were identified to *Lb. pentosus* by FTIR. These species are known to be very close to each other, so it is difficult to say which method (FTIR or RAPD) gives the right result. Therefore, with our method, *Lb. pentosus* and *Lb plantarum* are well discriminated (d = 124 , Tab. III).

– One strain identified to *Lb. plantarum* by RAPD was characterized by a peculiar

Table VIII. *Lc. lactis* subspecies calibration.
Tableau VIII. Calibration des sous-espèces *Lc. lactis.*

Strain		Distances to class		
		lactis cremoris	*lactis diacetyl.*	*lactis lactis*
Lc. lactis cremoris	**CNRZ 105T**	**0.75**	2.80	2.74
Lc. lactis lactis var diacetylactis	**NCFB 176T**	2.80	**0.75**	3.97
Lc. lactis lactis	**CNRZ 142T**	2.74	3.97	**0.75**

Table IX. *Ln. mesenteroides* subspecies calibration.
Tableau IX. Calibration des sous-espèces *Ln. mesenteroides*.

Strain		Distances to class		
		mesenteroides	*cremoris*	*dextranicum*
Ln. mesenteroides mesenteroides	**CIP 102305T**	**0.67**	43.13	3.45
Ln. mesenteroides cremoris	**CIP 103009T**	43.14	**0.67**	25.82
Ln. mesenteroides dextranicum	**CIP 102423T**	3.45	25.82	**0.67**

Table X. FTIR identification of wild strains.
Tableau X. Identification IRTF des souches sauvages.

Biochemical identification	RAPD identification	IRTF identification
Lb. paracasei (10)*	*Lb. paracasei* (10)	*Lb. paracasei* (10)
Lb. casei (1)	*Lb. casei* (1)	*Lb. zeae* (1)
Lb. plantarum (7)	*Lb. plantarum* (6)	*Lb. plantarum* (2)
		Lb. pentosus (4)
	Lb. plantarum ? (1)	*Lb. helveticus* (1)
Lb. acidophilus (1)	*Lb. delbrueckii* (1)	
Lc. lactis lactis (26)	*Lc. lactis lactis* (26)	*Lc. lactis lactis* (18)
		Lc. lactis/plantarum? (2)
		Lc. lactis/garvieae? (6)
Ln. lactis (1)	*Ln. lactis* (1)	*Ln. lactis* (1)
Ln. mesenteroides (2)		*Ln. mesenteroides* (2)

* Number of strains.

profile compared to the other strains of the species. This strain was identified to *Lb. helveticus* by FTIR.

– Eight strains identified *to Lc. lactis lactis* by RAPD were not well discriminated by FTIR. These strains were as close to *Lc. lactis* as to another class (*garvieae* or *plantarum*).

In conclusion, we obtained for 33 out of 48 strains (69%) the same identification as RAPD or phenotypic tests.

These results could probably be improved by the introduction of new strains in the classes where there are not yet collection strains (*Lb. plantarum, Lb. pentosus, and Lc. garvieae*). Besides, RAPD results are questionable, according to the fact that this method is not known as an identification method, but as a comparison method. Moreover, phenotypic methods are known to often be uncertain for the identification at the subspecies level.

3.4. Identification of spectra from other laboratories

To confirm the reproducibility of the method, 80 bacteria spectra corresponding to 17 collection strains (Tab. XI) performed by Lefier in two other laboratories (INRA in Poligny, France, and Hannach Research Institute in Scotland) were tested with our libraries. The spectra were recorded on two different spectrometers (Nicolet spectrometer in France and Mattson spectrometer in Scotland). They were transmitted to our laboratory by e-mail. The identification was performed using the method previously described. The results of the spectra identification are presented in Table XI. They agree with the official nomenclature at the genera level in 100% of the cases, and at the species level for 7 of the 17 strains (41%). Nevertheless, several results have to be discussed:

– For strains NCFB 2774^T, NCFB 363 ^{T} and CNRZ 62 ^{T}, we obtained for half of the spectra the correct identification, and for the other half another species. To improve these results, more spectra of these strains have to be tested.

– Strains CNRZ 205 and CNRZ 442 *(Lb. rhamnosus*) have been identified as *Lb. zeae*. The new species *Lb. zeae* has been described by Dicks et al. [9] from atypical strains of *Lb. rhamnosus* and *Lb. casei*. It is quite likely that other strains of these two species may be classified in *Lb. zeae* species. We can suppose that strain CNRZ 205 and CNRZ 442 are in this case.

– Two strains of *Lb. paracasei* have been identified to *Lb. zeae*. It is a fact that Mahalanobis distances between the type strains *paracasei* and *zeae* is not very significant (Tab. IV). To discriminate these species more efficiently, it would be necessary to determinate another spectral region.

– Strains CNRZ 211 and CNRZ 73 (*Lb. plantarum*) have been identified as *Lb. paraplantarum*, a new species described by Curk et al. [5], from strains of *Lb. plantarum* difficult to discriminate from *Lb. pentosus.* It is possible that CNRZ 73 will be in the future reclassified as *Lb. paraplantarum*, if this result is confirmed by other methods. Conversely, for CNRZ 211, identical to ATCC 14917^T and type strain of *plantarum* species, we must conclude that if the two strains are really the same, the differences in experimental conditions provokes discrepancies of the spectra. These two results have to be confirmed by the recording of spectra in the standardized conditions previously described.

4. CONCLUSION

The aim of this work was to specify the ability of FTIR to discriminate and identify LAB involved in the cheese industry. Our preliminary results indicate a good discrimination at the genus and species level, even at the subspecies level. The best spectral regions have been determined for each genus. The spectral database elaborated allows us to identify new strains, with a good percentage of correct results:

– 100% at the genus and species level for collection strains,

– 100% at the genus level and 69% at the species level for wild isolates.

The discrepancies between FTIR identification and official nomenclature have been discussed according to recent taxonomical changes. Results must be improved by

Table XI. Identification of spectra from different origin.
Tableau XI. Identification de spectres d'origine différente.

Official name		FTIR identification		
Strain		Genus	Species	Sub-species
Lb. brevis	**CNRZ 324**	*Lactobacillus*	*brevis*	
Lb. paracasei	**CNRZ 320**	*Lactobacillus*	*zeae*	
Lb. pentosus	***NCFB 363 T = LMG10755T**	*Lactobacillus*	*pentosus/paraplantarum*	
Lb. paracasei	**CNRZ 383**	*Lactobacillus*	*casei*	
Lb. paracasei	**CNRZ 62 T**	*Lactobacillus*	*casei/paracasei*	
Lb. paracasei	**CNRZ 763**	*Lactobacillus*	*zeae*	
Lb. plantarum	**CNRZ 211**	*Lactobacillus*	*paraplantarum*	
Lb. plantarum	**CNRZ 73**	*Lactobacillus*	*paraplantarum*	
Lb. rhamnosus	**CNRZ 205**	*Lactobacillus*	*zeae*	
Lb. rhamnosus	**CNRZ 442**	*Lactobacillus*	*zeae*	
Lb. rhamnosus	**CNRZ 212**	*Lactobacillus*	*rhamnosus*	
Lb. acidophilus	***NCFB 1748 T = CNRZ 204 T**	*Lactobacillus*	*acidophilus*	
Lb. rhamnosus	***NCFB 243 T = CNRZ 212 T**	*Lactobacillus*	*rhamnosus*	
Lb. paracasei ssp. tolerans	***NCFB 2774 T**	*Lactobacillus*	*casei/paracasei*	
Lb. delbrueckii ssp. delbrueckii	***NCFB 213 T = CNRZ 225 T**	*Lactobacillus*	*delbrueckii*	*delbrueckii*
Lc. lactis ssp. cremoris	**CNRZ 105T**	*Lactococcus*	*lactis*	*lactis*
Lc. lactis ssp. lactis	**CNRZ 142 T**	*Lactococcus*	*lactis*	*lactis*

recording spectra of new strains to increase the representation of each species. The greatest interests of the method are its speed, its easiness and its cheapness. These three characteristics are especially interesting in the dairy industry where strains have to be followed during all the processes. Identification of spectra received from other teams by e-mail is possible, nevertheless, the more standardized the experimental conditions, the better the results of identification.

ACKNOWLEDGEMENTS

We thank D. Lefier (INRA, Poligny, France) for his collaboration. The Conseil Regional of Basse Normandy and FEDER funds supported this work.

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