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Identification of seven species of the *Lactobacillus acidophilus* group by FT–IR spectroscopy

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Abstract

Fourier transform infrared (FT–IR) spectra of 102 strains of the seven species of the *Lactobacillus acidophilus* group were collected and investigated for their potential use in classification and identification on species level. The database built contains more than 370 spectra. Various procedures of pre-processing and classification methods have been compared with respect to their predictive ability. The most encouraging results were achieved with linear discriminant analysis (LDA) of the absorbance values of normalized spectra at selected wavenumbers. The rate of correct species assignment in cross-validation (Jackknife procedure with one spectrum left out for model building) were 95%, 95%, 69%, 100%, 88%, 100%, and 91% for *L. acidophilus, L. amylovorus*, *L. crispatus*, *L. gallinarum*, *L. gasseri*, *L. helveticus*, and *L. johnsonii*, respectively. Very distinct grouping was found for *L. gallinarum* and *L. helveticus*, the most difficult differentiation in LDA was between the pairs *L. crispatus*/*L. amylovorus* and *L. gasseri*/*L. johnsonii*.

Keywords: Infrared spectroscopy; *Lactobacillus acidophilus* group; Identification; Classification; Chemometrics

1. Introduction

Lactobacilli are very important in food industry since they are used as starter cultures and as probiotics in fermented milk products. Their taxonomy has always been a matter of vigorous discussions. The use of phylogenetic markers and the analysis of DNA sequences have brought a strong base into classification debates (Schleifer & Ludwig, 1995; Chavagnat, Haueter, Jimeno, & Casey, 2002).

In 1980, *Lactobacillus acidophilus* was recognized by DNA hybridization studies as a heterogeneous group of six species that have similar metabolic and functional properties. The members of this group include *L. acidophilus*, *L. amylovorus*, *L. crispatus*, *L. gallinarum*, *L. gasseri* and *L. johnsonii*. 16S rRNA sequences of lactobacilli were first described in 1991, which helped to build a picture of the phylogenetic relatedness of various species. Phylogenetically, *L. helveticus* is closely related to the *L. acidophilus* group (Gopal, 2002). This classification defined the choice of the species selected for our study.

Members of the *L. acidophilus* group are normal inhabitants of the mammalian gastrointestinal tract and of the skin. Some strains have been claimed to provide health benefits (Dellaglio, 2002). *L. acidophilus* is part of evaluation studies as an aid to prevent bacterial infections in the urogenital tract (Reid, Zalai, & Gardiner, 2001; Reid & Bocking, 2003). Antagonistic activities of *L. acidophilus* strains against microbial pathogens have been reviewed recently by Servin (2004).

With increasing interest in the health-promoting properties of lactobacilli, it is critically important to have reliable methods for identifying freshly isolated strains. Beyond quality aspects this includes safety and proprietary reasons.

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The identification of microorganisms by vibrational spectroscopic techniques has found increasing interest and is applied as a rapid and reliable method mainly in biomedicine (Maquelin et al., 2002a, b; Maquelin et al., 2003) but also in the characterization and classification of nonpathogenic bacteria such as lactic acid bacteria (Curk, Peladan, & Hubert, 1994; Amiel, Mariey, Curk-Daubie, Pichon, & Travert, 2000; Amiel, Mariey, Denis, Pichon, & Travert, 2001; Weinrichter, Luginbühl, Rohm, & Jimeno, 2001). Even the quantification of binary mixtures of lactic acid bacteria by Fourier transform infrared (FT–IR) spectroscopy was partly successful (Oberreuter, Mertens, Seiler, & Scherer, 2000). A concise review on these important applications of FT–IR spectroscopy combined with chemometrics has been published by Mariey, Signolle, Amiel, and Travert (2001).

2. Materials and methods

2.1. Bacterial growth, strains, media and reference identification

Bacterial strains from various collections were used in this study (Table 1). The unambiguous identification of these strains was achieved by partial *tuf* gene sequencing as recently developed and published by Chavagnat et al. (2002). Various dilutions were plated on MRS agar and the bacteria grown anaerobically at 37 °C up to a diameter of the colonies of about 1.5 mm.

2.2. Preparation of bacterial films on ZnSe optical plates

Well-grown colonies of a diameter of about 1.5 mm were dispersed in 100 µl of distilled water and mixed thoroughly. About 60 µl of these suspensions were transferred onto the ZnSe optical plates (Ø 9 mm) and dried in a preheated drying oven between 55 and 60 °C for 30 min. The ZnSe plates loaded with the dried bacterial film were mounted on an automated sampling system accommodating up to 37 samples (Pike MapplR, Pike Technologies, Madison, USA). For each strain, at least three and at most seven spectra from different colonies were collected.

2.3. Spectroscopy

Single beam spectra were collected with an FT–IR spectrometer (Bio-Rad FTS-7; tungsten IR source, DTGS detector, range 3100–690 cm^{-1}, resolution

Table 1
Species and strains of the *L. acidophilus* group selected for this study

Species and strains	Number of strains	Number of spectra[a]
L. acidophilus NCIMB 702472, NCIMB 701360, DSM 20079, La-5® (Chr. Hansen), FAM 1459, FAM 1626, FAM 1628, FAM 1459/3, FAM 1627, CIP 103 596, CIP 103598, CIP 103600, CIP 103601, NCIMB 1723, NCIMB 1899, NCIMB 8880, and two strains isolated from commercial fermented products	18	82
L. amylovorus NCIMB 702473, NCIMB 702658, NCIMB 702659, NCIMB 702660, NCIMB 702661, NCIMB 702662, NCIMB 702745, NCIMB 702663, DSM 20531T, CIP 103609, CIP 103610	11	40
L. crispatus NCIMB 702471, NCIMB 701417, NCIMB 702240, NCIMB 702172, NCIMB 4505, NCIMB 4504, DSM 20584T, DSM 20356, CIP 103602, CIP 103604, CIP 103606, NCIMB 8116, NCIMB 8821, NCIMB 4504, CIP 103608, NCIMB 4505	16	52
L. gallinarum CIP 103611T, CIP 103 650, CIP 103 612	3	9
L. gasserii NCIMB 702664, NCIMB 702173, NCIMB 702174, NCIMB 702470, NCIMB 8931, DSM 20243T, CIP 103605, NCIMB 8820, NCIMB 8819, CIP 103615, CIP 103616, CIP 103613, CIP 103617, CIP 62.18, CIP 103618, CIP 103619, CIP 103699, CIP 103784, CIP 103785, CIP 103786, NCIMB 8931 and one strain isolated from a commercial fermented product	22	72
L. helveticus FAM 1450, FAM 1475 and 13 isolates from local raw milk and cheese	15	51
L. johnsonii NCIMB 8795, NCIMB 702665, CIP 103620T, FAM 1939; DSM 20553, Nestlé-LJ1strain, FAM 1157, FAM 1147, FAM 1074, FAM 1158, FAM 1154, CIP 103 614, CIP 103652, CIP 103781, CIP 103782, NCIMB 8795 and two strains isolated from commercial fermented products	17	70

NCIMB—National Collections of Industrial Food and Marine Bacteria; DSM—Deutsche Sammlung von Mikroorganismen; FAM—Forschungsanstalt für Milchwirtschaft; and CIP—Collection de l'Institut Pasteur.
[a]Outlier spectra excluded.