Use of Group-Specific and RAPD-PCR Analyses for Rapid Differentiation of Lactobacillus Strains from Probiotic Yogurts

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Received: 22 January 2003 / Accepted: 24 February 2003

Abstract. The increasing interest in probiotic lactobacilli implicates the requirement of techniques that allow a rapid and reliable identification of these organisms. In this study, group-specific PCR and RAPD-PCR analyses were used to identify strains of the Lactobacillus casei and Lactobacillus acidophilus groups most commonly used in probiotic yogurts. Group-specific PCR with primers for the L. casei and L. acidophilus groups, as well as L. gasseri/johnsonii, could differentiate between 20 Lactobacillus strains isolated from probiotic yogurts and assign these into the corresponding groups. For identification of these strains to species or strain level, RAPD profiles of the 20 Lactobacillus strains were compared with 11 reference strains of the L. acidophilus and L. casei group. All except one strain could be attributed unambiguously to the species L. acidophilus, L. johnsonii, L. crispatus, L. casei, and L. paracasei. DNA reassociation analysis confirmed the classification resulting from the RAPD-PCR.

Strains of L. acidophilus and L. casei are increasingly being used in the manufacture of probiotic yogurts [12]. Taxonomically, those strains may not always be members of these two species, but of closely related lactobacilli grouped together in the L. acidophilus and L. casei complexes. The L. acidophilus group comprises six species (L. acidophilus, L. amylovorus, L. crispatus, L. gallinarum, L. gasseri, L. johnsonii) that resemble each other in their phenotypic features and which, therefore, can often not be unequivocally differentiated by physiological or biochemical properties, such as sugar fermentation profiles [6]. Similarly, the L. casei group includes a number of species which cannot unambiguously be distinguished by phenotypic properties [6]. These difficulties in the correct identification of probiotic lactobacilli have led to the misclassification of Lactobacillus strains in the past. For example, two human isolates selected for probiotic products were originally identified as L. acidophilus by Gilliland and coworkers [3, 5]. However, DNA–DNA hybridization studies revealed that one strain was L. crispatus and the other L. johnsonii [12]. DNA reassociation studies and molecular typing methods like PFGE or 16S rDNA sequencing are laborious, time-consuming, and are nonroutine techniques for the differentiation of closely related species. RAPD-PCR, on the other hand, is a rapid fingerprinting method that has already been used by several workers for Lactobacillus differentiation [4, 10, 13], and thus may represent a good technique for differentiation of probiotic yogurt strains. In this study, 18 Lactobacillus strains isolated from 15 different probiotic yogurts and two strains from a previous investigation identified as members of the L. acidophilus or L. casei group were investigated by group-specific PCR, RAPD-PCR, and DNA reassociation analysis.

Materials and Methods

Bacterial strains. Eighteen Lactobacillus strains were isolated from 15 different probiotic yogurts, using the methods described previously [12]—they are listed in Figs. 1 and 2. L. crispatus BFE 693 and L. casei BFE 728 identified during a previous study [12] and reference strains obtained from the German collection of microorganisms and cell cultures (DSMZ), were also used in this study (Figs. 1, 2).

For DNA reassociation analysis, L. acidophilus BFE 665 and BFE 682, L. johnsonii BFE 654, L. paracasei BFE 675 and BFE 687, and L. rhamnosus BFE 659 were used as reference strains. All strains were propagated in MRS broth at 37°C. All strains were also assessed for their ability to grow in MRS broth at 15°C. Stock cultures were kept in MRS broth at −20°C with 15% glycerol added.

Group-specific PCR. For group-specific PCR, specific regions of the
16S rDNA gene were amplified using primers and amplification conditions as reported by Roy et al. [11]. Total genomic DNA was isolated according to the method of Pitcher et al. [9] and was used as a template. Primers Lho and LBL R1 were used to identify members of the *L. acidophilus* group (except *L. gasseri* and *L. johnsonii*), primers LCS and LBL R2 were used to identify members of the *L. casei* group, while primers Lgj and LBL R1 were used to identify *L. gasseri* and *L. johnsonii* strains [11].

**RAPD-PCR.** RAPD analysis was performed using primer M13 (5′-GAG GGT GGC GGT TCT-3′) [7] in a 50-μL reaction volume. The PCR reaction mixture contained 100 ng of total genomic DNA, 200 μM of dNTPs, 50 μM of primer M13, 1 U Taq polymerase (Amersham Pharmacia, Freiburg, Germany), and 1 × Taq polymerase buffer (Amersham Pharmacia) containing 3 mM MgCl₂. DNA was amplified in 40 cycles (initial denaturation step at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min; annealing at 40°C for 20 s followed by ramping to 72°C at 0.6°C/s; extension at 72°C for 2 min). Amplification products were separated on a 1.5% agarose gel. Photographs of RAPD patterns were scanned and conversion, normalization, and further analysis of the scanned patterns were carried out using the Bionumerics software (Applied Maths, Kortrijk, Belgium). Similarity coefficients were calculated by using Pearson’s product-moment correlation coefficient, and strains were grouped by using the unweighted pair group method with arithmetical averages (UPGMA).

**DNA reassociation analysis.** Total genomic DNA was isolated and purified according to the guanidium thiocyanate method of Pitcher et al. [9], as described by Björkroth et al. [1]. DNA–DNA renaturation analysis was performed using a Gilford response spectrophotometer.