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# Comparison of Fructose-1,6-Bisphosphatase Gene (*fbp*) Sequences for the Identification of *Lactobacillus rhamnosus*

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**Abstract.** Comparative analysis of fructose-1,6-bisphosphatase gene (*fbp*) sequences was evaluated for the differentiation of reference and clinical strains of *Lactobacillus rhamnosus.* The sequences of 1,971 nucleotides of the *fbp* gene were determined on both DNA strands for 21 *L. rhamnosus* strains, representing reference, probiotic, and clinical strains. No PCR amplification of the *fbp* gene was observed for other species of the *Lactobacillus casei* complex (*L. casei* and *L. zeae*) or strains of *Lactobacillus acidophilus, Streptococcus thermophilus,* and *Escherichia coli.* Phylogenetic analysis of the *fbp* putative amino acid sequences of *L. rhamnosus* strains by the neighbor-joining method showed clear distinct positions of this species. The phylogenetic tree, derived from *fbp* nucleotide sequences, showed four clear divisions between strains of *L. rhamnosus.* From a taxonomic point of view, our results confirm for the first time that *fbp* gene sequences have high discriminating power for strains of *L. rhamnosus* that are difficult to differentiate.

Lactobacilli are members of the dense and diverse microbial community of the human gastrointestinal (GI) tract. This community includes both potentially beneficial and harmful microbes. In general, lactobacilli are considered to be beneficial for the host by inhibiting the growth of potential harmful bacteria in the intestinal tract and by exhibiting other beneficial effects. Administration of probiotics has been proposed to increase the number of lactobacilli in the GI tract. The widespread use of lactobacilli in fermented foods and dairy products has a long history, and most strains are considered commensal microorganisms with no pathogenic potential. However, some cases of local or systemic infections, including septicemia, meningitis, and endocarditis have occurred due to lactobacilli although they account for only 0.1% of bacteremia [1]. In case reports of bacteremia, the two most common species of lactobacilli identified have been *Lactobacillus casei* and *Lactobacillus rhamnosus*, both of which are also common in probiotic use. The widespread use of immunosuppressive therapy and antimicrobial agents ineffective against lactobacilli might increase their importance as possible pathogens among patients more vulnerable to infections. Furthermore, case reports of clinical infections connected with prior probiotic intake have been published [10–12]. However, in an epidemiological study of *Lactobacillus* bacteremia in Finland, no correlation between the increased probiotic use of *L. rhamnosus* GG (ATCC 53103) and the incidence of *Lactobacillus* bacteremia between 1990 and 2000 was found [19, 20]. *L. rhamnosus* GG is acid- and bile-stable and it attaches to and temporarily and effectively colonizes the human intestine [25]. These characteristics could increase the potential tendency of this species to cause infections [7]. Eleven clinical strains isolated were identical to *L. rhamnosus* GG without any temporally increasing trend that would suggest an association with the increase in probiotic use [19]. For safety reasons, it is crucial to be able to compare clinical isolates and probiotic strains. The difficulty in correctly identifying *L. rhamnosus* species and the increasing interest in some of their probiotic activities indicates the need for a simple and reliable molecular method for the definitive differentiation of strains.

Many typing methods have been proposed for lactobacilli strains, such as amplified ribosomal DNA restriction analysis, randomly amplified polymorphic

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DNA, and pulsed-field gel electrophoresis (PFGE) [15–17, 21]. Comparison of ribotyping, randomly amplified polymorphic DNA analysis, and PFGE in typing of *L. rhamnosus* and *L. casei* strains has already been performed. PFGE has been shown to be the most powerful method for strain typing and it is also used in epidemiological studies. However, it is a laborious and expensive method; therefore, only a limited number of samples can be analyzed [17, 25]. The discrimination of very closely related species, and especially of subspecies, is often critical. Thus the sequencing of several other genes has been used for the discrimination of lactobacilli [2, 4, 8, 24]. Nevertheless, to our knowledge, no study has reported on the potential to use a key protein-coding gene such as the fructose-1,6-bisphosphatase gene to identify *Lactobacillus rhamnosus* strains and to differentiate probiotic strains from clinical strains.

Fructose-1,6-bisphosphatase (EC 3.1.3.11) (FBPase) is a well-known enzyme involved in gluconeogenesis,, hydrolyzing D-fructose-1,6-bisphosphate to inorganic phosphate and D-fructose- 6-phosphate. The formation of D-fructose-1,6-bisphosphate from D-fructose-6-phosphate is catalyzed by the glycolytic key enzyme phosphofructokinase in an ATP-driven reaction. Simultaneous operation of FBPase and phosphofructokinase would result in cycling between fructose-6-phosphate and fructose-1,6-bisphosphate with concomitant hydrolysis of ATP. The *fbp* (formerly *fdp*) gene is required for FBPase synthesis. Based on the primary structure, four classes of FBPases (FBPase I–IV) have been identified: FBPase I, which in *Escherichia coli* is encoded by *fbp* [22]; FBPase II, which is encoded by *E. coli glpX* [3]; FBPase III, as present in *Bacillus subtilis* (*fbp* gene product; [6]); and FBPase IV, as identified in *Pyrococcus furiosus* (*fbpA* gene product; [26]). Eukaryal FBPases are orthologous to the bacterial FBPase I enzymes, since both contain typical FBPase domains (http://www.expasy.ch). The typical FBPase domain is absent in the bacterial FBPase II and FBPase III enzymes, suggesting that they are phylogenetically unrelated to FBPase I enzymes [13]. The presence of conserved domains in type I and type IV FBPases and I-1-Pases suggests that these enzymes share the same phylogenetic origin [26].

In this study, *fbp* gene sequences from *L. rhamnosus* strains were obtained and analyzed in order to infer a phylogenetic classification scheme. The accurate identification of clinical isolates of *L. rhamnosus* strains is accomplished using the sequences from the collections strains to construct a reference database.

## Materials and Methods

**Strains and media.** The bacterial strains used in this study are listed in Table 1. The strainXL1- Blue MRA P2 of *Escherichia coli* was grown in Luria–Bertani (LB) broth (Bioshop Canada, Burlington, ON, Canada) at 37°C with agitation at 200 rpm. Kanamycin (Sigma-Aldrich Canada, Oakville, ON, Canada) was added to the LB broth at a concentration of 50 μg/mL when required for the selection of transformants. Strains of *Lactobacillus* spp. were grown at 37°C in MRS broth (Difco Laboratories, Detroit, MI) and strains of *Streptococcus* spp. were grown in M17 broth (Difco) at 37°C. The bacterial strains were subcultured twice and incubated between 18 and 24 h. Stock cultures were stored at −80°C in BHI broth (Difco) with 15% (v/v) glycerol.

**DNA extraction.** Genomic DNA of bacterial strains was prepared according to Vincent et al. [27] from stationary-phase cultures in MRS, M17, or LB broth. The concentration of the purified DNA was determined by DyNA Quant 200 (Hoefer, San Francisco, CA) in capillary tubes using the Hoechst 33528 dye.

**Pulsed-field gel electrophoresis.** Preparation of cells and genomic DNA was performed as described by Roy et al. [14] with some modifications. Genomic DNA was digested with *Sma*I (Roche Applied Science, Laval, QC, Canada) at 25°C or *Apa*I (Roche Applied Science) at 30°C for 18 h. Samples were separated by using transverse alternating field electrophoresis (TAFE; Geneline II, Beckman Instruments, Mississauga, ON, Canada) under the following running conditions: (stage 1) 2 s pulse for 6 h at 350 mA; (stage 2) 5 s pulse for 6 h at 370 mA; (stage 3) 10 s pulse for 4 h at 390 mA; (stage 4) 15 s pulse for 4 h at 410 mA; (stage 5) 30 s pulse for 4 h at 430 mA; (stage 6) 60 s pulse for 3 h at 450 mA.

**PCR amplification.** A set of specific primers, FBP95MF1 (ATGAGTCAAAAATTGGTCTA) and FBP95MR1 (TTAGTCACCATTTCGTAACT), were designed from the *Lactobacillus rhamnosus* RW-9595M sequence (accession no. AF323526) to detect the presence of the fructose-1,6-bisphosphatase gene (*fbp*) in different strains of *L. rhamnosus.* PCR conditions were: 1× PCR buffer with 1.5 mM $MgCl_2$, 0.25 mM dNTP, 20 pmol of each primer, 1 unit of *Taq* polymerase (Pharmacia, Montreal, QC, Canada), and 25 ng of DNA in a 50 μL reaction volume overlaid with mineral oil. Reactions were performed in a Perkin-Elmer GeneAmp 9600 PCR System (Applied Biosystems, Foster City, CA). The PCR program consisted of 35 cycles, after an initial incubation at 94°C for 9 min. The cycles consisted of a denaturation step at 94°C for 30 s, an annealing step at 56°C for 30 s, an elongation at 72°C for 2 min, and a final elongation step at 72°C for 10 min. After amplification, all PCR products were conserved at 4°C. Amplified products were visualized by agarose gel electrophoresis, and fragments were purified by using the QIAquick gel extraction kit (Qiagen, Mississauga, ON, Canada) in accordance with the manufacturer's recommendations.

**Nucleotide sequencing.** Purified PCR products were cloned in the vector pCR 2.1 TOPO using the TOPO TA Cloning kit (Invitrogen Canada, Burlington, ON, Canada) with electrocompetent cells in accordance with the manufacturer's recommendations. Plasmids from transformants purified with the QIAprep Spin Miniprep Kit (Qiagen, Mississauga, ON, Canada) were used for automated sequencing. Sequencing of PCR products of both strands from clones was performed using the BigDye Terminator Cycle sequencing kit (Applied Biosystems) and M13 forward and reverse primers. The sequences were determined and analyzed with a 61 cm × 50 μm capillary in an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The PCR primers were also used for the cycle sequencing. For complete double-strand sequencing, 12 additional internal degenerate primers were used (Table 2). Forward and reverse primers were deduced from the alignment of sequences previously obtained the first time with M13 primers.

The alignments of the nucleotide and the translated amino acid sequences of the *fbp* gene sequences were performed with the program

Table 1. Source of lactic acid bacteria strains used in this study and the detection of the fructose-1,6-bisphosphatase gene

| Strain | Source or reference | Fructose-1,6-bisphosphatase gene |
|---|---|---|
| *S. thermophilus* Sfi6 | Nestlé Culture Collection | Negative |
| *S. thermophilus* NCFB 2393 | NCIMB | Negative |
| *S. thermophilus* TA-040 | Rhône-Poulenc Canada | Negative |
| *S. thermophilus* FYE-41 | Rhône-Poulenc Canada | Negative |
| *L. casei* ATCC 334 | ATCC | Negative |
| *L. casei* ATCC 335 | ATCC | Negative |
| *L. casei* ATCC 4007 | ATCC | Negative |
| *L. casei* ATCC 4646 | ATCC | Negative |
| *L. casei* ATCC 4913 | ATCC | Negative |
| *L. casei* ATCC 4940 | ATCC | Negative |
| *L. casei* ATCC 11578 | ATCC | Negative |
| *L. casei* ATCC 11582 | ATCC | Negative |
| *L. casei* ATCC 11974 | ATCC | Negative |
| *L. casei* ATCC 25180 | ATCC | Negative |
| *L. casei* ATCC 25302 | ATCC | Negative |
| *L. casei* ATCC 25303 | ATCC | Negative |
| *L. casei* ATCC 27092 | ATCC | Negative |
| *L. casei* ATCC 27216 | ATCC | Negative |
| *L. casei* ATCC 29599 | ATCC | Negative |
| *L. casei* ATCC 39392 | ATCC | Negative |
| *L. casei* ATCC 39539 | ATCC | Negative |
| *L. casei* DSM 20207 | DSMZ | Negative |
| *L. casei* DSM 20244 | DSMZ | Negative |
| *L. casei* Type V | Institut Rosell | Negative |
| *L. casei* RW-3703M | D. Roy (FRDC) | Negative |
| *L. zeae* ATCC 393 | ATCC | Negative |
| *L. zeae* ATCC 15820 | ATCC | Negative |
| *L. rhamnosus* ATCC 15008 | ATCC | Positive |
| *L. rhamnosus* ATCC 7469 | ATCC | Positive |
| *L. rhamnosus* ATCC 8530 | ATCC | Positive |
| *L. rhamnosus* ATCC 9595 | ATCC | Positive |
| *L. rhamnosus* ATCC 10863 | ATCC | Positive |
| *L. rhamnosus* ATCC 11443 | ATCC | Positive |
| *L. rhamnosus* ATCC 11981 | ATCC | Positive |
| *L. rhamnosus* ATCC 11982 | ATCC | Positive |
| *L. rhamnosus* ATCC 12116 | ATCC | Positive |
| *L. rhamnosus* ATCC 14957 | ATCC | Positive |
| *L. rhamnosus* ATCC 21052 | ATCC | Positive |
| *L. rhamnosus* ATCC 27773 | ATCC | Positive |
| *L. rhamnosus* ATCC 39595 | ATCC | Positive |
| *L. rhamnosus* ATCC 53103 | ATCC | Positive |
| *L. rhamnosus* RW-9595M | D. Roy (FRDC) | Positive |
| *L. rhamnosus* RW-6541M | D. Roy (FRDC) | Positive |
| *L. rhamnosus* R-011 | Institute Rosell | Positive |
| *L. rhamnosus* 25552 | CHUM | Positive |
| *L. rhamnosus* 42259 | CHUM | Positive |
| *L. rhamnosus* 61874-2 | CHUM | Positive |
| *L. rhamnosus* 76933-6 | CHUM | Positive |
| *L. acidophilus* ATCC 4356 | ATCC | Negative |
| *E. coli* XL1-Blue MRA-P2 | Stratagene | Negative |

Nestlé Culture Collection (Lausanne, Switzerland); NCIMB (National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland, UK); Rhône-Poulenc Canada (Ontario, Canada); Institut Rosell (Montreal, Quebec, Canada); ATCC (American Type Culture Collection, Rockland, MD, USA); DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany); D. Roy (Food Research and Development Center, St-Hyacinthe, Quebec, Canada); CHUM (Centre Hospitalier de l'Université de Montréal); Stratagene (La Jolla, CA, USA).

Table 2. Sequencing primers

| Primer | Sequence (5′–3′) |
|---|---|
| M13F | CGCCAGGGTTTTCCCAGTCACGAC |
| FBP-243F | ATGAGATGACCGAGACGAC |
| FBP-555F | AGTGGATTGAGGCGACGTG |
| FBP-900F | CGCTCGGATCGACCAATTAC |
| FBP-1084F | TACTTATCCCATCACCAAATGGC |
| FBP-1479F | CACGACATGACCACATTTGAGC |
| FBP-1529F | TGCAGAAGGCCGTAATGC |
| M13R | AGCGGATAACAATTTCACACAGGA |
| FBP-170R | TAAACGGCTGATGGGTGACC |
| FBP-596R | CTTCCAGCATGTCAAACAACTG |
| FBP-720R | ATACATACTGCCGCGATCC |
| FBP-1070R | GCCCCATTTTAGGCGAAAAG |
| FBP-1306R | CTCTCGACTACCTGATCTGG |
| FBP-1454R | GCCAATAATAAGCCAGCTTATC |

ClustalW 1.8 [23]. The phylogenetic tree was created by the neighbor-joining method of Saitou and Nei [18] and the p-distance measure and pairwise deletion as implemented in the MEGA program [9]. The bootstrap method was employed to determine the statistical confidence of the phylogenetic relationships [5]. A total of 1,000 bootstrap trees were generated for each data set.

**Nucleotide sequence accession numbers.** The sequences obtained in this study are available under GenBank accession numbers AY572931 to AY572950.

## Results and Discussion

The sequences of 1971 nucleotides of the *fbp* gene were determined on both DNA strands for 21 *Lactobacillus* strains, representing the type strain (ATCC 7469) and related strains of *L. rhamnosus,* using primers FBP95MF1 and FBP95MR1. The fragments did not reveal any overlapping or ambiguous peaks, thus indicating the presence of a single gene per genome in these species (not shown). Table 1 indicates that no PCR amplification of *fbp* gene was observed for other species of the *Lactobacillus casei* complex (*L. casei* and *L. zeae*) or strains of *Lactobacillus acidophilus, Streptococcus thermophilus,* and *Escherichia coli.* These results confirm that the primers were specifics for the *fbp* gene sequence of *L. rhamnosus.* The 1,971 nucleotide sequences were translated into 656 amino acid sequences. These sequences displayed similarity with the YydE of *Bacillus subtilis* (55% identity), which is a unique fructose-1,6-bisphosphatase involved in gluconeogenesis showing no significant similarity to other FBPases in protein sequence databases [6]. Completion of the sequencing of the *B. subtilis* genome allowed the *yydE* gene to be identified as *fbp.* The *B. subtilis* FBPase gene might have arisen by convergent evolution independently of other members of the FBPase family [6].

Phylogenetic analysis of the *fbp* putative amino acid sequences of *L. rhamnosus* strains by the neighbor-joining method showed clear distinct positions of this species (Fig. 1). Different sequences from genera exhibiting similarity with the FBPase III of *B. subtilis* were included in this analysis for completeness. The tree shows five major clusters representing *L. rhamnosus* strains, *B. subtilis* and closely related sequences of the *fbp* genes of species of the *Bacillus- Staphylococcus* group, and three other clusters. Two of these corresponding to *Lactococcus-Enterococcus* and *Lactococcus-Streptococcus-Enterococcus* were identified. These results indicate that the amino acid sequence of the *fbp* gene can be used to infer phylogenies between distantly related taxa.

The phylogenetic tree, derived from *fbp* nucleotide sequences showed four clear divisions between strains of *L. rhamnosus* (Fig. 2). Synonymous nucleotide mutations, which may occur in the *fbp* gene without modifying the translated product but nevertheless confer a higher degree of variability of nucleotide *fbp* sequences between species, may explain the difference between phylogenetic trees (Fig. 1, 2). High bootstrap values were obtained for the *fbp* sequences of identical clinical and reference strains. Clinical strains 61874-2 and 42259 were identical whereas 25552 and 76933-6 exhibited different nucleotide sequences. *L. rhamnosus* ATCC 53103 (strain GG) and ATCC 8530 were grouped in the same cluster as strain 25552. The other reference and probiotic strains of *L. rhamnosus* comprised two different clusters.

The resolution powers of gene sequences of *fbp* were compared with those of the corresponding putative amino acid sequences. The putative amino acid sequences share very high identity, so the lengths of branches in the amino acid tree are practically null (Fig. 1). Percentage identity values for the nucleotide (upper right) and amino acid (lower left) sequences are given in Table 3. Identity values for gene sequences are variable, while little variability was observed for amino acid sequences. Comparative analysis of gene sequences and those of the corresponding amino acids indicates that nearly all nucleotide mutations are synonymous substitutions. A total of 14 nucleotide substitutions resulted in amino acid replacements. Eight nucleotide substitutions occurred for clinical isolates of *L. rhamnosus* and strain ATCC 53103, which produced the following amino acid replacements for strain 25552: lysine instead of phenylalanine at amino acid position 50, methionine instead of valine at position 206, valine instead of arginine at position 336; for strain 76933-6: alanine instead of aspartic acid at position 435, aspartic acid instead of asparagine at position 560, valine instead of alanine at position 608; and for strains 25552 and ATCC 53103: threonine in-

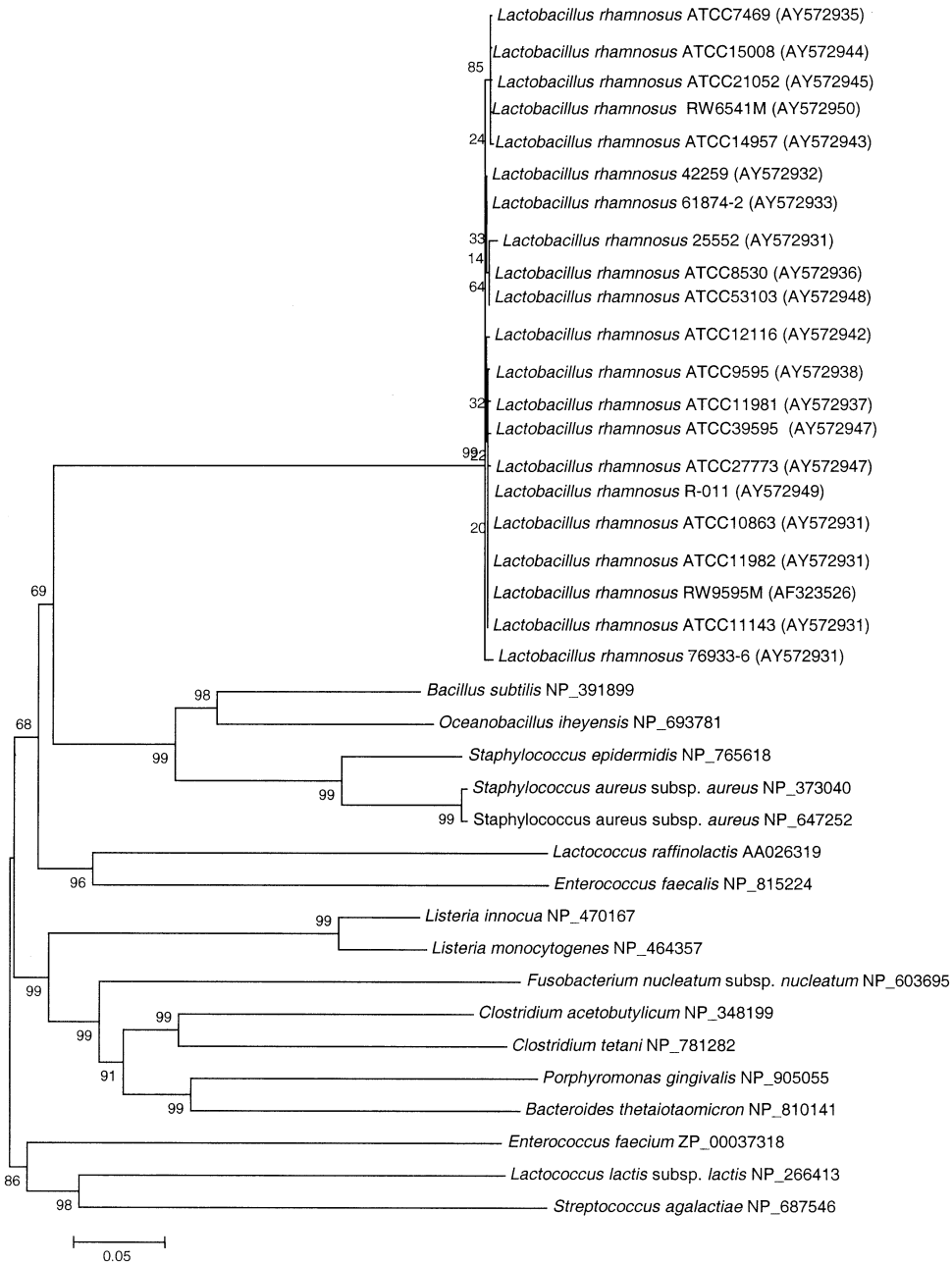


Fig. 1. Neighbor-joining tree, showing the phylogenetic relationships between bacteria based on a comparison of 695 *fbp* amino acid sequences. Bootstrap values were based on 1000. The bar represents 5% sequence divergence. GenBank accession numbers are given in parentheses.

stead of alanine at position 104. Finally, proline replaced glutamic acid in all clinical strains and ATCC 53103 at position 384.

The profiles of genomic DNA from *L. rhamnosus* strains obtained after digestion with the restriction enzymes *Apa*I and *Sma*I gave similar genotypings to those obtained with the nucleotide sequences of *fbp* (Table 4). The strains of clinical origin show distinct profiles which make it possible to differentiate them. *L. rhamnosus* ATCC 53103 (strain GG) was regrouped with the other clinical strains.

From a taxonomic point of view, our results confirm for the first time that *fbp* gene sequences have high discriminating power for strains of *L. rhamnosus* that are difficult to differentiate. At present, the most reliable method for the typing of a strain is the PFGE profile. In light of our results, the *fbp* gene can be proposed as a new method for inferring relationships among very

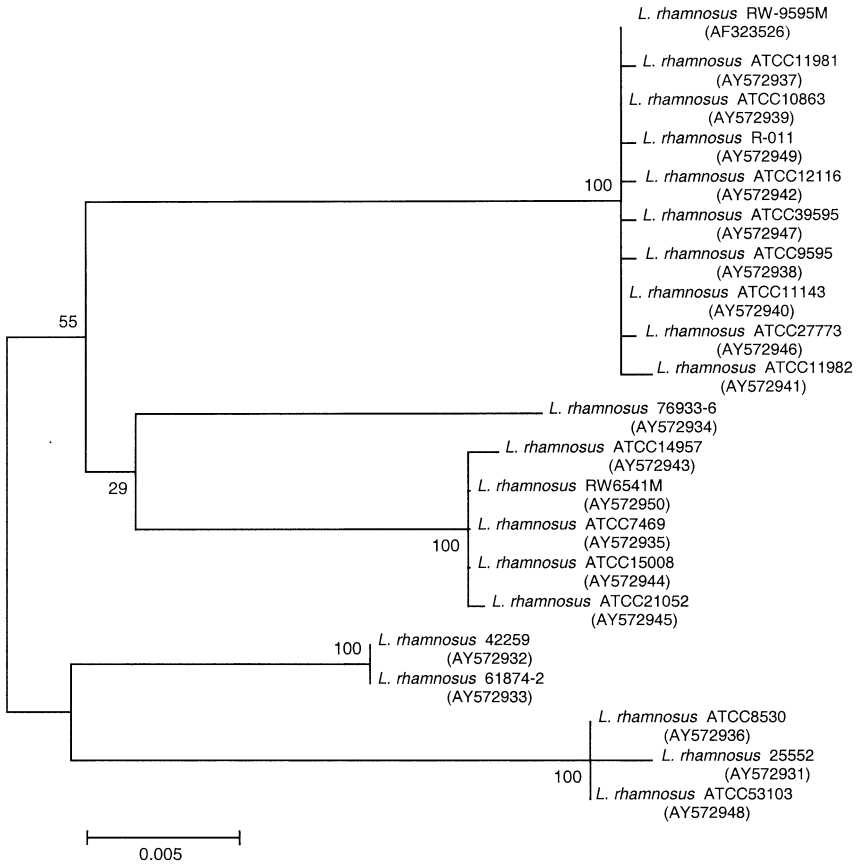


Fig. 2. Neighbor-joining tree, showing the phylogenetic relationships between *Lactobacillus rhamnosus* strains based on a comparison of 1971 *fbp* nucleotide sequences. Bootstrap values were based on 1000 replications. The bar represents 0.5% sequence divergence. GenBank accession numbers are given in parentheses.

Table 3. Identity values calculated for 1,971 bp nucleotide sequences of *fbp* gene sequences and putative 656 residue amino acid sequences

| | Identity (%)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | *L. rhamnosus* ATCC 7469$^T$ | *L. rhamnosus* 25552Cli | *L. rhamnosus* 42259Cli | *L. rhamnosus* 61874-2Cli | *L. rhamnosus* 76933-6Cli | *L. rhamnosus* ATCC 9595 | *L. rhamnosus* ATCC 53103 | *L. rhamnosus* RW-9595M |
| *L. rhamnosus* ATCC 7469$^T$ | | **97** | **96** | **96** | **97** | **96** | **97** | **96** |
| *L. rhamnosus* 25552Cli | 99 | | **96** | **97** | **96** | **95** | **99** | **95** |
| *L. rhamnosus* 42259Cli | 99 | 99 | | **99** | **96** | **96** | **97** | **96** |
| *L. rhamnosus* 61874-2Cli | 99 | 99 | 100 | | **96** | **96** | **97** | **96** |
| *L. rhamnosus* 76933-6Cli | 99 | 98 | 99 | 99 | | **96** | **96** | **96** |
| *L. rhamnosus* ATCC 9595 | 99 | 99 | 99 | 99 | 99 | | **95** | **99** |
| *L. rhamnosus* ATCC 53103 | 99 | 100 | 99 | 99 | 99 | 99 | | **95** |
| *L. rhamnosus* RW-9595M | 99 | 99 | 99 | 99 | 99 | 99 | 99 | |

[a] Identity values are shown for *fbp* gene sequences (bold face) and amino acid sequences.

Table 4.Abilities of nucleotide *fbp* gene sequences and PFGE to differentiate *Lactobacillus rhamnosus* strains

| Bacterial strain | Genotype by: | |
|---|---|---|
| | nt-*fbp* | PFGE[a] |
| **L. rhamnosus** | | |
| RW-9595M | F1 | P1 |
| ATCC 11981 | F1 | P1 |
| ATCC 10863 | F1 | P1 |
| R-011 | F1 | P4 |
| ATCC 12116 | F1 | P1 |
| ATCC 39595 | F1 | P1 |
| ATCC 9595 | F1 | P1 |
| ATCC 11143 | F1 | P1 |
| ATCC 27773 | F1 | P1 |
| ATCC 11982 | F1 | P1 |
| 76933-6 | F2 | P2 |
| ATCC 14957 | F3 | P3 |
| RW-6541M | F3 | P4 |
| ATCC 7469 | F3 | P1 |
| ATCC 15008 | F3 | P4 |
| ATCC 21052 | F3 | P4 |
| 42259 | F4 | P5 |
| 61874-2 | F4 | P5 |
| ATCC 8530 | F5 | P1 |
| 25552 | F5 | nd |
| ATCC 53103 | F5 | P6 |

nd: not determined.
[a] Combines the separate results obtained with *Apa*I and *Sma*I.

closely related strains of *L. rhamnosus. fbp* gene analysis can have a double potential as a phylogenetic marker: its amino acid sequence can be used to infer phylogenies between distantly related taxa and the nucleotide sequence can be used to evaluate taxonomic positions of probiotic and clinical strains of this species. At the nucleotide level, mutations on the *fbp* gene do not or only slightly alter its product. Many of the differences observed in DNA sequences among species were silent in terms of their effects on the encoded amino acid sequences. Most of the reference and probiotic strains exhibited identical protein sequences whereas the encoding DNAs of clinical isolates and GG strain exhibited significant divergence.

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## Literature Cited

1. Asahara T, Takahashi M, Nomoto K, Takayama H, Onoue M, Morotomi M, et al. (2003) Assessment of safety of *Lactobacillus* strains based on resistance to host innate defense mechanisms. Clin Diagn Lab Immunol 10:169–173
2. Chavagnat F, Haueter M, Jimeno J, Casey MG (2002) Comparison of partial *tuf* gene sequences for the identification of lactobacilli. FEMS Microbiol Lett 217:177–183
3. Donahue JL, Bownas JL, Niehaus WG, Larson TJ (2000) Purification and characterization of glpX-encoded fructose 1,6-bisphosphatase, a new enzyme of the glycerol 3-phosphate regulon of *Escherichia coli.* J Bacteriol 182:5624–5627
4. Felis GE, Dellaglio F, Mizzi L, Torriani S (2001) Comparative sequence analysis of a recA gene fragment brings new evidence for a change in the taxonomy of the *Lactobacillus casei* group. Int J Syst Evol Microbiol 51:2113–2117
5. Felsenstein J (1985) Confidence limit on phylogenies: An approach using bootstrap. Evolution 39:783–791
6. Fujita Y, Yoshida K, Miwa Y, Yanai N, Nagakawa E, Kasahara Y (1998) Identification and expression of the *Bacillus subtilis* fructose-1,6-bisphosphatase gene (*fbp*). J Bacteriol 180:4309–4313
7. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S (1992) Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. Dig Dis Sci 37:121–128
8. Groisillier A, Lonvaud-Funel A (1999) Comparison of partial malolactic enzyme gene sequences for phylogenetic analysis of some lactic acid bacteria species and relationships with the malic enzyme. Int J Syst Bacteriol 49:1417–1428
9. Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245
10. Mackay AD, Taylor MB, Kibbler CC, Hamilton-Miller JM (1999) *Lactobacillus* endocarditis caused by a probiotic organism. Clin Microbiol Infect 5:290–292
11. Presterl E, Kneifel W, Mayer HK, Zehetgruber M, Makristathis A, Graninger W (2001) Endocarditis by *Lactobacillus rhamnosus* due to yogurt ingestion? Scand J Infect Dis 33:710–714
12. Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkynen S, et al. (1999) Liver abscess due to a *Lactobacillus rhamnosus* strain indistinguishable from *L. rhamnosus* strain GG. Clin Infect Dis 28:1159–1160
13. Rittmann D, Schaffer S, Wendisch VF, Sahm H (2003) Fructose-1,6-bisphosphatase from *Corynebacterium glutamicum:* Expression and deletion of the *fbp* gene and biochemical characterization of the enzyme. Arch Microbiol 180:285–292
14. Roy D, Ward P, Champagne G (1996) Differentiation of bifidobacteria by use of pulsed- field gel electrophoresis and polymerase chain reaction. Int J Food Microbiol 29:11–29
15. Roy D, Ward P, Vincent D, Mondou F (2000) Molecular identification of potentially probiotic lactobacilli. Curr Microbiol 40: 40–46
16. Roy D, Sirois S, Vincent D (2001) Molecular discrimination of lactobacilli used as starter and probiotic cultures by amplified ribosomal DNA restriction analysis. Curr Microbiol 42:282–289
17. Roy D, Ward, P, Vincent D (1999) Strain identification of probiotic *Lactobacillus casei*-related isolates with randomly amplified polymorphic DNA and pulsed-field gel electrophoresis methods. Biotechnol Techn 13:843–847
18. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
19. Salminen MK, Tynkkynen S, Rautelin H, Saxelin M, Vaara M, Ruutu P, et al. (2002) *Lactobacillus* bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. Clin Infect Dis 35:1155–1160
20. Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, et al. (2004) *Lactobacillus* bacteremia, clinical significance, and patient outcome, with special focus on probiotic *L. rhamnosus* GG. Clin Infect Dis 38:62–69

21. Satokari RM, Vaughan EE, Smidt H, Saarela M, Matto J, de Vos WM (2003) Molecular approaches for the detection and identification of bifidobacteria and lactobacilli in the human gastrointestinal tract. Syst Appl Microbiol 26:572–584
22. Sedivy JM, Daldal F, Fraenkel DG (1984) Fructose bisphosphatase of *Escherichia coli:* Cloning of the structural gene (*fbp*) and preparation of a chromosomal deletion. J Bacteriol 158:1048–1053
23. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
24. Torriani S, Felis GE, Dellaglio F (2001) Differentiation of *Lactobacillus plantarum, L. pentosus,* and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with recA gene-derived primers. Appl Environ Microbiol 67:3450–3454
25. Tynkkynen S, Satokari R, Saarela M, Mattila-Sandholm T, Saxelin M (1999) Comparison of ribotyping, randomly amplified polymorphic DNA analysis, and pulsed-field gel electrophoresis in typing of *Lactobacillus rhamnosus* and *L. casei* strains. Appl Environ Microbiol 65:3908–3914
26. Verhees CH, Akerboom J, Schiltz E, de Vos WM, van der Oost J (2002) Molecular and biochemical characterization of a distinct type of fructose-1,6-bisphosphatase from *Pyrococcus furiosus.* J Bacteriol 184:3401–3405
27. Vincent D, Roy D, Mondou F, Dery C (1998) Characterization of bifidobacteria by random DNA amplification. Int J Food Microbiol 43:185–193