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Isolation of Potential Probiotic *Lactobacillus rhamnosus* Strains from Traditional Fermented Mare Milk Produced in Sumbawa Island of Indonesia

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Isolation of Potential Probiotic *Lactobacillus rhamnosus* Strains from Traditional Fermented Mare Milk Produced in Sumbawa Island of Indonesia

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To explore potential probiotics in the traditional foods of Indonesia, fermented mare milk produced in Sumbawa Island was investigated in this study. Gram stain, catalase activity, gas production, cell morphology, carbohydrate utilization pattern, and 16S rDNA sequencing were performed to identify isolated lactic acid bacteria. To assess their probiotic ability, tolerance of low pH, bile salts, artificial gastrointestinal fluids, and adhesion properties to extracellular matrices, were examined. In total 27 strains, 25 *Lactobacillus rhamnosus* and two *Lactobacillus fermentum*, were obtained. Among the isolated lactobacilli, three *Lb. rhamnosus* strains, FSMM15, FSMM22, and FSMM26, were selected as candidates for probiotics, using *Lb. rhamnosus* GG as index. *In vitro* binding assay of the three strains against several extracellular matrix proteins revealed that FSMM15 and FSMM26 gave greater binding ratios of mucin/bovine serum albumin (BSA) and significantly higher adhesive abilities to fibronectin than *Lb. rhamnosus* GG. FSMM22 showed significantly higher adhesion to laminin than *Lb. rhamnosus* GG.

Key words: *Lactobacillus rhamnosus*; laminin adhesive ability; probiotic properties; Sumbawa mare; traditional fermented milk

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host.[1)] They can reach the gastrointestinal (GI) tract alive, and exhibit their health promoting effects in the host, even though they colonize the GI tract only temporarily. Hence their ability to adhere constituents of the GI mucosal layer such as mucin[2)] and also to extracellular matrix (ECM) components including fibronectin,[3)] laminin,[4)] and collagen[4)] is a key function of probiotics in promoting beneficial health effects, as well as their antibacterial[5)] and immunomodulatory[6)] activities.

Probiotics can protect the host defensive mechanism against pathogenic infection in the gut lumen. Rapid formation of microbial communities is considered to reduce pH and to compete with pathogenic bacteria for adhesion sites, resulting in prevention of pathogenic colonization.[7)] Secretion of antibacterial substances, *e.g.*, acetate[8)] and bacteriocin,[9)] also prevents the growth of pathogens. Some lactobacilli[10)] and probiotic *Escherichia coli*[11)] stimulate human intestinal barrier functions through induction of epithelial β-defensin.

Several glycoproteins are localized on the surface of the basement membrane (BM), a thin layer surrounding epithelial tissues, nerves, fat cells, and muscles.[12,13)] These including laminin, type IV collagen, perlecan, and entactin/nidogen which assemble into fibrils or other complex macromolecular arrays. Their bind ability to adhesion receptors enables a tight association with the cell surface.[14)] They are frequently targeted by pathogenic bacteria that express surface proteins with affinity for ECM proteins.[15–17)] In this context, the ability to adhere ECM proteins expressed on the surface of BM is one of the most important criteria in selecting probiotics, which potentially interfere with infection pathogenic bacteria in the GI tract.[18)]

Fermented dairy products are believed to be promising sources of probiotics because of their history in the human diet and functionality. From *dadih*, a traditional Indonesian fermented buffalo milk, *Lactococcus lactis* IS-16183 and *Lb. rhamnosus* IS-7257 were isolated as potential probiotics inhibiting the adhesion of *E. coli* O157:H7 to human mucin *in vitro*.[19)] *Lb. casei* Zhang, *Lb. helveticus* ZL12-1, and *Lb. plantarum* BX6-6 were isolated from *koumiss*, a traditional fermented alcoholic beverage prepared from mare milk in Inner Mongolia, as showing antimicrobial activities.[20)] *Lb. salivarius*, *Lb. buchneri*, and *Lb. plantrum* I were also found in *koumiss* by Danova *et al.*[21)] *Lb. paracasei* UI14 and *Weissella*

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Abbreviations: ATCC, American Type Culture Collection; BM, basement membrane; BSA, bovine serum albumin; CFU, colony forming unit; DDBJ, DNA Data Bank of Japan; ECM, extracellular matrix; FSMM, fermented Sumbawa mare milk; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG ATCC53103; MRS, de Man-Rogosa-Sharpe; PBS, phosphate-buffered saline; PCR, polymerase chain reaction

confusa UI7 were isolated from whey and cheese respectively from Nigerian cow's milk.[22] Furthermore, *Lb. acidophilus* E2 and *Lb. casei* G12 were selected as potential probiotics from traditional fermented yak milk.[23]

Mare milk is traditionally utilized as a dairy product in Central Asia, Mongolia, and the former Soviet Union, where it provides a critical nutritional source.[24] In addition, Sumbawa Island, located in the middle of the Lesser Sunda Islands of Indonesia, is a production area for mare milk and fermented products of it, which are believed to possess beneficial functionalities. Literature on Sumbawa mare milk and its fermented products is scarce, although it is an attractive resource for exploring novel beneficial compounds and microorganisms. For example, Sujaya *et al.* isolated *Lb. rhamnosus* SKG34 and SKG49, which showed tolerance of acidic conditions at pH 2 and 3, from raw milk of the Sumbawa mare,[25] but nothing has been reported to date on the isolation of lactobacilli from fermented Sumbawa mare milk (FSMM). In this study, we performed screening of potential probiotics from FSMM, focusing mainly on lactobacilli, in relation to their ability to adhere the colonic mucin and two ECM proteins, including fibronectin and laminin. To our knowledge, this is the first report of the isolation of potential probiotic *Lb. rhamnosus* strains from FSMM produced in Indonesia.

Materials and Methods

Sample collection. Fermented mare milk (approximately 300 mL) was donated by a farmer on Sumbawa Island of Indonesia. Mature mare milk was collected manually in plastic containers in December 2010, and then kept at ambient temperature for 1 week at the farm, which allowed spontaneous fermentation by microorganisms that persisted from previous fermentation in the same container, without any additives. After fermentation, the fermented milk was transported at ambient temperature to Udayana University in Bali and immediately subjected to procedures to isolate lactobacilli.

Isolation of lactobacilli from FSMM. A 100 µL-aliquot of FSMM were mixed with 5 mL of de Man-Rogosa-Sharpe (MRS; Oxoid, Basingstoke, UK) broth and incubated statically at 37 °C for 48 h under aerobic conditions. A 10-fold dilution series (10^2–10^3) of the mixtures was performed to give 30–300 of colony forming units (CFUs), and each diluted mixture was spread on MRS agar plates supplemented with 1.1 mM bromocresol purple, and was incubated anaerobically at 37 °C for up to 48 h.[26] Single yellow colonies were randomly selected from the MRS agar plates, transferred into test tubes containing 5 mL of MRS broth, and incubated statically at 37 °C for 24 h under aerobic conditions. The culture broth was again subjected to dilution and was streaked onto MRS agar plates for purification. Single colony isolation was performed, and the resulting pure isolates were stored at 4 °C in stab agar or at −80 °C in 30% glycerol for further investigation. Gram staining properties, catalase activity, gas production with glucose as carbon source, and cell morphology were confirmed for initial characterization of all isolates.[27,28]

Identification of the isolated lactobacilli. The isolated lactobacilli were characterized by carbohydrate utilization test using an API 50 CH kit (bioMérieux, Marcy I'Etoile, France) following the manufacturer's instructions, and by 16S rDNA sequence analysis.[29] Two type strains, *Lb. fermentum* American Type Culture Collection (ATCC) 14931^T and *Lb. rhamnosus* Japan Collection of Microorganisms (JCM) 1136^T, and *Lb. rhamnosus* GG ATCC53103 (LGG), one of the most intensively investigated probiotics, isolated from the GI tract of healthy humans,[30] were used as controls. The isolates were grown in 5 mL of MRS broth at 30 °C or 37 °C for 24 h. One mL of the broth culture was centrifuged at 12,000 × *g* for 5 min at 4 °C, and then the cells mass was washed twice with phosphate-buffered saline (PBS). The cell mass was resuspended in PBS, and then an aliquot of the culture broth was mixed with API CHL medium to give the equivalent of 2 McFarland turbidity standard. Inoculated medium (100 µL) was applied to the API strips and covered with mineral oil. Fermentation was observed after incubation for 24 and 48 h anaerobically at 30 °C and 37 °C. Carbohydrates fermentation profiles were analyzed by Apiweb (https://apiweb.biomerieux.com/servlet/Authenticate?action=prepareLogin).

Genetic identification was done by sequencing of the variable area, V1–V3, of the 16S rDNA.[31] The isolates were amplified by colony-polymerase chain reaction (PCR) using 16S_27F (5′-AGAGTTTGA-TCCTGGCTCAG-3′) and 16S_520R (5′-ACCGCGGCTGCTGGC-3′) as universal primers.[29] ExTaq DNA polymerase (Takara Bio, Ohtsu, Japan) was used following the manufacturer's instructions. PCR reaction was performed as follows: first denaturation at 95 °C for 2 min, 35 cycles of 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 30 s (extension), and the final extension at 72 °C for 7 min. After colony-PCR amplification, the PCR products were purified using a GenElute PCR clean-up kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. Sequencing reaction was performed using approximately 40 ng of the amplicon as template, 16S_27F as primer, and a BigDye terminator v1.1 cycle sequencing ready reaction mix kit (Life Technologies, Carlsbad, CA) following the manufacturer's instructions. The reaction products were purified by ethanol precipitation and analyzed using an automated 310 DNA sequencer (Life Technologies). Identification to the species level was defined as 16S rDNA sequence similarity with the prototype strain sequences in the DNA Data Bank of Japan (DDBJ) and the National Center for Biotechnology Information. When the two databases gave different results, identification was done by the criteria of Dobson *et al.*[32]

Tolerance of low pH and bile salts of the isolated lactobacilli. The isolated strains were tested for their ability to resist low pH and bile salts. LGG was used as control. Because the pH value of gastric acid varies in a range of about 1.5–4.5 over a period of 2 h depending on a food's entering time and the gastric contents,[33] the pH value of the MRS broth was adjusted to 2.0, 3.0, and 4.0 by 1 M HCl. Cells were pre-cultured in 5 mL of MRS broth at 37 °C for 18 h, and then a 1-mL aliquot of the culture broth was harvested by centrifugation at 15,000 × *g* for 5 min and washed twice with PBS. The cells were suspended in 100 µL of PBS and incubated in 5 mL of fresh MRS broth at various pHs at 37 °C for 3 h. After incubation, 50 µL of the culture broth was appropriately diluted with PBS and then streaked on MRS agar plates. Viable cell numbers were counted after anaerobic incubation at 37 °C for 36 h. Tolerance of bile salts was verified by inoculating 100 µL of cells pre-cultured for 18 h into 5 mL of MRS broth containing 0.3, 0.5, and 1% bile salts (Oxoid). After 4 h of incubation at 37 °C, viable cells were counted as described above.

Transit tolerance of the isolated lactobacilli as against artificial gastric and intestinal fluids. The transit tolerance of the isolated strains as against simulated gastric and intestinal fluids was tested as described by Fernández *et al.*,[34] with minor modifications. LGG was used as control. One mL of 18-h culture-broth was harvested by centrifugation at 15,000 × *g* for 5 min at 4 °C, washed with sterilized PBS, and suspended in 100 µL of PBS. The cell suspension was added to 900 µL of artificial gastric fluid (125 mM NaCl, 7 mM KCl, 45 mM $NaHCO_3$, 3 g/L pepsin, pH 2 and 3 adjusted with 1 M HCl). The bacterial suspensions were incubated at 37 °C for up to 180 min with agitation (160 rpm). Aliquots of the mixture (50 µL) were taken at 0 and 180 min of incubation, an appropriate dilution of the aliquot was streaked on MRS agar plates, and then this was incubated at 37 °C for 36 h under anaerobic conditions, followed by counting of viable cells. The resistance of the strains to intestinal fluids was determined as follows: The resting cell suspension, exposed to artificial gastric fluid for 180 min, was centrifuged at 15,000 × *g* for 5 min at 4 °C. The cells were washed using PBS buffer and then resuspended into 850 µL of simulated intestinal fluid (0.1% pancreatin, Sigma-Aldrich, 0.15% bile salts, pH 8.0 adjusted with 1 M NaOH). The suspension was incubated anaerobically at 37 °C with agitation (160 rpm) for 180 min. After incubation, a 50-µL aliquot was subjected to counting of viable cells as described above.

Adhesion properties of the isolated lactobacilli to two ECM proteins. Out of 27 isolated lactobacilli, 14 strains which were reculturable from the stab agar transferred from Indonesia to Japan were checked for their adherence to several ECM proteins. LGG was used as control. Porcine colonic mucin was purified from the porcine large intestine by gel filtration and density-gradient centrifugation by the method of Kodaira *et al.*[35] Other ECM proteins, fibronectin and laminin, were purchased from Collaborative Biomedical Products (Bedford, MA) and Upstate Biotechnology (Lake Placid, NY) respectively. The ECM proteins with bovine serum albumin (BSA, Sigma-Aldrich) as negative control were immobilized on a 96-well plate as follows: Each 1 mg/mL of mucin, 100 nM fibronectin, 100 nM laminin, and 1 mg/mL BSA in 50 mM phosphate buffer (pH 7.5) was incubated at 4 °C for 12 h, and then blocking was done with 1.0% BSA in PBS at ambient temperature for 1 h. Each of the isolated strains and LGG grown in MRS broth at 37 °C until the absorbance at 600 nm reached 1.0 was washed twice with PBS, and then 100 µL of bacterial suspension (1×10^9 CFU/mL) was applied to the ECMs-coated 96-well plate. After incubation at 37 °C for 1 h, these were washed 3 times with 0.1% BSA in PBS. Attached cells were collected with 100 µL of 0.01% Triton X-100 in PBS. The suspensions were plated on MRS agar to determine numbers of adhering bacteria by colony plate counting.

Statistical analysis. Results were expressed as means and standard deviation. Data for five independent replicates were subjected to one-way analysis of variance (ANOVA) with Dunnett's multiple comparison of means test, using XLSTAT 2011 and Statcel2. A p value of less than 0.05 was regarded as indicating a significant difference.

Results and Discussion

Isolation, characterization, and identification of lactobacilli in FSMM

In total of 27 pure isolates were obtained from the FSMM following the procedures described above. All the isolates were primarily assigned lactobacilli, since they appeared as Gram-positive rods without catalase activity (data not shown). Of the 27 isolates, 24 homofermenter rods and one gas producing isolate were tentatively identified as *Lb. rhamnosus* and *Lb. fermentum* respectively, and two were not clearly assigned to the species level based on their carbohydrate utilization profiles (Tables 1 and 2). For further identification, a homology search using the 16S rDNA sequence was conducted. The sequence data were deposited at DDBJ under consecutive accession nos. AB703579 to AB703605 (Table 2). All the strains, except for the two strains identified as *Lb. fermentum*, were highly similar (97–100% homology) to *Lb. rhamnosus* by database search (Table 2). FSMM9 was *Lb. fermentum* based on high 16S rDNA sequence homology (99%) to *Lb. fermentum* strain VB1 (accession no. JQ073735), although their carbohydrate metabolism patterns were not coincident.

Among traditional fermented milks, *Lb. rhamnosus* has to date been found in *kule naoto* (made from zebu cow's milk in Kenya)[36] and *gariss* (made from camel's milk in Sudan)[37] as minor species, and in *dadih* (made from buffalo's milk in Indonesia)[19,38] as major species together with *Leuconostoc paramesenteroides* and *Lc. lactis* ssp. *lactis*. In the case of FSMM, most of the isolated lactobacilli were *Lb. rhamnosus* under our experimental conditions. These results clearly indicate that mare milk and fermented products of it are not the sole source of *Lb. rhamnosus* among livestock animals. On the other hand, *Lb. rhamnosus* was not isolated from any fermented milks of cow, yak, goat, or mare in Mongolia[39] or from *koumiss*, a low alcohol beverage made from mare's or camel's milk in Central Asia.[21] Therefore, the presence or predominance of *Lb. rhamnosus* strains in the traditional fermented dairy products is likely to stem from environmental factors such as temperature favorable to growth. Most of the lactobacilli isolated from FSMM metabolize neither sucrose nor melibiose (Table 1). The latter can be obtained only by invertase-catalyzed hydrolysis of raffinose, which distributed widely in the plant world,[40] and hence most is lactobacilli in FSMM are not likely to have originated in plants.

Probiotic properties of lactobacilli isolated from FSMM

The tolerance of all the lactobacilli isolates of an acidic environment was found to be similar to that of LGG. A continuous decrease in cell viability was observed for all the strains at lower pH, but they maintained more than 10^6 CFU/mL at pH 2 (Table 3). In the bile test, all the isolates except for FSMM23 showed resistance at varied concentrations (0.3%–1.0%) of bile salts. In general, the survival rate was constant with increasing concentrations of bile salts, but FSMM1, FSMM19, and FSMM20 showed a tendency to decrease in viable cell numbers (Table 3). LGG proliferated for 3 h in the artificial gastric fluid at pH 3. In contrast, none of the FSMM isolates grew under the same conditions, although they maintained high cell viability. FSMM strains showing values not greater than 0.8 of the CFU mean ratio (b/a in Table 3) of the artificial gastric fluid treatment were eliminated from selection. Subsequent treatment with artificial intestinal fluid damaged LGG, giving a CFU ratio of 0.8. FSMM11 and FSMM26 exhibited cell survival rates comparable to LGG, but most of the FSMM strains were severely damaged by this treatment. In selection, strains giving the CFU ratio of less than 0.7 were eliminated. Consequently, FSMM2, FSMM8, FSMM11, FSMM15, FSMM21, FSMM22, FSMM25, and FSMM26 were selected as probiotic candidates in terms of cell viability in the artificial GI fluids. It was confirmed that no lactobacilli strains, including LGG, proliferated after exposure to the artificial gastric and intestinal fluids at pH 2 by measurement of the absorbance of the culture media at a wavelength of 660 nm (data not shown).

All 14 lactobacilli strains subjected to adhesion assay against several ECM proteins and LGG exhibited adhesive properties against colonic mucin of varied strength (Fig. 1). The adhesive properties of FSMM6, FSMM15, FSMM22, FSMM24, and FSMM26 as to the colonic mucin were comparable to that of LGG. Except for FSMM22, all of these strains showed significantly lower adhesiveness against BSA than LGG. The adhesive properties of the FSMM strains as to fibronectin and laminin were also various (Fig. 1). When the adhesive properties of the FSMM strains as to fibronectin were compared with LGG, FSMM1, FSMM2, FSMM4, FSMM6, FSMM10, FSMM11, and FSMM20 showed significantly lower; FSMM5, FSMM9, FSMM22, and FSMM24 were similar; and FSMM15, FSMM21, and FSMM26 were significantly higher. Except for FSMM2, FSMM9, FSMM11, and FSMM15, the lactobacilli strains showed good adhesion to laminin,

Table 1. Carbohydrate Utilization Patterns of Two LAB Type Strains and Strains Isolated from FSMM

Carbohydrates	Type strains		Isolated strains from FSMM																										
	Rha	Fer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
CTRL	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−
GLY	±	−	±	±	−	−	±	±	±	±	±	±	±	−	±	±	+	±	±	±	+	±	±	±	−	±	±	±	±
ERY	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-ARA	−	−	±	±	±	±	±	±	±	±	±	+	+	±	±	±	+	±	±	±	+	±	±	±	−	±	±	±	±
L-ARA	−	−	−	−	±	±	−	−	−	−	±	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−
D-RIB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-XYL	±	−	−	−	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L-XYL	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-ADO	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Mβ DX	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-GAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-FRU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+
D-MNE	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	+	+	±	+	+	+	+
L-SBE	+	−	−	−	±	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−
L-RHA	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
DUL	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
INO	±	−	±	±	±	±	±	±	−	±	±	−	±	±	±	±	−	±	±	±	−	−	±	±	−	±	±	±	±
D-MAN	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
D-SOR	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
MαDM	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±
MαDG	+	−	−	−	±	−	−	±	±	−	−	−	−	±	−	−	−	±	−	±	−	±	±	−	±	−	±	±	±
NAG	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+
AMY	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
ARB	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
ESC	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
SAL	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	+	+	−	+	+	+	+
D-CEL	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
D-MAL	+	+	±	±	−	±	−	±	−	±	±	±	±	±	±	±	−	±	−	±	−	±	±	±	+	±	±	±	±
D-LAC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	+	+	±	+	+	+	+
D-MEL	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
D-SUC	±	+	±	−	−	±	−	±	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	±	+	±	±	−	±
D-TRE	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+
INU	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−
D-MLZ	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	±	+	+	+	+
D-RAF	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Starch	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GLG	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XLT	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GEN	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
D-TUR	+	−	±	−	−	±	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−
D-LYX	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-TAG	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
D-FUC	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L-FUC	−	−	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
D-ARL	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L-ARL	−	−	+	+	−	+	+	+	+	+	+	+	+	±	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+
GNT	±	+	±	±	−	−	±	+	±	+	+	+	+	±	−	+	±	±	+	+	±	±	±	+	−	+	±	±	+
2KG	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−
5KG	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−

Rha, *Lb. rhamnosus* JCM1136^{T}; Fer, *Lb. fermentum* ATCC14931^{T}.

CTRL, control; GLY, glycerol; ERY, erythritol; D-ARA, D-arabinose; L-ARA, L-arabinose; D-RIB, D-ribose; D-XYL, D-xylose; L-XYL, L-xylose; D-ADO, D-adonitol; Mβ DX, methyl-β-D-xylopyranoside; D-GAL, D-galactose; D-GLU, D-glucose; D-FRU, D-fructose; D-MNE, D-mannose; L-SBE, L-sorbose; L-RHA, L-rhamnose; DUL, dulcitol; INO, inositol; D-MAN, D-mannitol; D-SOR, D-sorbitol; MαDM, methyl-α-D-mannopyranoside; MαDG, methyl-α-D-glucopyranoside; NAG, *N*-acetyl glucosamine; AMY, amygdalin; ARB, arbutin; ESC, esculin ferric citrate; SAL, salicin; D-CEL, D-cellobiose; D-MAL, D-maltose; D-LAC, D-lactose; D-MEL, D-melibiose; D-SUC, D-sucrose; D-TRE, D-trehalose; INU, inulin; D-MLZ, D-melezitose; D-RAF, D-raffinose; GLG, glycogen; XLT, xylitol; GEN, gentiobiose; D-TUR, D-turanose; D-LYX, D-lyxose; D-TAG, D-tagatose; D-FUC, D-fucose; L-FUC, L-fucose; D-ARL, D-arabitol; L-ARL, L-arabitol; GNT, gluconate; 2KG, 2-keto gluconate; 5KG, 5-keto gluconate.

+, positive; −, negative; ±, undetermined.

and FSMM22 showed highest adhesion, significantly higher than LGG.

Finally, three strains, FSMM15, FSMM22, and FSMM26, were selected as comparable to LGG in respect to their abilities *in vitro* to survive under artificial GI conditions and to adhere to colonic mucin (Fig. 1 and Table 3). Among these, FSMM15 and FSMM26 are likely to be advantageous for colonization of the intestinal tract, because they showed higher binding ratios of mucin/BSA than LGG (Fig. 1). Probiotics that can adhere to ECM proteins are assumed to interfere competitively with infection by enteric pathogens in that the pathogens also bind to ECM proteins.[18] We found that FSMM15/26 and FSMM22 show

Table 2. Identification of Lactobacilli Strains Isolated from FSMM

Strains (accession no.)	API identification	16S rDNA identification	Sequence length[a)] (bp)	Homology[b)] (%)	Closely related strains (accession no.)
FSMM1 (AB703579)	*Lb. rhamnosus*	*Lb. rhamnosus*	416	99	*Lb. rhamnosus* GYB9 (AF375918)
FSMM2 (AB703580)	*Lb. rhamnosus*	*Lb. rhamnosus*	413	98	*Lb. rhamnosus* JSW10 (AF375896)
FSMM3 (AB703581)	*Lb. rhamnosus*	*Lb. rhamnosus*	370	98	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM4 (AB703582)	*Lb. rhamnosus*	*Lb. rhamnosus*	450	99	*Lb. rhamnosus* MAB22 (AF375897)
FSMM5 (AB703583)	*Lb. rhamnosus*	*Lb. rhamnosus*	442	97	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM6 (AB703584)	*Lb. rhamnosus*	*Lb. rhamnosus*	445	100	*Lb. rhamnosus* NBRC14710 (AB680649)
FSMM7 (AB703585)	*Lb. rhamnosus*	*Lb. rhamnosus*	371	98	*Lb. rhamnosus* ATCC7469 (AB008211)
FSMM8 (AB703586)	*Lb. rhamnosus*	*Lb. rhamnosus*	462	100	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM9 (AB703587)	*Lb. rhamnosus*	*Lb. fermentum*	449	99	*Lb. fermentum* VB1 (JQ073735)
FSMM10 (AB703588)	*Lb. rhamnosus*	*Lb. rhamnosus*	373	99	*Lb. rhamnosus* LrJ3 (HQ418482)
FSMM11 (AB703589)	*Lb. rhamnosus*	*Lb. rhamnosus*	455	100	*Lb. rhamnosus* RB4 (AF375898)
FSMM12 (AB703590)	*Lb. rhamnosus*	*Lb. rhamnosus*	443	100	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM13 (AB703591)	*Lb. rhamnosus*	*Lb. rhamnosus*	454	99	*Lb. rhamnosus* X211 (JN415185.1)
FSMM14 (AB703592)	*Lb. rhamnosus*	*Lb. rhamnosus*	419	100	*Lb. rhamnosus* X211 (JN415185.1)
FSMM15 (AB703593)	N.I.	*Lb. rhamnosus*	331	99	*Lb. rhamnosus* LrJ3 (HQ418482)
FSMM16 (AB703594)	*Lb. rhamnosus*	*Lb. rhamnosus*	413	99	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM17 (AB703595)	*Lb. rhamnosus*	*Lb. rhamnosus*	403	99	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM18 (AB703596)	*Lb. rhamnosus*	*Lb. rhamnosus*	445	97	*Lb. rhamnosus* LrJ3 (HQ384288)
FSMM19 (AB703597)	N.I.	*Lb. rhamnosus*	419	97	*Lb. rhamnosus* Lr18 (HQ418480)
FSMM20 (AB703598)	*Lb. rhamnosus*	*Lb. rhamnosus*	480	99	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM21 (AB703599)	*Lb. rhamnosus*	*Lb. rhamnosus*	327	98	*Lb. rhamnosus* ChPR-II-str56 (HM462427)
FSMM22 (AB703600)	*Lb. rhamnosus*	*Lb. rhamnosus*	492	99	*Lb. rhamnosus* MAB22 (AF375897)
FSMM23 (AB703601)	*Lb. fermentum*	*Lb. fermentum*	441	100	*Lb. fermentum* NS9 (JQ013298)
FSMM24 (AB703602)	*Lb. rhamnosus*	*Lb. rhamnosus*	378	97	*Lb. rhamnosus* MAB22 (AF375897)
FSMM25 (AB703603)	*Lb. rhamnosus*	*Lb. rhamnosus*	451	100	*Lb. rhamnosus* MAB22 (AF375897)
FSMM26 (AB703604)	*Lb. rhamnosus*	*Lb. rhamnosus*	377	100	*Lb. rhamnosus* ATCC8530 (CP003094)
FSMM27 (AB703605)	*Lb. rhamnosus*	*Lb. rhamnosus*	495	99	*Lb. rhamnosus* 38-180a (HQ697635)

N.I., not identified.
[a)]Sequence length of amplicon for 16S rDNA identification.
[b)]Sequence homology of the strain in the leftmost column against the closely related strain rightmost.

Table 3. Resistance of Isolated Lactobacilli Strains to Low pH, Bile Salt, and Artificial Gastric and Intestinal Fluids

Strains	pH			Bile salt			Artificial gastric fluid			Artificial intestinal fluid		
	2	3	4	0.3%	0.5%	1.0%	0 h (a)	3.0 h (b)	b/a	0 h (a)	3.0 h (b)	b/a
LGG	6.7 ± 0.1	7.6 ± 0.1	7.5 ± 0.3	8.7 ± 0.1	8.7 ± 0.1	8.5 ± 0.2	7.1 ± 0.1	8.3 ± 0.1	1.2	7.4 ± 0.3	5.6 ± 0.1	0.8
FSMM1	6.7 ± 0.2	7.2 ± 0.1	7.5 ± 0.1	8.1 ± 0	7.2 ± 0.2	7.2 ± 0.2	7.7 ± 0.4	6.7 ± 0.4	0.9	6.1 ± 0.6	3.2 ± 0.4	0.5
FSMM2	6.7 ± 0.1	6.9 ± 0.1	7.1 ± 0.2	7.7 ± 0.4	6.8 ± 0.5	7.2 ± 0.2	7.7 ± 0.2	7.0 ± 0.3	0.9	5.5 ± 0.1	3.8 ± 0.1	0.7
FSMM3	6.8 ± 0	7.1 ± 0.3	7.1 ± 0.1	8.0 ± 0.4	7.1 ± 0.1	7.3 ± 0.1	7.6 ± 0.3	7.0 ± 0.3	0.9	6.0 ± 0.3	3.5 ± 0.3	0.6
FSMM4	6.8 ± 0.1	7.0 ± 0.1	7.4 ± 0	7.3 ± 0.1	7.3 ± 0.2	7.6 ± 0.2	7.8 ± 0.1	7.0 ± 0.5	0.9	6.4 ± 0.3	3.7 ± 0.6	0.6
FSMM5	6.8 ± 0.2	6.8 ± 0.1	7.2 ± 0.3	7.3 ± 0.1	7.2 ± 0.4	7.3 ± 0.2	7.7 ± 0.1	6.8 ± 0.2	0.9	5.1 ± 0.6	2.2 ± 1.8	0.4
FSMM6	6.5 ± 0.3	6.7 ± 0.2	7.1 ± 0.1	7.6 ± 0.2	7.4 ± 0.1	7.4 ± 0.1	8.0 ± 0.1	7.5 ± 0.3	0.9	6.4 ± 0.4	3.3 ± 0.4	0.5
FSMM7	6.6 ± 0	6.9 ± 0.1	7.2 ± 0	7.6 ± 0.2	7.3 ± 0	7.3 ± 0.1	7.8 ± 0.3	7.2 ± 0.3	0.9	6.5 ± 0.1	3.7 ± 0.1	0.6
FSMM8	6.7 ± 0.1	6.9 ± 0.2	7.2 ± 0.2	7.0 ± 0.3	7.4 ± 0	7.3 ± 0.2	7.9 ± 0	6.8 ± 0.2	0.9	5.5 ± 0.4	3.7 ± 0.2	0.7
FSMM9	6.6 ± 0.1	6.6 ± 0.1	7.0 ± 0.1	7.3 ± 0.4	7.2 ± 0.2	7.3 ± 0.1	7.8 ± 0.1	6.5 ± 0.2	0.8	5.2 ± 0.1	N.D.	N.D.
FSMM10	6.8 ± 0.2	6.7 ± 0.1	7.1 ± 0.1	7.5 ± 0.4	7.3 ± 0.1	7.2 ± 0.2	7.9 ± 0.3	6.7 ± 0.6	0.8	4.7 ± 0.7	3.2 ± 0.3	0.7
FSMM11	6.8 ± 0.1	7.0 ± 0.2	7.1 ± 0.2	7.6 ± 0.2	7.3 ± 0.1	7.4 ± 0.3	7.6 ± 0	6.8 ± 0.3	0.9	5.6 ± 0.9	4.2 ± 0.3	0.8
FSMM12	6.5 ± 0.3	7.0 ± 0.1	7.1 ± 0.1	7.5 ± 0.1	7.1 ± 0.2	7.4 ± 0.2	7.8 ± 0.2	6.3 ± 0.1	0.8	5.6 ± 0.4	4.0 ± 0.3	0.7
FSMM13	6.3 ± 0.1	7.0 ± 0.3	7.0 ± 0.1	7.4 ± 0.2	7.1 ± 0.2	7.3 ± 0	7.8 ± 0.3	6.9 ± 0.3	0.9	5.5 ± 0.5	N.D.	N.D.
FSMM14	6.6 ± 0.3	7.1 ± 0.1	7.8 ± 0	7.5 ± 0	7.4 ± 0.1	7.5 ± 0.1	8.2 ± 0.1	6.9 ± 0.3	0.8	5.6 ± 0.6	3.8 ± 0.3	0.7
FSMM15	6.3 ± 0.1	7.4 ± 0.1	8.1 ± 0.1	7.6 ± 0.1	7.9 ± 0.3	8.1 ± 0.2	8.7 ± 0.2	8.4 ± 0.3	1.0	6.3 ± 0.7	4.1 ± 0.3	0.7
FSMM16	6.6 ± 0.1	7.2 ± 0.1	7.9 ± 0.8	7.7 ± 0.3	7.6 ± 0.3	7.6 ± 0.2	8.0 ± 0.3	7.2 ± 0.5	0.9	5.7 ± 0.7	N.D.	N.D.
FSMM17	6.5 ± 0	7.2 ± 0.1	7.5 ± 0	7.9 ± 0.3	7.4 ± 0.4	7.4 ± 0.1	8.6 ± 0.1	6.7 ± 0.6	0.8	5.7 ± 0.6	N.D.	N.D.
FSMM18	6.6 ± 0.1	7.0 ± 0.2	7.2 ± 0	7.7 ± 0.2	7.6 ± 0.1	7.2 ± 0	7.6 ± 0.4	6.2 ± 0.9	0.8	5.1 ± 0.6	3.2 ± 0.2	0.6
FSMM19	6.8 ± 0	6.8 ± 0	7.1 ± 0.1	8.1 ± 0.2	7.7 ± 0.6	7.3 ± 0.2	7.7 ± 0.2	6.9 ± 0.4	0.9	5.2 ± 0.7	3.0 ± 0.2	0.6
FSMM20	6.2 ± 0.3	6.8 ± 0.1	7.2 ± 0.1	8.0 ± 0.2	7.8 ± 0.1	7.3 ± 0.1	8.1 ± 0.1	7.3 ± 0.1	0.9	6.0 ± 0.3	3.9 ± 0.3	0.7
FSMM21	6.5 ± 0.2	7.0 ± 0.1	7.1 ± 0.1	8.1 ± 0.4	8.0 ± 0.1	7.5 ± 0.1	7.6 ± 0.2	7.3 ± 0.6	1.0	6.1 ± 0.8	4.2 ± 0.2	0.7
FSMM22	6.6 ± 0.2	7.1 ± 0.1	7.3 ± 0	7.8 ± 0.3	8.0 ± 0.2	7.4 ± 0.1	7.5 ± 0.1	7.0 ± 0.4	0.9	6.3 ± 0.1	4.1 ± 0.3	0.7
FSMM23	7.1 ± 0.4	6.8 ± 0.2	7.9 ± 0.3	8.8 ± 0.1	8.0 ± 0.1	N.D.	8.7 ± 0.2	8.3 ± 0.4	1.0	7.7 ± 0.1	5.6 ± 0.3	0.7
FSMM24	6.6 ± 0.2	6.9 ± 0.2	7.2 ± 0.1	7.9 ± 0.5	7.3 ± 0.2	7.2 ± 0	7.5 ± 0.1	7.0 ± 0.4	0.9	6.1 ± 0.4	3.7 ± 0.1	0.6
FSMM25	6.4 ± 0	7.0 ± 0.1	7.3 ± 0.1	7.3 ± 0.2	7.7 ± 0.1	7.3 ± 0.1	7.5 ± 0.2	7.4 ± 0.1	1.0	6.2 ± 0.1	4.6 ± 0.3	0.7
FSMM26	6.4 ± 0.3	7.0 ± 0	7.6 ± 0.2	7.2 ± 0.2	7.3 ± 0.2	7.4 ± 0.3	7.3 ± 0.3	7.1 ± 0.7	1.0	5.5 ± 0.1	4.3 ± 0.3	0.8
FSMM27	6.6 ± 0.1	6.9 ± 0.2	7.7 ± 0.2	7.4 ± 0.4	8.1 ± 0.1	7.5 ± 0.2	7.6 ± 0.1	6.9 ± 0.3	0.9	6.5 ± 0.2	4.2 ± 0.3	0.6

LGG, *Lb. rhamnosus* GG ATCC53103 as positive control.
N.D., not detectable.

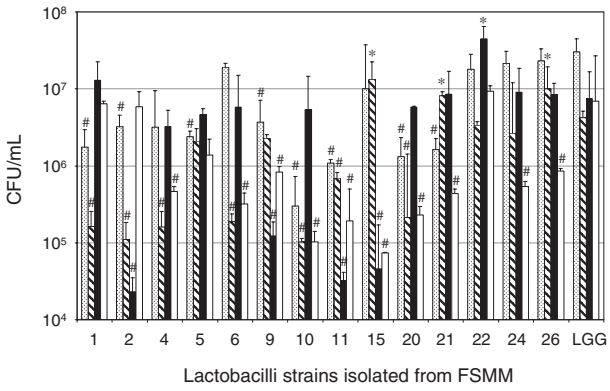

Fig. 1. Adhesion of Lactobacilli Strains to BSA and ECM Proteins. FSMM numbering was applied. LGG, *Lb. rhamnosus* ATCC53103. Hollow bars, cells adhering to BSA; solid bars, to laminin; shaded bars, to fibronectin; dotted bars, to colonic mucin. *Significantly high, $p < 0.05$ ($n = 5$), *versus* LGG; #significantly low, $p < 0.05$ ($n = 5$), *versus* LGG.

significantly higher adhesion *in vitro* to fibronectin and laminin respectively as compared to LGG, and hence these strains should have good potential for probiotics. In contrast, Vankerckhoven *et al.* pointed out the susceptibility and potential pathogenicity of clinically isolated and potential probiotic *Lb. rhamnosus* strains.[41] This appears to be unrelated to their binding properties as to ECM proteins, but further studies *in vivo* are requisite to clarify the probiotic abilities of FSMM15, FSMM22, and FSMM26.

Evidence that adhesion of probiotics to ECM proteins can inhibit adhesion by and colonization of enteric pathogens in the GI tract is very scarce to date. Since at least 12 proteins of *Lb. plantarum* WCFS1 are predicted to be directly involved in adherence to the host,[42] the multiplicity of a probiotic's recognition patterns for ECM proteins probably interferes with further investigation. The use of the two isolated *Lb. rhamnosus* strains, FSMM15 and FSMM22, which showed discriminative adhesion to laminin, might provide a way to clarify the role of laminin in the prevention of pathogenic infection in the GI tract.

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References

1) Araya M, Morelli L, Reid G, Sanders ME, and Stanton C, FAO/WHO report, p. 8 (2002).
2) Juntunen M, Kirjavainen PV, Ouwehand AC, Salminen SJ, and Isolauri E, *Clin. Diagn. Lab. Immunol.*, **8**, 293–296 (2001).
3) Muñoz-Provencio D, Pérez-Martínez G, and Monedero V, *J. Appl. Microbiol.*, **108**, 1050–1059 (2010).
4) Toba T, Virkola R, Westerlund B, Bjorkman Y, Sillanpaa J, Vartio T, Kalkkinen N, and Korhonen TK, *Appl. Environ. Microbiol.*, **61**, 2467–2471 (1995).
5) Plummer S, Weaver MA, Harris JC, Dee P, and Hunter J, *Int. Microbiol.*, **7**, 59–62 (2004).
6) Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, and Langella P, *Proc. Natl. Acad. Sci. USA*, **105**, 16731–16736 (2008).
7) Nava GM, Bielke LR, Callaway TR, and Castañeda MP, *Anim. Health Res. Rev.*, **6**, 105–118 (2005).
8) Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, and Ohno H, *Nature*, **469**, 543–547 (2011).
9) Jack RW, Tagg JR, and Ray B, *Microbiol. Rev.*, **59**, 171–200 (1995).
10) Schlee M, Harder J, Köten B, Stange EF, Wehkamp J, and Fellermann K, *Clin. Exp. Immunol.*, **151**, 528–535 (2008).
11) Möndel M, Schroeder BO, Zimmermann K, Huber H, Nuding S, Beisner J, Fellermann K, Stange EF, and Wehkamp J, *Mucosal Immunol.*, **2**, 166–172 (2009).
12) Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, and Martin GR, *J. Biol. Chem.*, **254**, 9933–9937 (1979).
13) Martin GR and Timpl R, *Annu. Rev. Cell Biol.*, **3**, 57–85 (1987).
14) Gumbiner BM, *Cell*, **84**, 345–357 (1996).
15) Lopes JD, dos Reis M, and Brentani RR, *Science*, **229**, 275–277 (1985).
16) Konkel ME, Garvis SG, Tipton SL, Anderson DE Jr, and Cieplak W Jr, *Mol. Microbiol.*, **24**, 953–963 (1997).
17) Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, and Telford JL, *Proc. Natl. Acad. Sci. USA*, **102**, 15641–15646 (2005).
18) Horie M, Ishiyama A, Fujihira-Ueki Y, Sillanpää J, Korhonen TK, and Toba T, *J. Appl. Microbiol.*, **92**, 396–403 (2002).
19) Dharmawan J, Surono IS, and Kun LY, *Asian Austral. J. Anim. Sci.*, **19**, 751–755 (2006).
20) Wu R, Wang LP, Wang JC, Li HP, Menghe B, Wu JR, Guo MR, and Zhang HP, *J. Basic Microbiol.*, **49**, 318–326 (2009).
21) Danova S, Petrov K, Pavlov P, and Petrova P, *Int. J. Dairy Technol.*, **58**, 100–105 (2005).
22) Ayeni FA, Sánchez B, Adeniyi BA, de Los Reyes-Gavilán CG, Margolles A, and Ruas-Madiedo P, *Int. J. Food Microbiol.*, **147**, 97–104 (2011).
23) Zhang L, Yu QL, Han L, Zhang M, Yang LL, and Li YP, *J. Food Agric. Environ.*, **9**, 18–26 (2011).
24) Park YW, Zhang H, Zhang B, and Zhang L, "Handbook of Milk of Non-Bovine Mammals," Blackwell Publishing, Oxford, pp. 275–296 (2006).
25) Sujaya IN, Dwipayanti NMU, Suariani NLP, Widarini NP, Nocianitri KA, and Nursini NW, *Jurnal Veteriner*, **9**, 33–40 (2008).
26) Sujaya IN, Amachi S, Yokota A, Asano K, and Tomita F, *World J. Microbiol. Biotechnol.*, **17**, 349–357 (2001).
27) Kandler O, Andler O, and Weiss N, "Bergey's Manual of Systematic Bacteriology," Williams and Williams, Baltimore, pp. 1208–1234 (1986).
28) Kozaki M, Uchimura T, and Okada S, "Experimental Manual of Lactic Acid Bacteria," Asakurasyoten, Tokyo, pp. 34–37 (1992).
29) Mori K, Yamazaki K, Ishiyama T, Katsumata M, Kobayashi K, Kawai Y, Inoue N, and Shinano H, *Int. J. Syst. Bacteriol.*, **47**, 54–57 (1997).
30) Gorbach SL and Goldin BR, U.S. Patent, 4839281 (Jun. 13, 1989).
31) Neefs JM, van de Peer Y, Hendriks L, and de Wachter R, *Nucleic Acids Res.*, **18**, 2237–2317 (1990).
32) Dobson CM, Chaban B, Deneer H, and Ziola B, *Can. J. Microbiol.*, **50**, 482–488 (2004).
33) Mitchell DJ, McClure BG, and Tubman TRJ, *Arch. Dis. Child.*, **84**, 273–276 (2001).
34) Fernández MF, Boris S, and Barbés C, *J. Appl. Microbiol.*, **94**, 449–455 (2003).
35) Kodaira H, Ishihara K, Hotta K, Kagoshima M, Shimada H, and Ishii K, *Biol. Pharm. Bull.*, **23**, 1173–1179 (2000).
36) Mathara JM, Schillinger U, Kutima PM, Mbugua SK, and Holzapfel WH, *Int. J. Food Microbiol.*, **94**, 269–278 (2004).

37) Ashmaig A, Hasan A, and El Gaali E, *Afr. J. Microbiol. Res.*, **3**, 451–457 (2009).
38) Zakaria Y, Ariga H, Urashima T, and Toba T, *Milchwissenschaft*, **53**, 30–33 (1998).
39) Yu J, Wang WH, Menghe BL, Jiri MT, Wang HM, Liu WJ, Bao QH, Lu Q, Zhang JC, Wang F, Xu HY, Sun TS, and Zhang HP, *J. Dairy Sci.*, **94**, 3229–3241 (2011).
40) French D, "Advances in Carbohydrate Chemistry," Academic Press, NY, pp. 149–181 (1954).
41) Vankerckhoven V, Moreillon P, Piu S, Giddey M, Huys G, Vancanneyt M, Goossens H, and Entenza JM, *J. Med. Microbiol.*, **56**, 1017–1024 (2007).
42) Boekhorst J, Wels M, Kleerebezem M, and Siezen RJ, *Microbiology*, **152**, 3175–3183 (2006).