# Phenotypic Differences between Commercial *Lactobacillus rhamnosus* GG and *L. rhamnosus* Strains Recovered from Blood

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**The isolation of clinical isolates that are indistinguishable, by molecular methods, from the probiotic strain of *Lactobacillus rhamnosus* GG has been reported. We compared the virulence potential of these clinical isolates with that of the probiotic strain. It was observed that all isolates differed significantly in 1 or more phenotypic properties.**

*Lactobacillus rhamnosus* GG (ATCC 53103) is one of the most widely used probiotics in the world and has several scientifically documented health benefits [1]. In Finland, the annual per capita consumption of *L. rhamnosus* GG–containing products has increased from ~1 L in 1995 to 6 L in 2000. A recent study did not indicate an increase in cases of *Lactobacillus* bacteremia in Finland, despite this strong increase in consumption of probiotics [2]. However, a number of *Lactobacillus* strains isolated from patients with bacteremia were identified and found to be indistinguishable from *Lactobacillus* GG by PFGE [3].

The aim of the current study was to assess and compare potential phenotypic properties associated with bacterial translocation [4], such as adhesion to intestinal mucus and extracellular matrix proteins, resistance to serum-mediated killing, induction of respiratory burst in PBMCs, hemolysis, and phosphatidylinositol-specific phospholipase C (PI-PLC) activity. The target was to further assess the potential similarities or differences between *Lactobacillus* GG–like isolates from cases of bacteremia and the original *Lactobacillus* GG strain.

***Materials and methods.*** *Lactobacillus* GG was provided by Valio. *Lactobacillus* GG–like isolates were obtained from clinical cultures of blood that was mainly collected from patients with severe underlying diseases [2] and identified by *L. rhamnosus*–specific PCR and PFGE [3]. The bacteria were grown anaerobically at a temperature of 37°C for 18 h in de Man, Rogosa, and Sharpe (MRS) broth and minimally subcultured to avoid adaptation to laboratory conditions.

Adhesion assays were performed in a manner similar to that described elsewhere [5]. In short, collagen type 4 and fibrinogen were purchased from Sigma. Intestinal mucus was isolated from surgically resected bowel tissue from patients who underwent surgery for colorectal cancer at Turku City Hospital (Turku, Finland). The use of human tissue was approved by the joint ethical committee of the University of Turku and Turku University Central Hospital, and informed written consent was obtained from the patients. Mucus was prepared as described elsewhere [5]. The substrata were passively immobilized in microtiter plate wells (0.5 mg/mL) by overnight incubation at a temperature of 4°C. Excess material was washed away with HEPES-buffered Hank's balanced salt solution (10 mmol/L HEPES; pH, 7.4). The bacteria were cultured as described above; to metabolically radiolabel the bacteria, 10 $\mu$L/mL [5′-³H]-thymidine (Amersham Biosciences) was added to the culture medium.

The optical density (at 600 nm) of the radiolabeled bacteria was adjusted to $0.5 \pm 0.01$ before use in the adhesion assay to standardize the concentration of bacteria at $2 \times 10^8$ cfu/mL. Bacteria were added to the wells and incubated for 1 h at 37°C. Nonbound bacteria were removed by washing with HEPES-buffered Hank's balanced salt solution. Bound bacteria were released and lysed with 1% sodium dodecyl sufate in 0.1 mol/L NaOH. Radioactivity was determined by liquid scintillation, and the adhesion valve was expressed as the percentage of radioactivity recovered after adhesion relative to the radioactivity in the bacterial suspensions added to the immobilized collagen, fibrinogen, or mucus.

Hemolysis was determined modified after Baumgartner et al. [6]. In short, strains were grown in MRS agar that was supplemented with 5% human blood (type O) and incubated for 48 h at 37°C under anaerobic conditions. *Bacillus cereus* (grown aerobically in Luria Bertani agar at 37°C) was included as a positive control.

Serum resistance was determined by the method described by Burns and Hull [7]. In brief, blood was collected from 13 healthy adult donors and was allowed to clot and pool. Part of the serum was heated to 56°C for 20 min to inactivate the complement system. Aliquots were frozen at −70°C until use. Bacteria were grown in the serum as described above and

**Table 1.** Comparison of phenotypic properties of *Lactobacillus rhamnosus* GG and *L. rhamnosus* GG–like isolates from cases of bacteremia.

| | Mean value ± SD[a] | | | | | |
|---|---|---|---|---|---|---|
| | Adhesion rate, % | | | Respiratory burst induction (mV/100,000 PMN) | Survival rate, %[b] | |
| *L. rhamnosus* strain | To mucus | To collagen | To fibrinogen | | In serum | In inactivated serum |
| GG | 17.9 ± 3.3 | 7.3 ± 1.7 | 13.1 ± 1.8 | 6202 ± 358 | 150 ± 50 | 190 ± 40 |
| T-17221 | 22.9 ± 2.1 | 10 ± 0.6 | 14.1 ± 2.2 | 10286 ± 725[c] | 90 ± 30 | 40 ± 20[c] |
| T-30914 | 21.5 ± 1.6 | 9.8 ± 1.8 | 16.7 ± 0.5[c] | 3876 ± 182[c] | 150 ± 30 | 270 ± 40[c] |
| T-31528 | 29.9 ± 3.2[c] | 11.1 ± 1.5[c] | 18.8 ± 4.1 | 3990 ± 73[c] | 110 ± 30 | 90 ± 50 |
| T-33721 | 29.1 ± 2.3[c] | 10.8 ± 1.3 | 15.9 ± 0.5[c] | 11163 ± 377[c] | 60 ± 30[c] | 60 ± 30[c] |

**NOTE.** None of the isolates had α- or β-hemolytic activity or phosphatidylinositol-specific phospholipase activity.

[a] Results are presented as the mean value for 3 independent experiments ± SD.

[b] Relative to PBS treated control.

[c] Value significantly different from that of *L. rhamnosus* GG ($P < .05$).

washed twice with PBS (10 mmol/L phosphate; pH, 7.2), and the optical density (at 600 nm) was adjusted to 0.5 ± 0.01 to standardize the concentration of bacteria at $10^7$–$10^8$ cfu/mL. The bacteria were mixed with serum, heat-inactivated serum (final serum concentration, 80%), or PBS and incubated aerobically for 90 min at 37°C. The reaction was stopped by incubation on ice for 10 min and by serial dilution in PBS. Dilutions were plated in MRS agar and incubated for 2 days at 37°C under anaerobic conditions. *Yersinia enterocolitica* 6471/76 grown in Luria Bertani broth was included as a positive control.

Bacteria were allowed to grow for 18 h in MRS broth and harvested in HEPES-buffered Hanks' balance salt solution and the optical density (at 600 nm) was adjusted to 0.5 ± 0.01. PBMCs were collected by lysing erythrocytes in freshly collected human blood with 0.8% NH$_4$Cl. PBMCs were washed and resuspended in Hank's balanced salt solution. The concentration of PBMCs was standardized to $5 \times 10^6$ cells/mL by flow cytometry. The measurement of the respiratory burst was performed in a manner similar to that described by Lilius and Marnila [8]. In short, 25 μL of Hanks' balanced salt solution containing 0.1% gelatin, 20 μL of 5-amino-2,3-dihydro-1,4-phthalazine-dione (luminol; 10 mmol/L), 40 μL of bacterial suspension, and 40 μL of PBMC suspension were added to gelatin-coated microtiter plate wells (Cliniplate; Labsystems). For background measurements, PBMCs were incubated without bacteria. The plates were incubated at a temperature of 37°C, and luminescence was measured for 2 h at 3-min intervals with a Victor multilabel counter (Perkin Elmer). Results are presented as the maximum signal (mV per 100,000 PBMCs) after subtraction of the background and as the average of at least 3 independent observations of PBMCs from different donors.

PI-PLC activity was determined as described by Rodriguez et al. [9]. In short, the 10 μL of the strains were spot-inoculated on appropriate solid media. After 24–48 h of growth at 37°C, depending on the strain, the plates were overlaid with 10 mL of 1.4% agarose-PBS containing 20 mg/L L-α-phosphatidylinositol (Sigma). The plates were then incubated for 5 days at 37°C, and the formation of a turbid halo was determined. *B. cereus* was included as a positive control. All results are presented as the average of at least 3 independent observations within the standard deviation. A nonpaired *t* test was used to evaluate the statistical significance ($P < .05$) of differences in values between the *Lactobacillus* GG probiotic strain and the clinical isolates.

***Results.*** The in vitro adhesion rate to human intestinal mucus, collagen, and fibrinogen of 3 of the tested clinical isolates was different from that of the original *Lactobacillus* GG strain. These same 3 isolates exhibited a significantly higher adhesion rate than the original strain (table 1; $P < .05$). All tested isolates exhibited a different induction of respiratory burst than the original strain. Two isolates, T-33721 and T-30914, induced a stronger respiratory burst and 2 other strains, T-30914 and T-31528, induced a weaker burst (table 1). Two isolates were found to have a lower rate of survival in human serum and in heat-inactivated serum than the original strain, and 1 isolate had a higher resistance to heat-inactivated serum than the original strain (table 1). None of the tested clinical isolates was found to have α- or β-hemolytic activity or PI-PLC activity (table 1).

***Discussion.*** Lactobacilli are generally considered safe for consumption on the basis of their long history of safe use in foods and their presence in the gastrointestinal tract. Despite a strong increase in the consumption of probiotic products in Finland, the low incidence of *Lactobacillus* bacteremia has not been affected [2]. However, some isolates that are genetically indistinguishable from the original strain of *Lactobacillus* GG were identified. In the current study, we investigated phenotypic properties possibly related to the translocation of these isolates and compared them with those of the original strain.

Despite the fact that the clinical isolates could not be distinguished from *Lactobacillus* GG on the basis of PFGE results,

significant differences were observed in their in vitro adhesion properties, resistance to serum-mediated killing, and induction of respiratory burst. In particular, resistance to a host's innate immunity has been suggested to be related to the infective potential of lactobacilli in a rabbit model of infective endocarditis [10].

Adhesion to the intestinal mucosa and extracellular matrix proteins is a first step in the pathogenesis of many microbes [11], and 3 of the 4 *Lactobacillus* GG—like isolates adhered to at least 1 of these substrata at higher levels than did the original strain of *Lactobacillus* GG.

All tested clinical isolates induced respiratory burst differently from *Lactobacillus* GG. Low induction of respiratory burst may contribute to prolonged survival after translocation. Also, resistance to the complement system contributes to this. Like most gram-positive microbes, the isolates exhibited a relatively strong resistance to serum-mediated killing.

Because of the variation observed between the tested isolates, it is unclear whether the tested properties, although important for pathogens, are relevant to the bacteremia caused by lactobacilli. The differences do, however, indicate that although strains could not be differentiated from *Lactobacillus* GG by the molecular methods we used, they are not identical to *Lactobacillus* GG on the basis of phenotypic properties.

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