

Supplement data

# Effects of *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 on Overweight and the Gut Microbiota in Humans: Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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## 1. Material and Method

### 1.1. Acid and bile tolerance

Acid and bile tolerance of *Lactobacillus* strains were evaluated, as described by Chung et al. [1]. Briefly, 100 µL of overnight bacterial cultures in MRS broth were inoculated into 10 mL sterile phosphate buffered saline (PBS). Acid resistance experiments were performed by incubation at 37 °C for 2 h with the addition of 1% pepsin in saline with a pH value of 2.5 (10M HCl, adjusted using Merck). Bile tolerance experiments were performed by incubation at 37 °C for 2 h in saline with a pH value of 8.0 (10 M HCl, adjusted using Merck) supplemented with 0.3% bile. In each experiment, the *Lactobacillus* strains incubated for 2 hours at 37°C in PBS with a pH of 6.2 was set as a control group. Counting of strain populations was performed by surface plating on MRS agar followed by incubation at 37 °C for 24 h. The survival rate was calculated as follows.

$$\text{Percent survival} = \frac{\text{final (cfu/mL)}}{\text{control (cfu/mL)}} \times 100.$$

### 1.2. Resistance to the Effects of the GI Tract

We evaluated *Lactobacillus* strains by measuring survival rate in physiological conditions similar to those of the human GIT, as described by Jung et al. [2]. To this end, in vitro digestion tests were performed to evaluate resistance to saliva, gastric juice, and bile salts. Simulated Salivary Fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were preheated to 37 °C prior to in vitro digestion of bacteria. Then, 5 mL *Lactobacillus* strain culture was poured into a 50 mL conical tube. The oral simulation was performed by adding 26 µL of 0.3 M CaCl<sub>2</sub> solution and 4 mL of 6.55 mg/mL α-amylase solution in SSF. The pH was then adjusted to 7.0 with 1 M NaOH, and the mixture was incubated at 37 °C for 2 min. The gastric fluid simulation was performed by adding 6 µL of 0.3 mol/L CaCl<sub>2</sub>, 694 µL distilled water, and 9.1 mL of 0.07 mg/mL pepsin to SGF. The pH was adjusted to 3 with 1 M HCl, and the mixture was incubated at 37 °C with continuous shaking for 2 h. The intestinal phase was simulated by adding 40 µL of 0.3 M CaCl<sub>2</sub>, 1.31 mL distilled water, 2.5 mL of 160 mM bile extract, and 16 mL of 22.15 mg/mL pancreatic solution. The pH was raised to 7.0 by adding 1 M NaOH, and the mixture was incubated at 37 °C for 2 h with shaking. For each step, a control group cultured in PBS was set. At the end of each simulation, aliquots of *Lactobacillus* strains were collected, and viable

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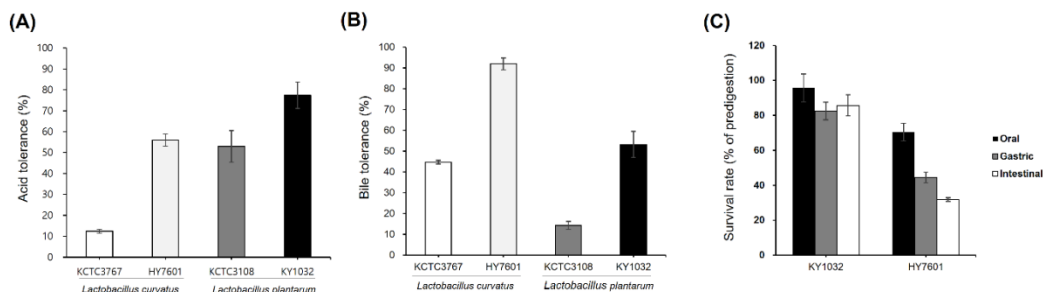
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cell counts were measured to determine the viability after simulated saliva, gastric fluid, and intestinal fluid digestions.

## 2. Result



**Figure S1.** Acid and bile tolerance of *Lactobacillus* strains. (A) Acid tolerance of *Lactobacillus* strains in PBS with pH value 2.5 and addition 1% pepsin and (B) bile tolerance of *Lactobacillus* strains in PBS with pH value 8.0 and addition 0.3% bile. (C) The survival rate of KY1032 and HY7601 during simulated gastrointestinal tract. Data are represented as the mean  $\pm$  standard deviation (SD) of three independent experiments. KCTC3767, *Lactobacillus curvatus* type strain; KCTC3108, *Lactobacillus plantarum* type strain.

## References

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