Supplementary material

Supplementary methods

1.1 Determination of gastric acid and bile salt tolerance

Weissella confusa FN015, *Lacicaseibacillus paracasei* FN016, *Lactiplantibacillus plantarum* FN029 and *Lacticaseibacillus rhamnosus* GG was cultured in MRS broth to reach the exponential phase of growth. The culture was transferred to MRS broth at a ratio of 10% and incubated at 37°C for 24 hours. The cells were harvested by centrifugation at 15,000g. The bacterial pellet was then resuspended in PBS to a concentration of 10^9 CFU/mL.

The strain's tolerance to gastric acid (pH=2.0) and bile salt (0.3%) was determined with reference to the method of Nagpal et al.[1] Sterile PBS 900 μL with pH 3.0 (experimental) and 7.0 (control) were prepared respectively, and bacterial solution 100 μL (10^9 CFU/mL) washed with sterile PBS was inoculated. After anaerobic culture at 37℃ for 4 h, it was thoroughly mixed and then gradient dilution was performed. 50 μL bacterial solution from each gradient was evenly coated on a solid medium plate, and colony counting was performed after anaerobic culture at 37℃ for 24 h.

Bile tolerance was measured, 900 μL sterile PBS (control) with 0.3% (w/v) bile salt added (experimental) and no bile salt added (control) were prepared, and 100 μL (10^9 CFU/mL) bacterial solution washed with sterile PBS was inoculated, respectively. After anaerobic culture at 37℃ for 4 h, it was thoroughly mixed for gradient dilution, and 50 μL bacterial solution for each gradient was evenly coated on solid medium plate, and colony counting was performed after anaerobic culture at 37℃ for 24 h.

Gastric acid or bile salt tolerance (%) = CFU1/CFU0 × 100.

CFU1: Number of viable bacteria in the experimental group, CFU/mL

CFU0: Number of viable bacteria in control group, CFU/mL

1.2 Mucin adhesion determination

The concentration of the bacterial suspension was adjusted so that the OD600 reached 1.0 ± 0.5, after washing the activated bacterial solution three times in sterile PBS.[2] A 200 μL mucin (Mucin II, sigma) solution (1 mg/mL) was added to each well of the 96-well plate, which was then sealed with a sealing film, and be placed in a wet box at 4℃ for 12 h. Mucin solution was sucked out. Wells were washed by PBS containing 0.1% (w/v) bovine serum albumin. Excessive washing

solution was removed. A 100 μL bacterial solution was added into well, and aforementioned incubation and washing step was repeated. The adherent cells were thermally fixed at 60℃ for 20 min. Each well was added with 200 μL of 0.1% crystal violet solution, and stained for 30 min. The microwells were washed and allowed to dry at room temperature. A 100 μL dimethyl sulfoxide was added to each well, incubated for 15 min, and the absorbance value of the stained hole was measured at 570 nm wavelength. The stained mucin-coated hole with bacteria was used as a blank control, and the blank control hole value was subtracted from the OD570 of the measured bacterial solution. 3 parallel experiments were set up for each strain.

1.3 Strain hydrophobicity determination

In the experimental procedure,[3] after the three generations of activation, the optical density (OD570 nm) value, A0, of the bacterial solution was determined. A 2 mL aliquot of the bacterial solution (10^9 CFU/mL) was combined with 2 mL of xylene in a test tube and mixed vigorously using a vortex mixer. After allowing the mixture to stand at room temperature for 30 minutes, the solution became stratified. The OD570 nm value of the aqueous phase was then measured and denoted as A1. The surface hydrophobicity of each strain was calculated based on the OD values using the following formula:

Surface hydrophobicity = [(A1 - A0) / A0] × 100%.

1. M. C. Arrieta, A. Arévalo, L. Stiemsma, P. Dimitriu, M. E. Chico, S. Loor, M. Vaca, R. C. T. Boutin, E. Morien, M. Jin, S. E. Turvey, J. Walter, L. W. Parfrey, P. J. Cooper and B. Finlay, Associations between infant fungal and bacterial dysbiosis and childhood atopic wheeze in a nonindustrialized setting, *J Allergy Clin Immunol*, 2018, **142**, 424-434.e410.
2. M. C. Arrieta, L. T. Stiemsma, N. Amenyogbe, E. M. Brown and B. Finlay, The intestinal microbiome in early life: health and disease, *Front Immunol*, 2014, **5**, 427.
3. M. C. Arrieta, L. T. Stiemsma, P. A. Dimitriu, L. Thorson, S. Russell, S. Yurist-Doutsch, B. Kuzeljevic, M. J. Gold, H. M. Britton, D. L. Lefebvre, P. Subbarao, P. Mandhane, A. Becker, K. M. McNagny, M. R. Sears, T. Kollmann, W. W. Mohn, S. E. Turvey and B. B. Finlay, Early infancy microbial and metabolic alterations affect risk of childhood asthma, *Sci Transl Med*, 2015, **7**, 307ra152.

Supplementary figure

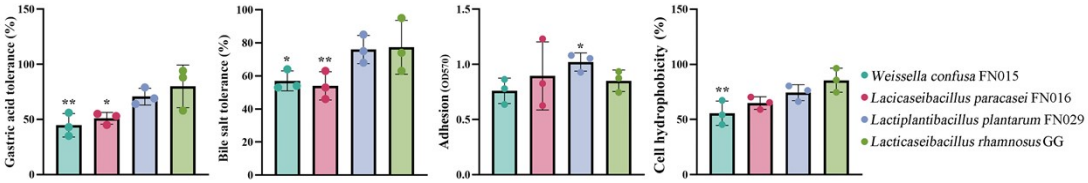

Fig.S1. (A) Strain acid resistance, (B) strain bile salt resistance, (C) strain adhesion, (D) strain surface hydrophobicity. *$p < 0.05$,**$p < 0.01$, vs. *Lactobacillus rhamnosus* GG group by student t test.