Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Material

Supplementary Figures

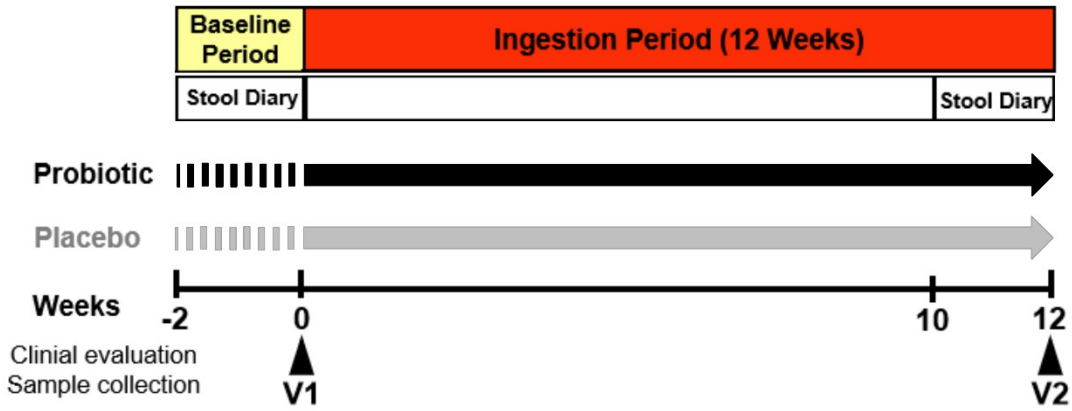

Fig S1. Study design.

Supplementary Information of Methods

Subjects

The subjects were not allowed to consume any fermented dairy products (except the provided study products) or antibiotics during the study period.

The exclusion criteria included the following: (1) secondary parkinsonism, Alzheimer's disease, or other central nervous system diseases; (2) the use of systemic antibiotics or antimycotics in the 30 days prior to the study; (3) a known or suspected allergy to probiotics or intolerance to lactose or gluten; (4) a history of cardiac disease, renal disease, diarrhoea, abdominal pain, inflammatory bowel disease, diabetes or hepatic conditions; and (5) an investigator's uncertainty about the willingness or ability of a subject to comply with the protocol requirements.

16S rRNA sequencing and the measurement of faecal metabolomics

16S rRNA gene amplicon and sequencing: We extracted faecal genomic DNA from the faecal samples by using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V3-V4 regions of the 16S rRNA gene were amplified using universal primers (341F and 806R) linked with indices and sequencing adaptors. The amplicons were sequenced on an Illumina MiSeq platform (Illumina Inc., CA, USA) to obtain 300-bp paired-end reads. Fastq files were demultiplexed by MiSeq Controller Software (Illumina Inc.). The operational taxonomic units (OTUs) were analysed by phylogenetic and OTU methods in Quantitative Insights into Microbial Ecology (QIIME) software version 2.0. The bacterial α-diversity was studied by biodiversity indices, computed from the number of OTUs by the Wilcoxon signed rank test. β-Diversity was displayed using principal coordinate analysis (PCoA) based on an unweighted UniFrac distance, and permutational multivariate analysis of variance (PERMANOVA) was performed to compare the global microbiota composition before and after the intervention in each group. The bacterial α-diversity was studied by biodiversity indices, computed from the number of OTUs by the Wilcoxon signed rank test. β-Diversity was displayed using principal coordinate analysis (PCoA) based on an unweighted UniFrac distance, and permutational multivariate analysis of variance (PERMANOVA) was performed to compare the global microbiota composition before and after the intervention in each group.

Targeted faecal metabolomics analysis: A targeted metabolomics approach based on a validated method was used to analyse the faecal samples, with a total of 198 metabolites quantified. Briefly, the faecal samples were thawed in an ice bath to diminish degradation. Five milligrams of each sample was weighed and transferred into a safety lock tube. Homogenization with 20 μL of ultrapure water was followed by extraction with 120 μL of methanol containing an internal standard solution, followed by centrifugation at 13500 g and 4 ℃ for 10 min. Thirty microlitres of the supernatant was transferred to a 96-well plate for derivatization. Thirty microlitres of a working standard solution and faecal extract was added to a 96-well plate. The following procedures were then performed on an Eppendorf epMotion workstation

(Eppendorf Inc., Hamburg, Germany). Twenty microlitres of a freshly prepared derivatization reagent was added to each well, and after derivatization at 30 ℃ for 60 min, 400 μL of an ice-cold 50% methanol solution was added to dilute the sample, which was then stored at -20 °C for 20 minutes followed by 20 min at -20 ℃. This was followed by centrifugation at 4000 g for 30 min at 4 ℃, and 135 μL of the supernatant from each well was transferred to a new 96-well plate with 10 μL internal standards in each well. An ultra-performance liquid chromatography coupled with a tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA) was used to quantitate the metabolite in this project. Internal standards were added to the test samples to monitor analytical variations during the entire sample preparation and analysis processes. The calibrators consisted of a blank sample (a matrix sample processed without internal standard), a zero sample (a matrix sample processed with internal standard), and a series of seven concentrations covering the expected range for the metabolites present in the specific biological samples. The raw data files generated by the UPLC-MS/MS system were processed using MassLynx software (v.4.1, Waters, Milford, MA, USA) to perform peak integration, calibration, and quantification for each metabolite. The self-developed platform iMAP (v1.0, Metabo-Profile, Shanghai, China) was used for statistical analyses. Due to the quality of the faecal samples, 118 samples (probiotic group n = 60; placebo group n = 58) were eventually undergo a multiomics analysis.

Plasma L-tyrosine analysis: Plasma L-tyrosine was measured using high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). Briefly, the plasma samples were thawed on ice at 4 °C and mixed thoroughly before preparation. Two hundred microlitres of the plasma sample was added to 4 times the volume of ethanol and incubated for 30 min at 4 °C after mixing well. Then, the samples were centrifuged at 13000 rpm and 0 °C for 25 min, filtered through a 0.45 μm membrane and blown dry by nitrogen. The samples were resolve using 100 μL 0.1 M hydrochloric acid. Then, 25 μL of a borate buffer and derivatization reagent were added to a 10 μL sample and fully mixed; 200 μL of a diluent was added and mixed for 2 min, and then the sample was injected. L-tyrosine analysis was performed using

liquid chromatography (Agilent, USA) with a diode array detector (DAD, G1313) and fluorescence detector (FLD, 1200). The mobile phases were A: 10 mM disodium hydrogen phosphate and 10 mM sodium borate and B: methanol/acetonitrile/water (45:45:10). The chromatographic column used was a YMC-C18-EXRS (150 mm × 4.6 mm, 3 μm). The injection volume was 1 μL per sample, the injection temperature was set at 4 °C, and the flow rate was 1 mL/min. The ultraviolet wavelengths were 338 nm and 236 nm, the excitation wavelength was 260 nm, and the emission wavelength was 325 nm. The limits of detection (LODs) and limits of quantification (LOQs) were the concentrations at signal-to-noise ratios (S/N) of 3/1 and 10/1 in the sample solution, respectively.