One water-soluble of polysaccharide from *Ginkgo biloba* leaves with antidepressant activities via modulation of the gut microbiome

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Materials and methods

Isolation of *Lactobacillus*

Lactobacillus is isolated and identified as described previously **[Wilck N. et al. "Salt-responsive gut commensal modulates TH17 axis and disease." *Nature* (2017)]**. Faecal samples from healthy mice were dissolved and diluted at a 1:10 dilution in anaerobic phosphate-buffered saline (PBS) (pH 7.6) containing L-cysteine HCl at 0.1% in a Coy Anaerobic Chamber (5% H_2, 20% CO_2, 75% N_2). Samples were diluted tenfold and each dilution spread on MRS agar. Plates were incubated at 37 °C under anaerobic conditions and examined for growth at 24 h. Individual colonies growing at the highest dilution were picked into MRS medium and grown for an additional 16 h. The single colonies isolated by streaking plating method were further identified by colony morphology, Gram staining and 16S rRNA sequenced. Liquid cultures were stored in 15% DMSO. For identification of isolates, DNA was extracted by adding 5 µl liquid culture to 20 µl sterile distilled water and storing at 4 °C overnight; 2 µl of this extract was amplified with Phusion HF polymerase in a 20-µl reaction using universal 16S primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR products were purified using Agencourt AMPure XP and submitted with the 27F primer for Sanger sequencing. An isolate for which the full-length 16S sequence shared 100% identity with the V4–V5 region of the *Lactobacillus* species identified in the 16S library was selected for further study. Then, the single colony was subcultured 3 times by streak plate method and identified by 16S rRNA sequenced as shown in fig. S4. The strain colonies were milky white, round, smooth and convex. All colonies are homogeneous, indicating that all strains on the plate are single clones (fig. S5A). The strain is Gram-positive and has a rod-like morphology. And there were no other bacteria observed in the photomicrograph, indicating that the cultured bacteria are pure (fig. S5B).

The 16S rRNA PCR amplification product was recovered and sequenced. The 16S rRNA sequence of the strain was aligned using the BLAST program in the NCBI database. The identities between the strain we isolated and the *L. reuteri* in the NCBI

database was found to be 100% (fig. S5C). So, we can confirm that the strain we isolated is 100% pure *Lactobacillus reuteri*. *Lactobacillus johnsonii* and *Lactobacillus murinus* were isolated and identified by the same method (fig. S6 and fig. S7). Frozen stocks of *Lactobacillus* including *L. reuteri*, *L. murinus*, and *L. johnsonii* (in PBS with 25% glycerol) were prepared, stored at −80 °C.

Bacterial culture conditions

Lactobacillus, including *L. reuteri*, *L. murinus* and *L. johnsonii*, were inoculated anaerobically in an anaerobic incubator (85% nitrogen, 10% carbon dioxide, and 5% hydrogen) as single colonies in 5 ml of MRS medium at 37°C, respectively.

Man Rogosa Sharpe Medium (MRS) containing (per liter): Peptone 10g, Beef Cream 5g, Yeast cream 4g, Glucose 5g, Sodium acetate 5g, Dipotassium hydrogen phosphate 2g, Tween-80 1.08g, Magnesium sulfate ($MgSO_4•7H_2O$) 0.2g, Triammonium citrate 2g, Manganese sulfate ($MnSO_4•4H_2O$) 0.05g were added.

Luria-Bertani Medium (LB) for *E. coli* containing (per liter): Yeast extact 5g, Peptone 10g, NaCl 10g were added.

YCFA **[Duncan, Sylvia H, et al. "Growth requirements and fermentation products of Fusobacterium prausnitzii, and a proposal to reclassify it as Faecalibacterium prausnitzii gen. nov. comb. nov." *International Journal of Systematic and Evolutionary Microbiology* (2002)]** (per liter): 10 g casitone, 5g glucose, 2.5 g yeast extract, 4 g $NaHCO_3$, 1 g cysteine, 450 mg K_2HPO_4, 450 mg KH_2PO_4, 900 mg NaCl, 90 mg $MgSO_2•7H_2O$, 90 mg $CaCl_2$, 1 mg resazurin, 10 mg haemin, 10 μg biotin, 10 μg cobalamin, 30 μg *p*-aminobenzoic acid, 50 μg folic acid and 150 μg pyridoxamine. Final concentrations of short-chain fatty acids (SCFA) in the medium were 33 mM acetate, 9 mM propionate and 1 mM each of isobutyrate, isovalerate and valerate. After autoclaved, Heat labile vitamins were added (per liter): 50 μg thiamine and 50 μg riboflavin.

***In vitro* fermentation and microbiota analysis**

A simulated intestinal model was used to explore the effect of GPS on a stabilized gut microbial community *in vitro*. The luminal chamber was continuously stirred at 220 rpm. and kept at 37°C. The luminal chamber was seeded with 50 ml of

broad-range bacteriological medium YCFA Medium in the presence or absence of GPS (5mg/ml). The simulated intestinal model system was inoculated with an aliquot of the fecal sample from each donor individually. A preculture was prepared anaerobically in a chamber (5% hydrogen, 10% carbon dioxide, and 85% nitrogen) by adding 2% fecal material to 5 ml of YCFA broth as described above **[Li, Xiaojun, et al. "Protein-Bound β-glucan from Coriolus Versicolor has Potential for Use Against Obesity." *Molecular Nutrition & Food Research* (2019).].** After ten hours of incubation, the culture solution was quickly frozen by liquid nitrogen and transported to Beijing Biomarker Technologies Co., Ltd. (Beijing, China) by dry ice for 16s rDNA sequencing.

Growth curve

Measurements were made based on a protocol reported previously **[Wu Hao, et al. "Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug." *Nature Medicine* (2017)]**. After incubation for 14 h, each preculture was inoculated in freshly prepared LB broth or MRS broth with or without 5 mg/ml GPS in a 10 ml Erlenmeyer flask with filter at a concentration (v/v) of 0.5% *E. coli* or 1% *L. reuteri* in a volume of 5 ml. The effect of GPS on bacterial-growth kinetics was analyzed by flat colony counting method. The growth-curve data over 10 h for *E. coli* and 13 h for *L. reuteri* were analyzed using GraphPad Prism software.

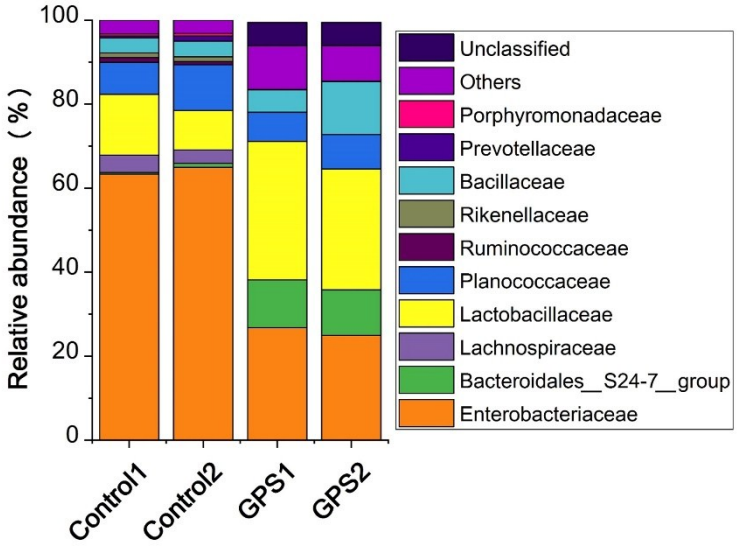

Fig. S1: Taxonomic distributions of bacteria in vitro fermentation experiments from 16S rDNA sequencing data.

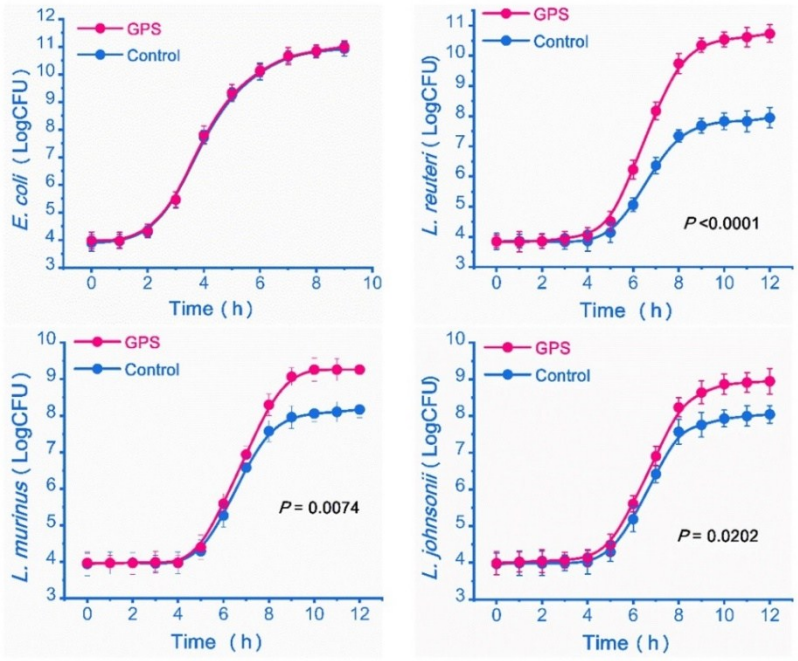

Fig. S2: Growth of *E. coli*, *L. reuteri*, *L. murinus*, *L. johnsonii* and *L. rhamnosus* as single cultures in the presence or absence of GPS (with six technical replicates and error bars represent ± SD).

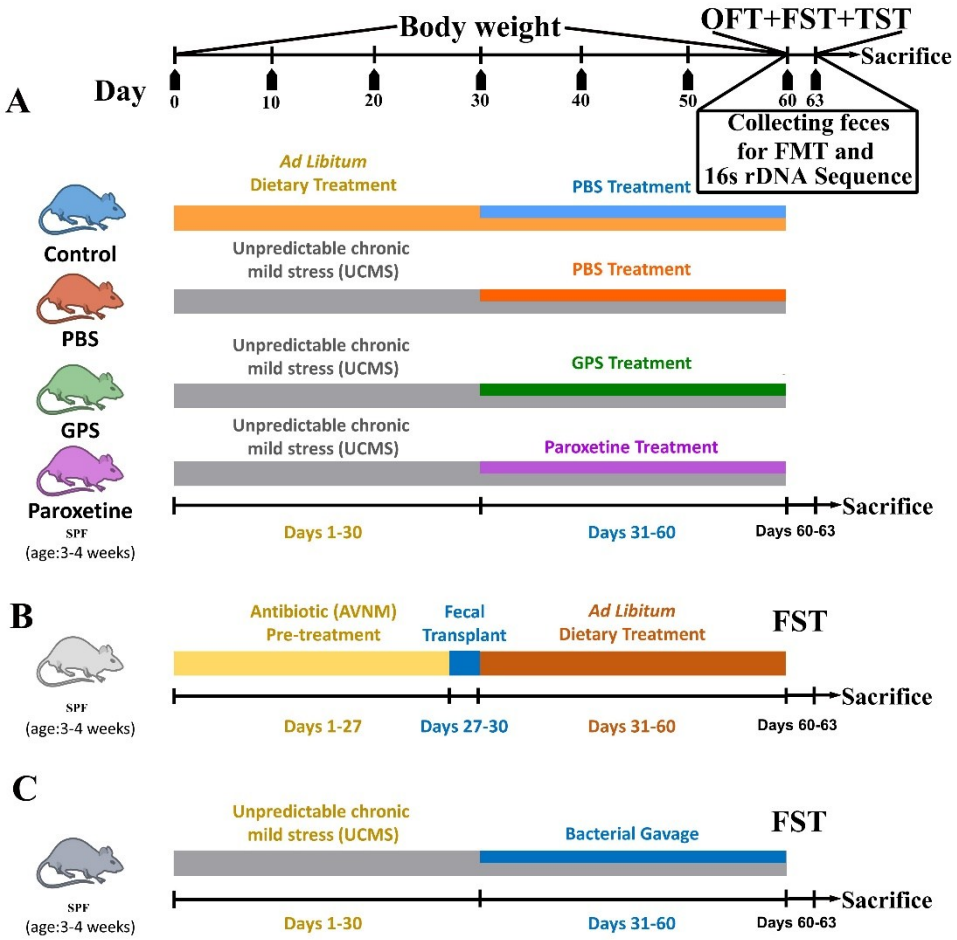

Fig. S3: Schematic diagram of the experimental procedure. (A) Depression modeling and GPS treatment flow chart. (B) Flow chart of fecal transplantation experiments. (C) Flow chart of *L. reuteri* supplement experiments.

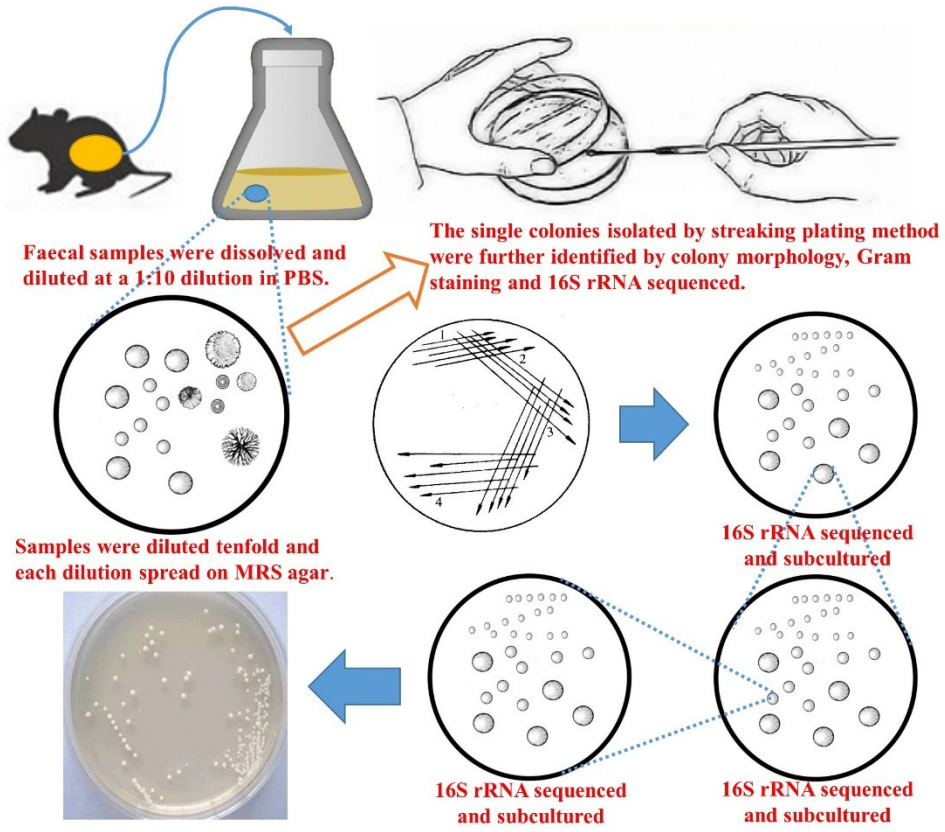

Fig. S4: Flow chart of purification and identification of *Lactobacillus*.

Fig. S5: Colony morphology (A) of *Lactobacillus reuteri* and its Gram staining (B) and BLAST comparison results (C).

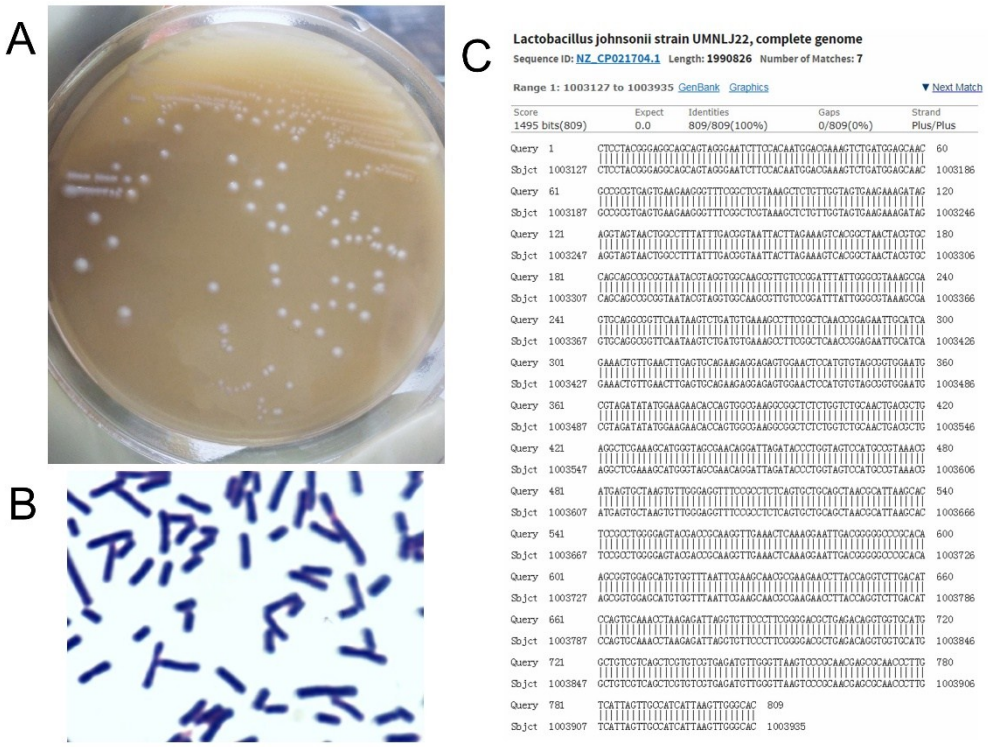

Fig. S6: Colony morphology (A) of *Lactobacillus johnsonii* and its Gram staining (B) and BLAST comparison results (C).

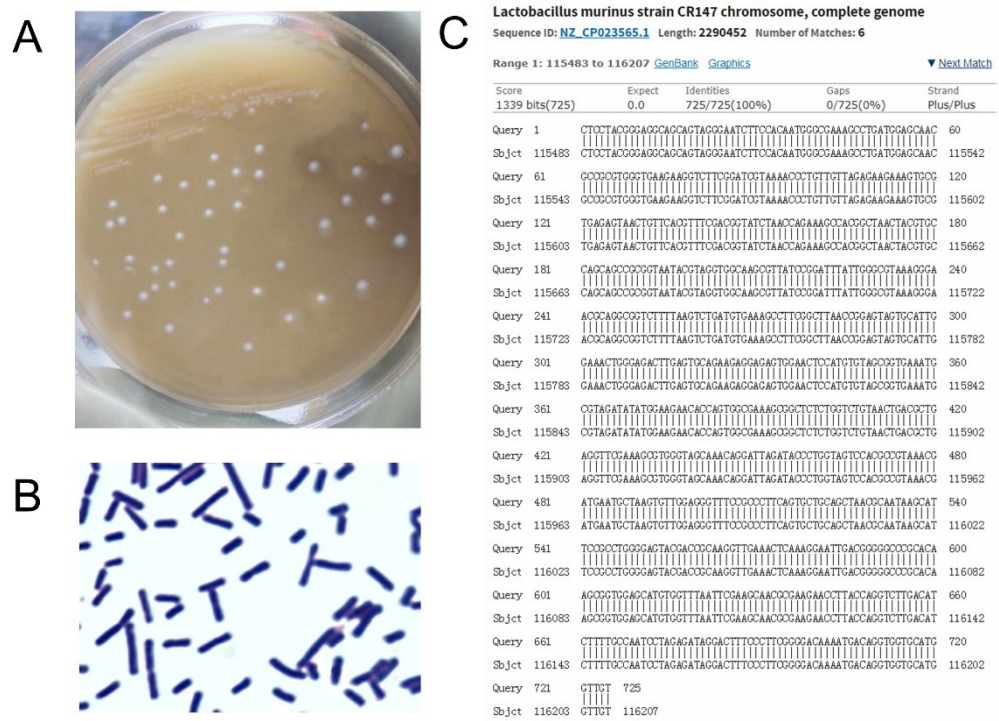

Fig. S7: Colony morphology (A) of *Lactobacillus murinus* and its Gram staining (B) and BLAST comparison results (C).

Table S1: The number of mice in different groups of depression modeling and GPS treatment

	Control	UCMS	GPS	Paroxetine	Total
Behavioral test	10	10	10	10	40
Elisa test	10	10	10	10	40
Immunofluorescence	5	5	5	5	20
Total	10	10	10	10	40

Table S2: The number of mice in different groups of FMT.

	Abx-UCMS-FMT	Abx-GPS-FMT	Total
Behavioral test	8	8	16
Elisa test	8	8	16
Immunofluorescence	4	4	8
Total	8	8	16

Table S3: The number of mice in different groups of *L. reuteri* supplement experiments.

	Control	UCMS	*L. reuteri*	Total
Behavioral test	8	8	8	24
Elisa test	8	8	8	24
Immunofluorescence	4	4	4	12
Total	8	8	8	24