# Supplemental Information

## Oral administration of *Lactococcus lactis* WHH2078 alleviates depressive and anxiety symptoms in mice with induced chronic stress

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## SUPPLEMENTARY METHODS

### Chronic stress-induced depressive model

In this study, the chronic unpredictable mild stress (CUMS) model was used, which is a classical model for inducing depression in rodents and is validated by various preclinical studies [1-4]. The CUMS stressors used in this study are mild and have no fatal risk.

**1. Tube restraint**

The mouse was placed gently in a 50 mL plastic tube with multiple holes at the far end to allow breathing, Bedding was added into the plastic tube to make the mouse unable to move, then the plastic tube was covered slightly. The mouse returned to its cage after 6 h of restraint.

**2. Food deprivation**

The food was removed from the mouse cage for 24 h.

**3. Water deprivation**

The bottle of water was removed from the mouse cage for 24 h.

**4. Wet bedding**

A hundred mL of sterile water was added to the bedding in the mouse cage and replaced with new dry bedding after 24 h.

**5. No bedding**

The bedding in the mouse cage was removed for 24 h.

**6. 45° cage tilt**

The mouse cage was tilted at 45° for 24 h.

**7. Clip tail**

The mouse tail was clamped by a paper clip at 2-3 cm from the tail tip for 1 min each time, 5 times per day.

**8. Continuous illumination**

The mouse cage was moved into an environment with continuous white light for 24 h. The illumination intensity is 500 lux.

**9. Swimming in cold water**

A glass tank (50 cm × 50 cm × 50 cm) with 30 cm deep water (12°C) was prepared. The mouse was placed into the glass tank for 5 min; after swimming, the mouse was returned to the cage with dry bedding immediately.

**Behavioral tests**

**1. Sucrose preference test (SPT)**

Sucrose preference test was performed following the procedures described previously with minor modification [5]. Briefly, before testing, mice were habituated in a cage with 1% sucrose and water bottles randomly assigned for 24 h. After the adaptation, mice were fasted for 24 h, then were separated in a single per cage with two drinking bottles for 24 h: one containing 1% sucrose and another containing sterile water. Each bottle was weighed before and after the test. The sucrose preference ratio (SPR) was calculated as follows: SPR (%) = sucrose intake/total fluid intake (including sucrose and water intake) ×100%

**2. Tail suspension test (TST)**

Tail suspension test was conducted according to a previous method [6]. Briefly, the mouse tail was taped with adhesive tape to a suspension bar, which is 50 cm high from the floor. Locomotor activity is monitored by the video tracking system (JLBehv-STGM-1, Yuyan instruments Co. Ltd., Shanghai, China) for 5 min [7-9]. The immobility time was scored. Immobility is defined as the absence of voluntary or escape-orientated movement.

**3. Forced swim test (FST)**

Forced swim test was performed according to a previous method [10]. Briefly, a glass cylinder filled with 30 cm deep water at 25 ± 2 ℃ was prepared. One day before testing, mice were placed into the glass cylinder to train swimming for 15 min. On the testing day, mice were placed into the same glass cylinder to swim for 5 min [7-9]. Locomotor activity is monitored using a video tracking system (JLBehv-FSM-1, Yuyan instruments Co. Ltd., Shanghai, China), which calculates the immobility time of each mouse during the test period. Immobility is defined as the motionless situation except floating motions required to keep the head above the water [3].

**4. Open field test (OFT)**

Open field test was carried out following the procedures described previously [11]. Briefly, a cube with a square arena (50 cm × 50 cm × 50 cm) enclosed by continuous black walls made of plexiglass was prepared. Mice were placed into the middle of the area facing the wall, and locomotor activity was monitored using a video tracking system (JLBehv-LAM-1, Yuyan instruments Co. Ltd., Shanghai, China) for 5 minutes. The time spent in the central area (25 cm × 25 cm) and the distance traveled were measured.

## SUPPLEMENTARY TABLES

**Table S1.** The basic information of candidate *Lactococcus lactis* strains

| Strains | Species | Origin | Collection Location | Isolation Time | Storage Condition |
|---|---|---|---|---|---|
| WHH695 | *Lactococcus lactis subsp. cremoris* | Yogurt | Xining, Qinghai, China | June 2013 | -80°C in glycerol |
| WHH822 | *Lactococcus lactis subsp.lactis* | Yogurt | Bayannaoer, Inner Mongolia, China | June 2014 | -80°C in glycerol |
| WHH826 | *Lactococcus lactis subsp.lactis* | Yogurt | Xining, Qinghai, China | October 2014 | -80°C in glycerol |
| WHH853 | *Lactococcus lactis subsp. cremoris* | Yogurt | Bayannaoer, Inner Mongolia, China | June 2015 | -80°C in glycerol |
| WHH879 | *Lactococcus lactis subsp.lactis* | Cheese | Linhe, Inner Mongolia, China | June 2015 | -80°C in glycerol |
| WHH888 | *Lactococcus lactis subsp.lactis* | Yogurt | Bayannaoer, Inner Mongolia, China | October 2015 | -80°C in glycerol |
| WHH889 | *Lactococcus lactis subsp. cremoris* | Cheese | Bayannaoer, Inner Mongolia, China | October 2015 | -80°C in glycerol |
| WHH893 | *Lactococcus lactis subsp.lactis* | Yogurt | Bayannaoer, Inner Mongolia, China | October 2015 | -80°C in glycerol |
| WHH912 | *Lactococcus lactis subsp.lactis* | Yogurt | Linhe, Inner Mongolia, China | June 2016 | -80°C in glycerol |
| WHH924 | *Lactococcus lactis subsp.lactis* | Yogurt | Linhe, Inner Mongolia, China | June 2016 | -80°C in glycerol |
| WHH925 | *Lactococcus lactis subsp.lactis* | Cheese | Bayannaoer, Inner Mongolia, China | June 2017 | -80°C in glycerol |
| WHH932 | *Lactococcus lactis subsp.lactis* | Yogurt | Bayannaoer, Inner Mongolia, China | June 2017 | -80°C in glycerol |
| WHH1034 | *Lactococcus lactis subsp. cremoris* | Cheese | Linhe, Inner Mongolia, China | October 2017 | -80°C in glycerol |
| WHH2078 | *Lactococcus lactis subsp.lactis* | Yogurt | Shigatse, Tibet, China | June 2018 | -80°C in glycerol |
| WHH2080 | *Lactococcus lactis subsp.lactis* | Cheese | Shigatse, Tibet, China | June 2018 | -80°C in glycerol |
| WHH3683 | *Lactococcus lactis subsp. cremoris* | Yogurt | Yili, Xinjiang, China | October 2018 | -80°C in glycerol |
| WHH3684 | *Lactococcus lactis subsp.lactis* | Yogurt | Yili, Xinjiang, China | October 2018 | -80°C in glycerol |
| WHH3823 | *Lactococcus lactis subsp. cremoris* | Yogurt | Yili, Xinjiang, China | October 2018 | -80°C in glycerol |
| WHH3831 | *Lactococcus lactis subsp.lactis* | Yogurt | Yili, Xinjiang, China | October 2018 | -80°C in glycerol |

**Table S2.** The sensitivity, precision, and accuracy of the HPLC and ELISA methods used for neurochemical factors detection.

| Items | Sensitivity[1] | Precision (%)[2] | Accuracy (%)[3] |
|---|---|---|---|
| **HPLC** | | | |
| RIN14B cell supernatant 5-HTP | 50.61 | 1.71 | 4.88 |
| Hippocampal 5-HTP | 43.20 | 2.97 | 6.29 |
| Serum 5-HTP | 39.62 | 1.43 | 5.19 |
| Colonic 5-HTP | 64.21 | 4.17 | 6.24 |
| **ELISA** | | | |
| Hippocampal 5-HT | 44.30 | 2.88 | 5.22 |
| Hippocampus mBDNF | 50.62 | 3.15 | 4.50 |
| Serum corticosterone | 82.27 | 3.24 | 5.51 |

[1] Neurochemical factors standards in the range of 1 pg/mL to 10 μg/mL and the assays are linear between 100 pg/mL and 1 μg/mL for 5-HTP ($r^2 = 0.9996$), 5-HT ($r^2 = 0.9992$), mBDNF ($r^2 = 0.9991$), and corticosterone ($r^2 = 0.9996$). The sensitivity of the method is indicated by the slope of the neurochemical factor standard curve.

[2] The precision (agreement between replicate measurements) of the method, as evaluated by the relative deviation (mean of absolute deviation/mean of replicate measurements × 100%). A value below 5 % indicates the method is highly precise.

[3] The accuracy (the closeness of an experimental value to the true value) of the method, as determined with known amounts of neurochemical factors standards and expressed as the relative errors [(measurement value – true value)/true value × 100%)]. A value below 10 % indicates the method is highly accurate.

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