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Original Article

Multi-Probiotics ameliorate Major depressive disorder and accompanying gastrointestinal syndromes via serotonergic system regulation

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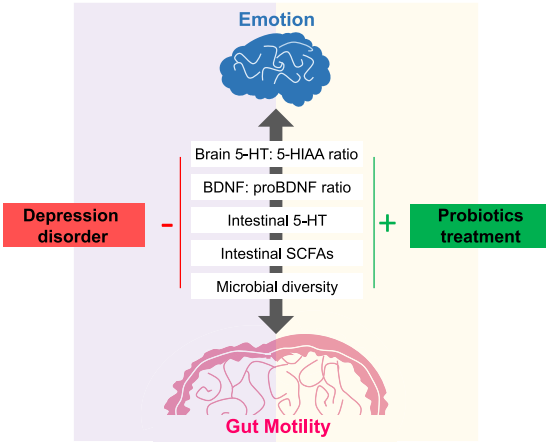
HIGHLIGHTS

- Probiotics reduced the depression rating scores of MDD patients.
- Probiotics ameliorate the gastrointestinal problems of MDD patients.
- Probiotics caused slight perturbation on the patients' gut microbiome.
- Probiotics improved the gut motility of stressed mice.
- Probiotics' gut-brain beneficial effect correlates to their regulation of serotonin.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Major depressive disorder (MDD) is a leading global psychiatric disease. MDD is highly comorbid with gastrointestinal abnormalities, such as gut motility dysfunction. An effective strategy to manage depression and its accompanying gastrointestinal symptoms is warranted.
Objectives: Three probiotic strains (*Bifidobacterium breve* CCFM1025, *Bifidobacterium longum* CCFM687, and *Pediococcus acidilactici* CCFM6432) had previously been validated in mice to possess antidepressant-like potential. This study investigated the potential psychotropic effects of a combined three-strain probiotic intervention for human MDD patients. The mechanism of action was further investigated in the stress-induced depression mice model.

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Probiotics
Serotonin
Stress

Methods: MDD patients were given a freeze-dried, mixed probiotic formula for four weeks. The patients' psychometric and gastrointestinal conditions were evaluated using clinical rating scales before and after treatment. Their gut microbiome was also analysed using 16S rRNA gene amplicon sequencing. The mechanisms underlying the beneficial probiotic effects were determined using a chronic stress-induced depressive mouse model.

Results: Multi-probiotics significantly reduced depression scores, and to a greater extent than the placebo (based on the Hamilton Depression Rating, Montgomery-Asberg Depression Rating, and Brief Psychiatric Rating Scales). Multi-probiotics also significantly improved the patients' gastrointestinal functions (based on self-evaluation using the Gastrointestinal Symptom Rating Scale). Serotonergic system modification was demonstrated as the key mechanism behind the probiotics' benefits for the brain and the gut.

Conclusion: Our findings suggest a novel and promising treatment to manage MDD and accompanying gut motility problems, and provide options for treating other gut-brain axis-related disorders.

Introduction

Major depressive disorder (MDD) is a leading global psychiatric problem. MDD is a heterogeneous disease and its pathogenesis is correlated with genetic background, lifestyle, and environmental influences [1]. MDD is generally characterised by long-term emotional distress, sleep disorders, and sexual dysfunction, which in turn causes social functioning problems [2]. MDD also occurs with many chronic diseases or severe bodily injury, and leads to high rates of suicide or suicide attempts [3,4]. To date, over 350 million people worldwide have suffered from depressive disorders, and an explosion of MDD cases has occurred during the global COVID-19 pandemic, which further worsens the disease burden [5,6]. The demand for novel and efficient therapeutic solutions for MDD is therefore urgent, regardless of the pandemic.

MDD is highly comorbid with gastrointestinal abnormalities, especially gut motility dysfunction. An epidemiological survey based on 9,000 patients with depression or anxiety showed that the occurrence of constipation was approximately 29.8% [7]. Stress, which is a common cause of depression, has also been proven to induce defecation disorders [8]. Stress can significantly affect the neuroendocrine system's function, including impairing the secretion of hormones involved in indigestion and peristalsis, inhibiting the parasympathetic nerve's innervation on the colon, and inducing gut microbiome dysbiosis, which are collectively represented as clinical constipation symptoms [9,10]. In addition, constipation is a primary side effect of psychotropic drugs because of the reduction in serotonin-selective reuptake transporter (SERT) activity [11]. Due to the high incidence of constipation, many depressed patients have to use adjunctive laxatives [12]. However, the long-term use of laxatives forms drug dependence, severely damages intestinal barrier function, and even causes colon melanosis [13,14]. Considering the lag in medicinal development, new therapies that can synergistically treat depression and constipation should be developed.

In recent years, the gut microbiota has been well investigated for its role in regulating enteric and central nervous system functions [15,16]. The gut microbiota also links with the host's immune system and hormonal system to yield a long-distance effect on the brain via microbial metabolites [17]. With growing understanding of the microbiome-gut-brain axis, various gut microbiome-oriented nutritional supplements such as probiotics and prebiotics have been developed to adjunctly solve both psychological and gastrointestinal problems [18]. In our previous study, *Bifidobacterium breve* CCFM1025, *Bifidobacterium longum* CCFM687, and *Pediococcus acidilactici* CCFM6432 were isolated from healthy human faeces and individually shown to have anti-depressive and anxiolytic effects in mice [19–21]. The mechanisms of action were correlated with serotonergic system and gut microbiome modulations [19,20]. However, the gastrointestinal effects of these probiotics have not been thoroughly investigated. The combined mixture of these three probiotic strains was further investigated in this study to verify their effect on both mental and gastrointestinal symptoms in MDD patients, and the probiotics' mechanism of action was further validated via animal experiment. This study aimed to demonstrate the psychotropic potential of probiotics and provide a guide for their translational and clinical applications.

Material and methods

Ethics statement

This study involved both human and animal experiments. The human trial was approved by the Research Ethics Committee of the Tinghu People's Hospital (Yancheng, China; Approval Code: ET2020076) and documented in the Chinese Clinical Trial Registry (No. ChiCTR2100046321). All patients provided written informed consent before enrolment. All experiments involving animals were conducted according to the ethical policies and procedures approved by the Experimental Animal Ethics Committee of Jiangnan University (approval number: JN.No2020930c0841204 [242]), following the guidelines of the European Union's *Directive 2010/63/EU*.

Clinical trial

Participants

The inclusion criteria were mild to moderate MDD in patients aged over 18 years without restrictions on antidepressant drugs (medication information of each patient is shown in Table S1). The exclusion criteria were the co-occurrence of other mental disorders as diagnosed according to I DSM-IV criteria, refractory depression, depression with severe suicide and self-injury tendencies, schizophrenia, bipolar disorder, neurodegenerative diseases, severe physical diseases (such as AIDS, epilepsy, heart disease, and hyperthyroidism), and pregnant females or those who were breastfeeding.

Power analysis

A priori sample size estimation was calculated using the G*Power 3.1 program (Kiel, Germany). Anticipated effect sizes were derived from a previous probiotic clinical intervention study in which treatments for severe depression were compared using the Hamilton Depression Rating Scale (the effect size was 0.41) [22]. In total, at least 20 participants per group were calculated as necessary to detect a significant interaction effect using two-way ANOVA with standard alpha (0.05) and beta (0.8) values. However, due to the COVID-19 caused lockdown of the hospital, only 28 participants completed the trial successfully.

Experimental design

The study was a two-arm parallel design, placebo-controlled, double-blinded randomised controlled trial (RCT). Forty MDD patients volunteered to participate in this study initially and were randomly assigned into *Placebo* (n = 13) and *Multi-probiotics* (n = 15) groups. The demographic characteristics of participants are shown in Table 1.

Interventions

Patients in the probiotics group consumed a probiotic formula (supplied in the form of a sachet) produced by Shisheng Yisheng Co. Ltd. (Yangzhou, China). The formula contained a mixture of freeze-dried *B. breve* CCFM1025, *B. longum* CCFM687, and *P. acidilactici* CCFM6432. Each strain had a viable bacteria count of 4×10^9 CFU/g, with a proportion of 1:1:1 in the mixture. Maltodextrin was used as the excipient and placebo, and the taste, colour, and size of the placebo sachet were matched to the probiotic sachet. The intervention persisted for four weeks (Fig. 1A).

Psychometric and gastrointestinal evaluation

Three psychometric questionnaires were completed by the doctors during patient examination, including the Hamilton Depression Rating Scale (HAMDS) [23], the Montgomery-Asberg Depression Rating Scale (MADRS) [24], and the Brief Psychiatric Rating Scale (BPRS) [25]. Patients completed the Gastrointestinal Symptom Rating Scale (GSRS), a self-completed questionnaire of 16 items in a multiple-choice format [26]. Items were classified into 'Bowel dysfunction syndrome', 'Indigestion syndrome', 'Dyspeptic syndrome', and 'Abdominal pain syndrome' [27].

Faecal 16S rRNA sequencing and bioinformatic analyses

Fresh faecal samples were collected from all participants at the baseline and endpoint. 16S rRNA gene sequencing and bioinformatic analysis were performed as previously reported [19,28]. Specifically, the gut microbial alpha-diversity was evaluated using the Shannon index and observed operational taxonomic units (OTUs). In addition, Aitchison distance was used to describe the compositional nature of microbiome datasets (beta diversity), and perturbations caused by the placebo or probiotics on the gut microbiome were determined by volatility as previously described [29].

Animal experiment

Grouping, behavioural tests, and gut motility tests

The animal experiment design is shown in Fig. 3A. The depressed animal model was established using chronic unpredictable mild stress (CUMS) with male C57BL/6 mice (6 weeks old, ~18–20 g, purchased from the Shanghai Laboratory Animal Center (SLAC), Experimental Animal Co., Ltd, Shanghai, China) as previously described [20,30]. *B. breve* CCFM1025, *B. longum* CCFM687, and *P. acidilactici* CCFM6432 (1:1:1) were given at a dosage of 10^9 CFU (in 0.4 mL) per day. Behavioral tests, including the forced swim test and tail suspension test, were performed as previously reported [20,30]. Gut motility tests, including gastrointestinal transit and the time of the first defecation, were performed using the methods previously described [31,32].

Table 1
Groups and demographic characteristics of participants.

Measure	Placebo group	Multi-probiotics group
Age	48.08 ± 18.61	38.87 ± 17.62
Female percentage	76.92%	66.67%
Antidepressants user	69.23%	73.33%
Sample size	13	15

5-Hydroxytryptamine (5-HT) turnover

5-HT (serotonin) and 5-hydroxyindoleacetic acid (5-HIAA) in the prefrontal cortex (PFC), brainstem, and colon were quantified using high-performance liquid chromatography (HPLC) as previously described [20,30]. The 5-HT and 5-HIAA standards were purchased from Sigma-Aldrich, Shanghai, China. The 5-HT turnover was defined as the 5-HIAA: 5-HT ratio.

Endocrine hormones

Hypothalamus corticotropin-releasing factor (CRF) and serum corticosterone were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (R&D Systems, Minnesota, USA).

Hippocampal brain-derived neurotrophic factor (BDNF)

Immunohistochemical detection of proBDNF (1:100, sc-65514, Santa Cruz Biotechnology Inc., Texas, USA), and immunofluorescence detection of mature BDNF (1:200, A4873, ABclonal Technology, Wuhan, China) on the hippocampal sections (4 μm) were performed as previously described. The target proteins were quantified using Image-Pro Plus software (Media Cybernetics Inc., Rockville, Maryland, USA).

Stool moisture

Fresh stool samples were collected in pre-weighed tubes (t). After weighing [w1], the opened tubes were placed in a hot air oven at 85 °C for 24 h. The tube was once again weighed [w2], and stool moisture was calculated as follows: (w1 – w2)/(w1 – t) × 100%.

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA extraction and the determination of *Tph1* and *Slc6a4* gene expression were performed as previously reported [13].

Caecal short-chain fatty acids (SCFAs)

Caecal SCFAs were determined by gas chromatography–mass spectrometry (GC–MS) using SCFA standards as references. Detailed methods have been previously reported [20,30].

Statistical analysis

Data were expressed as means with 95% confidence intervals (CI). Each biological data point was generated from three replicates. All data were checked for normality using the Shapiro-Wilk test before further analyses. For the clinical data, two-way analysis of variance (ANOVA) was performed between the pre-treatment and post-treatment dataset in each group, followed by Sidak's multiple comparisons. For the score change dataset, an unpaired Student's *t*-test was performed between the *Placebo* and *Multi-probiotics* groups, with Cohen's d to evaluate the effect size. For the animal experiment data, an unpaired Student's *t*-test was performed between *Non-stressed* and *Stressed*, and *Stressed* and *Multi-probiotics*. *P*-values are shown on the graphs. All *P*-values for multiple comparisons were adjusted by family-wise significance, and a 95% CI and a $P < .05$ were considered as statistically significant in all comparisons. Cohen's d and *post-hoc* power of each comparison in the animal experiment were listed in Table S4. Statistical analyses were performed using SPSS 22.0 and Prism 8.0.

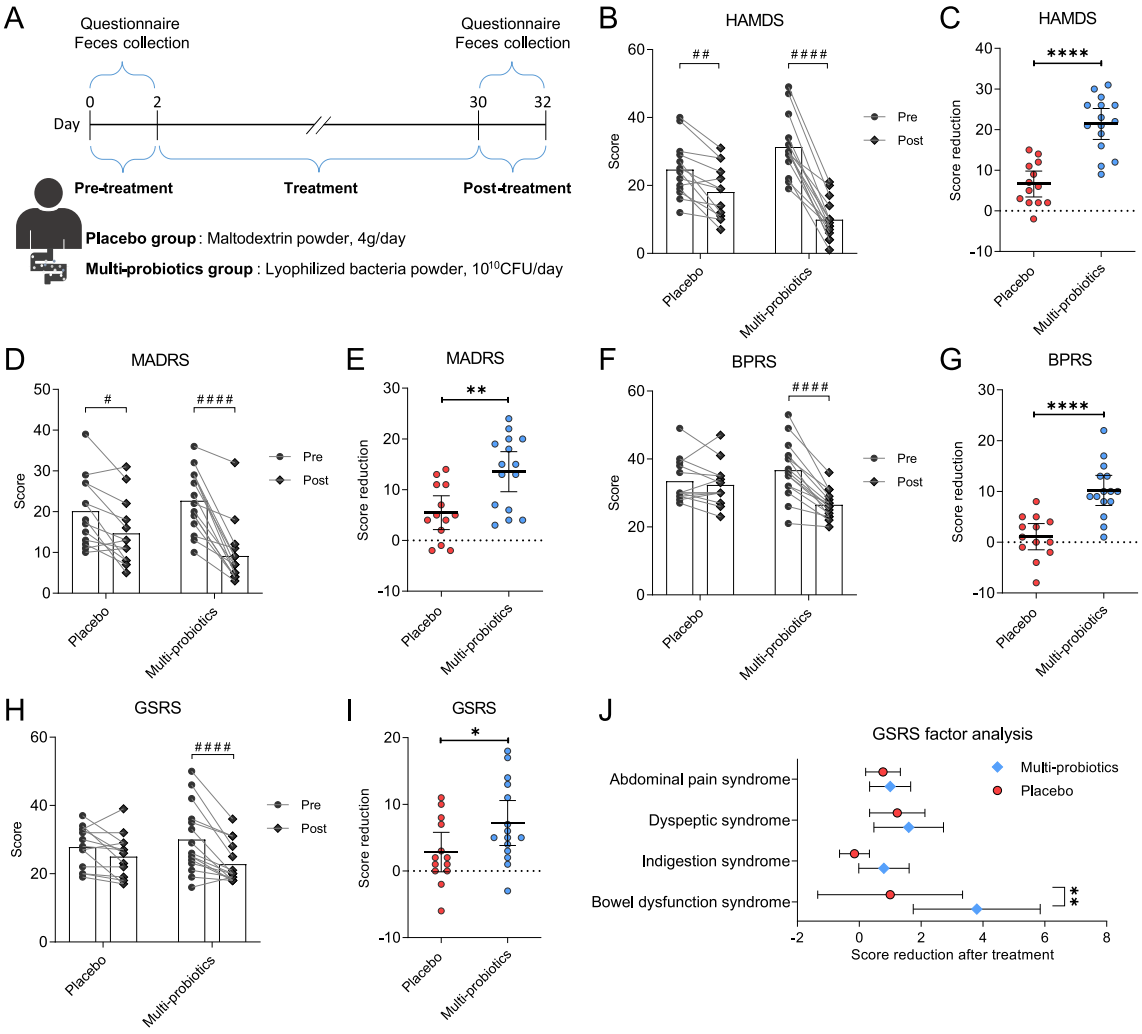

Fig. 1. Psychometric and gastrointestinal changes after multi-probiotics treatment. (A) Clinical experimental design. (B) Score changes of the Hamilton Depression Rating Scale (HAMDS) in the placebo and probiotic groups. (C) Score reduction of the HAMDS from the baseline. (D) Score changes of the Montgomery-Asberg Depression Rating Scale (MADRS) in the placebo and probiotic groups. (E) Score reduction of the MADRS from the baseline. (F) Score changes of the Brief Psychiatric Rating Scale (BPRS) in the placebo and probiotic groups. (G) Score reduction of the BPRS from the baseline. (H) Score changes of the Gastrointestinal Symptom Rating Scale (GSRS) in the placebo and probiotic groups. (G) Score reduction of the GSRS from the baseline. (J) GSRS factor analysis. $^{\#}P$ <.05, $^{\#\#}P$ <.01, $^{\#\#\#\#}P$ <.0001 in Sidak's multiple comparisons test after two-way ANOVA. $^{*}P$ <.05, $^{**}P$ <.01, $^{****}P$ <.0001 in the unpaired t-tests.

Results

Multi-probiotics ameliorate the emotional and gastrointestinal symptoms in MDD patients

The experiment schedule is shown in Fig. 1A. The HAMDS score significantly decreased after treatment in both the placebo (P =.001) and multi-probiotics (P <.001) groups (Fig. 1B). However, the score reduction in the multi-probiotics group was greater than that in the placebo group (d = 0.553, P <.001; Fig. 1C). Similarly, the MADRS score significantly decreased from the baseline ($P_{\text{-Placebo}}$ = 0.679, $P_{\text{-Probiotics}}$ < 0.001; Fig. 1D), and to a greater extent for the multi-probiotics group than the placebo group (d = 0.319, P =.003; Fig. 1E). The placebo failed to lower the BPRS score from the baseline (P =.679; Fig. 1F), while the multi-probiotics did significantly lower the BPRS score from the baseline (P <.001), and to a greater extent than the placebo group (d = 0.473, P <.001; Fig. 1G). Gastrointestinal abnormalities were self-evaluated using the GSRS. Multi-probiotics decreased the score from the baseline (P <.001; Fig. 1H), and the score reduction was significantly greater than in the placebo group (d = 0.198, P =.049; Fig. 1I). Factor analysis indicated that the 'bowel dysfunction syndrome' score change primarily contributed to the BPRS score difference (d = 0.184, P =.006; Fig. 1J). All statistical information is provided in Supplementary Tables S2 and S3, including the mean, standard deviation (SD), 95% CI of the mean, and the unpaired t-test results.

Multi-probiotics caused no significant perturbation in the participants' gut microbiome

The faecal microbiome of the placebo and multi-probiotics groups at the baseline and endpoint was determined by 16S rRNA sequencing. A significant difference in Shannon's alpha diversity was only observed between the *Probiotics-pre* and *Probiotics-post* group (P =.038; Fig. 2A). The number of observed OTUs was significantly increased by both placebo and probiotics ($P_{\text{-Placebo}}$ = 0.002, $P_{\text{-Probiotics}}$ < 0.001; Fig. 2B). However, no significant difference was detected in the pre-treatment vs post-treatment changes of alpha diversity between the placebo and probiotics groups ($P_{\text{-Shannon}}$ = 0.843; $P_{\text{-observed OTUs}}$ = 0.629; Fig. S1). Changes in the microbial beta diversity were determined by the volatility [23], which is defined as the Aitchison distance travelled over the inter-

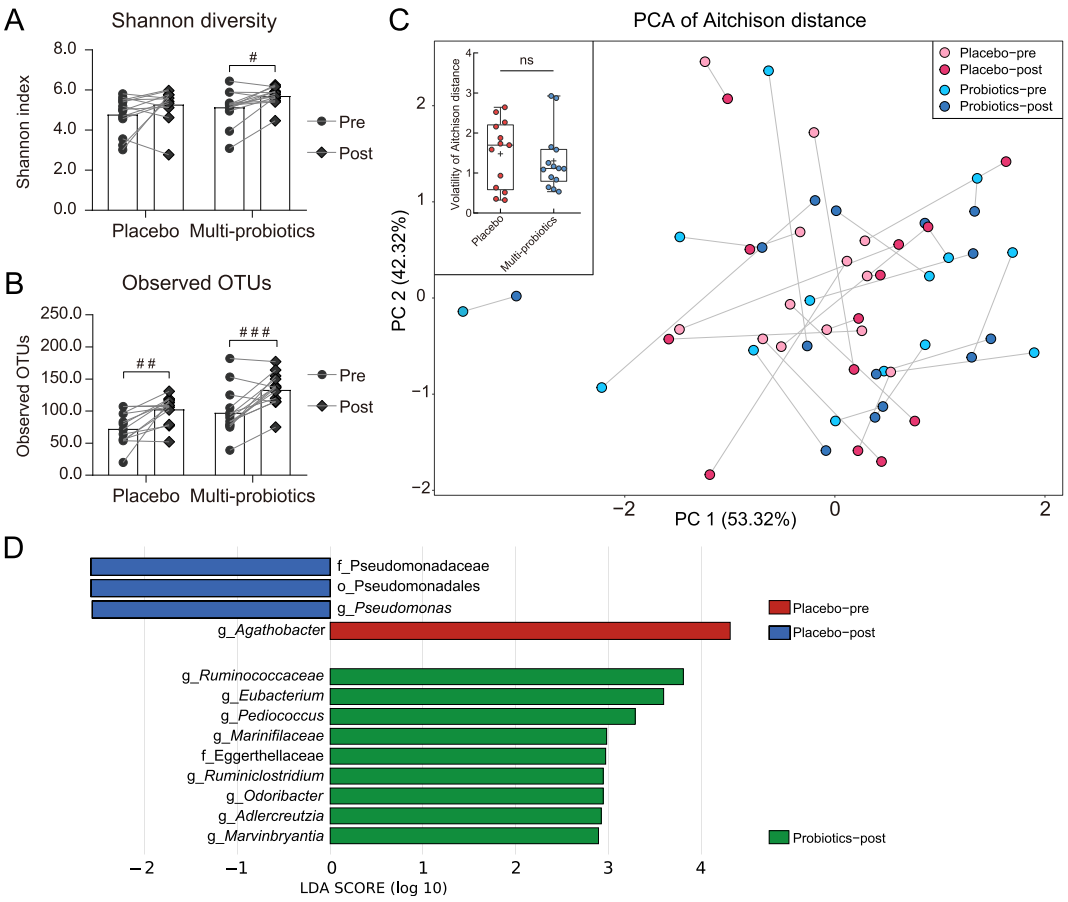

Fig. 2. Gut microbial alterations. (A) Shannon diversity. (B) Observed operational taxonomic units (OTUs). $^{\#}P$ <.05, $^{\#\#}P$ <.01, $^{\#\#\#}P$ <.001 in Sidak's multiple comparisons test after two-way ANOVA. (C) Principal component analysis (PCA) showing the microbial compositions of patients before and after the treatment. Lines link the same subject and show the distance travelled over time. (D) Differential microbial taxa before and after the treatment between placebo and probiotic groups, identified by linear discriminant analysis (LDA) effect size (LEfSe) analyses. Data were computed with an LDA score above 2.00 and a *P*-value below 0.05 for the factorial Kruskal-Wallis test.

vention period. No significant difference was observed (P =.568; Fig. 2C). Linear discriminant analysis effect size (LEfSe) analyses were performed to determine the differences in microbial features after treatment. Four taxa in the placebo group and nine taxa in the probiotics group were identified to have changed after treatment (Fig. 2D). In particular, *Ruminococcaceae* was previously reported to be deficient in MDD patients [33], and was increased by the probiotic treatment here.

Multi-probiotics induced antidepressant-like effects in chronically stressed mice

The mechanisms of the multi-probiotics' antidepressant-like effects were further investigated in stress-induced depressed mice (Fig. 3A). Similar to the results in human MDD patients, multi-probiotics significantly reduced the mice's depressive-like behaviour in the forced swim test (Fig. 3B) and tail suspension test (Fig. 3C). To evaluate the neuroendocrine changes under stress, the hypothalamic–pituitaryadrenal (HPA) axis related hormones were tested. The serum corticosterone (Fig. 3D) and hypothalamus corticotropin-releasing factor (CRF; Fig. 3E), which were elevated in the depressed mice, were normalised by probiotic treatment. As the most important neurotransmitter in regulating emotion, 5-HT level in the brain was also measured. The probiotic treatment reversed the enhanced 5HT turnover in the prefrontal cortex (Fig. 3F) and brainstem (Fig. 3G). *B. breve* CCFM1025 previously was proven with effect on regulating the neuronal plasticity [19]. Here the proBDNF and BDNF levels in hippocampus were detected to evaluate the multi-probiotics' correspondence effect. The depressed mice exhibited overexpressed proBDNF (Fig. 3H) and insufficient BDNF (Fig. 3I) in the hippocampus, and the probiotic intervention balanced the proBDNF/BDNF levels. The over expression of proBDNF- has also been proven to be correlated with long-term depression and may induce many psychiatric disorders [34,35]. The present results are similar with our previous findings, multi-probiotics could protect against the neuronal plasticity problems through suppress the expression of proBDNF in hippocampus.

Multi-probiotics improved the gut motility of depressed mice

The gut motility of the depressed mice was evaluated by measuring the gastrointestinal transit time of food and the time of the first defecation. As shown in Fig. 4A and B, gut motility was significantly impaired in the depressed mice, consistent with the MDD patients (Fig. 1J). Probiotics consumption significantly improved the defecation function. The depressed mice also had a lower colonic 5-HT level than healthy mice (Fig. 4C), which may have been caused by the enhanced gene expression of the serotonin transporter (*Slc6a4*; Fig. 4D) rather than by a biosynthesis deficit (i.e., low expression of *Tph1*, encoding the tryptophan hydroxylase 1; Fig. 4E). Probiotics reduced *Slc6a4* transcription without affecting *Tph1* (Fig. 4D and E). In addition, the probiotic treatment recovered the decreased stool moisture of the depressed mice (Fig. 4F), which may have been caused by the increased intestinal SCFA levels (Fig. 4G).

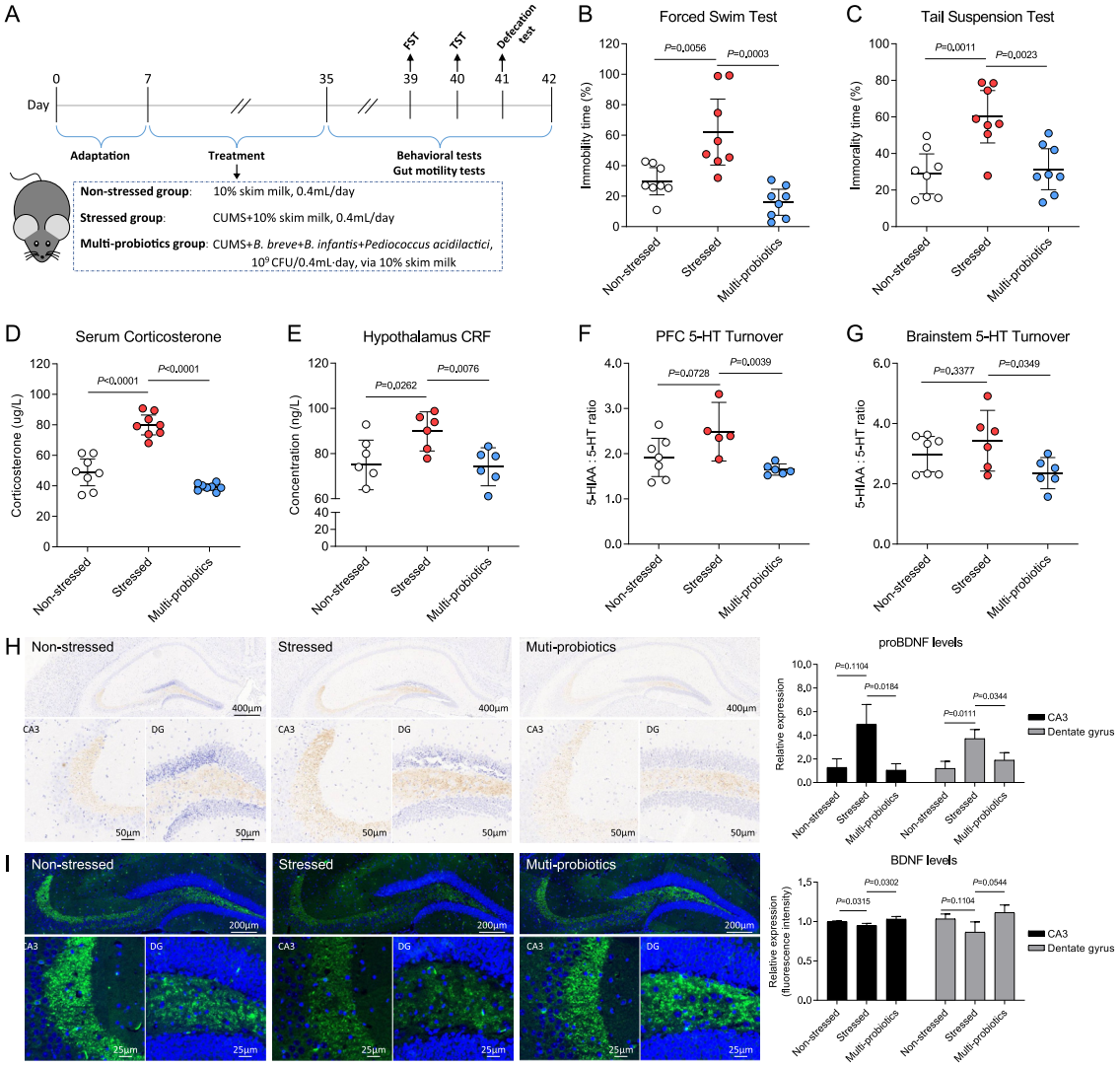

Fig. 3. Antidepressant-like effects and possible mechanisms of the probiotics. (A) Animal experimental schedule. (B) Forced swim test (n = 8 in each group). (C) Tail suspension test (n = 8 in each group). (D) Basal serum corticosterone levels (n = 8 in each group). (E) Hypothalamus corticotropin-releasing factor (CRF) levels (n = 6 in each group). (F) 5-HT turnover in the prefrontal cortex (n = 5–7 in each group). (G) 5-HT turnover in the brainstem (n = 6–7 in each group). (H) Immunohistochemistry examination of hippocampal proBDNF levels. The left panel shows representative staining of the target protein. Histogram bars indicate the relative expression of proBDNF to the *Non-stressed* group (n = 3 in each group). (I) Immunofluorescence staining of mature BDNF levels in the hippocampus. The nucleus was stained with 4′,6-diamidino-2-phenylindole (DAPI, blue), and BDNF was labelled with fluorescein (FITC, green). Histogram bars indicate the relative expression of BDNF to the *Non-stressed* group (n = 3 in each group).

Discussion

A combination of three probiotics was investigated in this study for their clinical antidepressant-like capacities. Multi-probiotics showed a better antidepressant-like effect than placebo. Based on further investigation in animals, the mechanisms possibly correlated to the modulation of HPA axis-related hormones, brain serotonergic systems, and neuronal plasticity. The findings are consistent with our previous report of each strain's mechanism of action [19–21].

The majority of previous clinical trials evaluating the mood-regulating effects of probiotics have been performed in healthy cohorts or patients with irritable bowel syndrome. The effects of probiotics on MDD-comorbid gastrointestinal dysfunction has rarely been investigated [36,37]. Unlike previous studies, the efficacy of combined probiotics was manifested in this study, as shown by their ability to reduce the self-rating scale scores (Fig. 1C, E, G, I, and J). From the differences in statistical significance (*P*-values) and effect size (Cohen's d) between the probiotics and placebo groups, it is clear that probiotics demonstrated a better efficacy to mitigate both depressive symptoms and gastrointestinal dysfunction than the placebo. Considering limitations in the invasive sampling of patients, the underlying mechanisms were further investigated in a stress-induced mouse model. Our findings indicated that alterations in the brain's 5-HT turnover and the BDNF, intestinal 5-HT, and SCFA levels were involved in the beneficial effects of probiotics.

Since the mid-20th century, a lack of 5-HT has been hypothesised as a cause of depression [38]. First-line antidepressants developed based on this theory, such as fluoxetine, paroxetine, and citalopram, are still in use [39]. Our previous study demonstrated that *B. breve* CCFM1025 and *B. longum* CCFM687 could stimulate 5-hydroxytryptophan and 5-HT production in the gut, facilitating the whole body's 5-HT circulation [20,30]. Previous animal results indicated that microbiome changes are key consequences of probiotic treatment, and they may causally affect colonic *Tph1* expression [19]. However, in this study we found limited perturbation of the human faecal microbiome by probiotics.

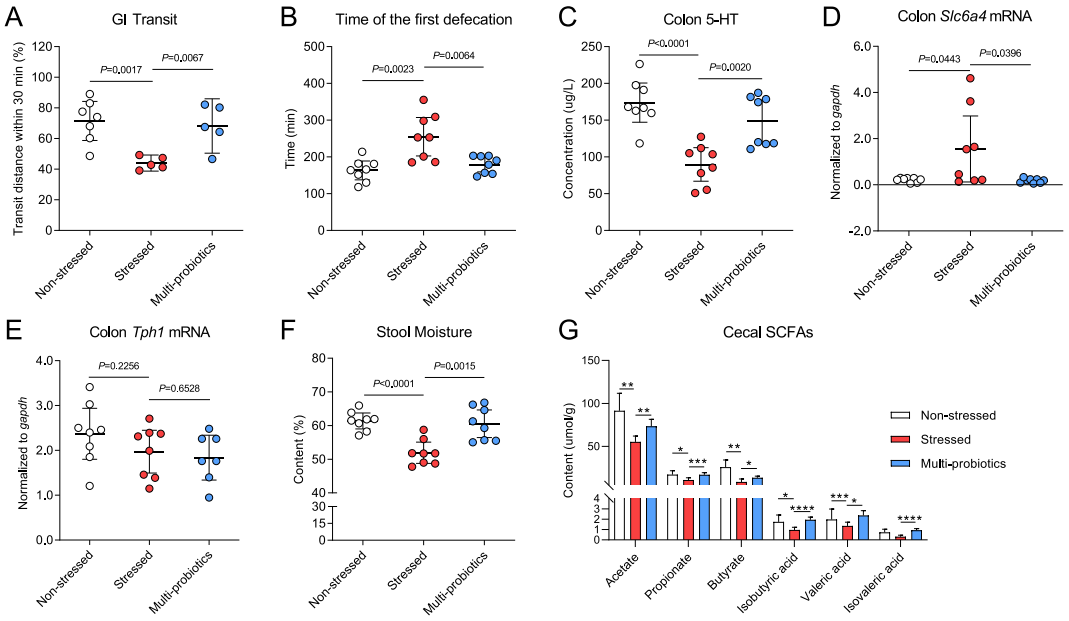

Fig. 4. Gastrointestinal effects and possible probiotic mechanisms. (A) Gastrointestinal (GI) transit test. Bars indicate the food transiting distance (percentage of the whole intestine) within 30 min after gavage (n = 5–7 in each group). (B) Time of the first coloured defecation after gavage (n = 8 in each group). (C) 5-HT levels in colon tissue (n = 8 in each group). (D) Transcriptional levels of the colonic serotonin transporter gene (*Slc6a4*; n = 7–8 in each group). (E) Transcriptional levels of the colonic tryptophan hydroxylase 1 gene (*Tph1*; n = 8 each group). (F) Stool moisture. (G) Caecal short-chain fatty acid levels (n = 8 each group; $^{*}P$ <.05, $^{**}P$ <.01, $^{***}P$ <.001, $^{****}P$ <.0001 in the unpaired t-tests).

The volatility, measured by the Aitchison distance, indicated that the alterations of the faecal microbiome caused by the placebo and probiotics were not significantly different (Fig. 2C). Similar results have been reported in other studies. Zhang et al. found that the consumption of mixed probiotics selectively changed sailors' gut microbial structures during long sea voyages. A statistical difference in beta diversity was observed. However, the authors considered it more likely that the probiotics played a role in 'maintaining intestinal microbiome homeostasis' [40]. Another study from the same lab indicated that *Lactobacillus plantarum* P-8 induced a significantly lower Aitchison distance change ($P < 0.001$) than the placebo, and the anxiolytic effect may have been caused by changes in neuroactive metabolite levels (based on predicted intestinal metabolomes) [41]. We assume that probiotics demonstrate a more noticeable effect on the mouse gut microbial structure than on that of humans, because the human gut microbiome is dominated by the overall diet, not just the probiotics. In contrast, the experimental animals' diet was highly uniform and more easily challenged by the probiotics. In addition, the amounts of gut microorganisms in mice and humans are highly different. The probiotics' gut microbial effect observed in mice may have been diminished in humans due to the larger human microbiome.

Although probiotics appeared to maintain the patients' gut microbiology, their gut motility significantly changed. This beneficial effect was reproduced in the depressed mice, and we believe the mechanisms involve the following two processes. First, the probiotics elevated the intestinal SCFA levels. The SCFAs increased the faecal osmotic pressure (Fig. 4F and G) and promoted moisture absorption, which then facilitated the defecation function [42–44]. Second, the probiotics increased the colonic 5-HT level. Intestinal 5-HT plays a crucial role in stimulating intestinal peristalsis. Numerous studies have demonstrated that gut 5-HT biosynthesis is gut microbe-dependent, and SCFAs may play an essential role during this process [31,45,46]. Unlike in previous reports, we found that the level of 5-HT biosynthesis did not change in either group even when the SCFA levels changed. However, the colonic serotonin transporter gene (*Slc6a4*) was overexpressed in the depressed mice. By reducing colonic *Slc6a4* expression, the over-reuptake of synaptic 5-HT was normalized in the probiotics-treated mice. Our previous study also indicated that *B. breve* CCFM1025 could reverse the brain's *Slc6a4* overexpression, although the mechanisms are largely unknown [19]. It is known that gut microbes or probiotics profoundly affect nerve transmission physiology. The above two mechanisms appear to work in parallel; however, more work needs to be performed to verify whether they interact with each other.

Several limitations of this study must be considered when interpreting the results. First, although the effect sizes of all comparisons in the clinical results were convincing, the sample size for microbial analysis was insufficient to draw further conclusions. A further evaluation based on a larger sample size should be performed in the future. Additionally, more physiological indicators of patients should be collected, such as serology changes or neuroimaging, to monitor their recovery from depression, instead of relying only on psychometric rating scores. Moreover, the dose–effect relationship between probiotic treatment and depression recovery should be further investigated. Considering the gut microbiome background difference, the heterogeneity of probiotics' effect in different cohorts should be investigated using targeted experimental design, which would offer precise clinical medication guidance.

Conclusions

Overall, this study provided evidence that probiotic treatments can mitigate psychiatric symptoms and the comorbid gastrointestinal symptoms of MDD patients. Serotonergic system modulation is a key mechanism driving the probiotics' benefits for both the brain and gut. These findings suggest a novel and promising treatment for managing depression or other gut-brain axis-related disorders. Future studies are needed to confirm the probiotics' effect on a larger population, and the clinical medication

guidance of specified probiotic therapies for different population are warranted.

Compliance with Ethics Requirements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

All Institutional and National Guidelines for the care and use of animals (fisheries) were followed.

CRediT authorship contribution statement

Peijun Tian: Conceptualization, Writing – original draft, Funding acquisition. **Renying Zou:** Investigation, Formal analysis. **Luyao Wang:** Software, Visualization, Formal analysis. **Ying Chen:** Methodology, Investigation. **Xin Qian:** Methodology, Investigation. **Jianxin Zhao:** Resources, Software, Data curation. **Hao Zhang:** Conceptualization, Validation. **Long Qian:** Resources, Investigation. **Qun Wang:** Resources, Investigation. **Gang Wang:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Wei Chen:** Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2022.05.003.

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