Food Science of Animal Resources

Food Sci. Anim. Resour. 2023 November 43(6):1044~1054
DOI https://doi.org/10.5851/kosfa.2023.e54

pISSN : 2636-0772 eISSN : 2636-0780
http://www.kosfaj.org

SHORT COMMUNICATION

Protective Effects of *Lacticaseibacillus rhamnosus* IDCC3201 on Motor Functions and Anxiety Levels in a Chronic Stress Mouse Model

Jae Gwang Song[1,†], Daye Mun[2,†], Bomi Lee[1], Minho Song[3], Sangnam Oh[4], Jun-Mo Kim[5], Jungwoo Yang[6], Younghoon Kim[2,*], and Hyung Wook Kim[1,*]

[1]College of Life Sciences, Sejong University, Seoul 05006, Korea
[2]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Korea
[3]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea
[4]Department of Functional Food and Biotechnology, Jeonju University, Jeonju 55069, Korea
[5]Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea
[6]Ildong Bioscience, Pyeongtaek 17957, Korea

OPEN ACCESS

Received July 28, 2023
Revised September 1, 2023
Accepted September 13, 2023

***Corresponding authors** :
Younghoon Kim
Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul 08826, Korea
Tel: +82-2-880-4808
Fax: +82-2-880-2271
E-mail: ykeys2584@snu.ac.kr

Hyung Wook Kim
College of Life Sciences, Sejong University, Seoul 05006, Korea
Tel: +82-2-3408-3202
Fax: +82-2-3408-4334
E-mail: kimhyung@sejong.ac.kr

***ORCID**
Jae Gwang Song
https://orcid.org/0000-0002-6015-9393
Daye Mun
https://orcid.org/0000-0002-3470-7632
Bomi Lee
https://orcid.org/0000-0002-3998-8701
Minho Song
https://orcid.org/0000-0002-4515-5212
Sangnam Oh
https://orcid.org/0000-0002-2428-412X
Jun-Mo Kim
https://orcid.org/0000-0002-6934-398X
Jungwoo Yang
https://orcid.org/0000-0003-3836-729X
Younghoon Kim
https://orcid.org/0000-0001-6769-0657
Hyung Wook Kim
https://orcid.org/0009-0003-4839-3827

† These authors contributed equally to this work.

Abstract Growing evidence indicates a crucial role of the gut microbiota in physiological functions. Gut-brain axis imbalance has also been associated with neuropsychiatric and neurodegenerative disorders. Studies have suggested that probiotics regulate the stress response and alleviate mood-related symptoms. In this study, we investigated the effects of the probiotic *Lacticaseibacillus rhamnosus* IDCC3201 (L3201) on the behavioral response and fecal metabolite content in an unpredictable chronic mild stress (UCMS) mouse model. Our study shows that chronic stress in mice for three weeks resulted in significant changes in behavior, including lower locomotor activity, higher levels of anxiety, and depressive-like symptoms, compared to the control group. Metabolomic analysis demonstrated that disrupted fecal metabolites associated with aminoacyl-tRNA biosynthesis and valine, leucine, and isoleucine biosynthesis by UCMS were restored with the administration of L3201. Oral administration of the L3201 ameliorated the observed changes and improved the behavioral alterations along with fecal metabolites, suggesting that probiotics play a neuroprotective role.

Keywords chronic stress, psychobiotics, *Lacticaseibacillus rhamnosus*, gut-brain axis, metabolome

Introduction

Long-term exposure to stress exerts detrimental effects on an individual's overall well-being, serving as a predisposing factor for severe conditions, including anxiety

© Korean Society for Food Science of Animal Resources. This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licences/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

and depressive disorders (Ataallahi et al., 2022). Chronic stress is characterized by symptoms such as impaired focus, cognitive decline, sleep disturbances, weight loss, profound exhaustion, and depression (Jianguo et al., 2019). From the perspective of the gut-brain axis, which has been actively studied in recent years, emerging evidence highlights a deep association between gut microbiota and depressive symptoms (Karl et al., 2018). The gut microbiota exerts substantial influence not only on nutrient metabolism but also on the modulation of the immune system, integrity of the intestinal barrier, and homeostasis of the central nervous system (Cryan and Dinan, 2012; Lee et al., 2022; Patra and Kar, 2021). Interestingly, recent studies underscore the association between dysbiosis, characterized by perturbed intestinal microbiota balance, and the onset of several metabolic diseases and psychological disorders (Cai et al., 2022; Jeong et al., 2021; Li et al., 2019). Hence, ongoing studies are actively investigating the potential of modulating the gut microbiome to improve anxiety and depressive disorders induced by chronic stress.

A growing body of research continues to report on specific probiotics, called psychobiotics, that have beneficial effects on the gut-brain axis and are suggested to alleviate psychological disorders by improving inflammatory responses, alleviating gut mucosal defects, and modulating neurotransmitters (Chen et al., 2021; Li et al., 2018b; Zareie et al., 2006). According to Li et al. (2018b), *Lactobacillus helveticus*, *Lactiplantibacillus plantarum*, and *Bifidobacterium longum* alleviated depressive behaviors and anxiety by improving interferon-γ, tumor necrosis factor-α, and indoleamine 2,3-dioxygenase-1 levels in the hippocampus of chronic mildly stressed mice (Li et al., 2018b). Additionally, it has been reported that treatment with *Lacticaseibacillus rhamnosus* and *L. helveticus* could improve chronic stress-induced gut barrier dysfunction and protect against bacterial translocation to the mesenteric lymph nodes (Zareie et al., 2006). However, there is still insufficient evidence regarding how chronic stress and psychobiotics affect the metabolism of the host and gut microbiota.

Fecal metabolomics is an analysis method that detects small molecules such as amino acids, sugars, fatty acids, and organic acids generated by the metabolic interactions between the microbiome and the host, providing insight into altered metabolic pathways (Vasquez et al., 2022; Zhang et al., 2023). Fecal metabolome analysis offers the possibility of identifying metabolites that can be used as indicators or biomarkers for the function of specific microbes, providing information to determine the role of the gut microbiota in health and disease (Zierer et al., 2018). Specifically, fecal metabolome analysis in stress models provides important evidence for understanding the physiological and biochemical changes in the gut in response to stress, which could give a perspective on how stress affects the various biological pathways. Furthermore, it could help us understand the impact of stress on the complex interactions in the gut-brain axis (Konjevod et al., 2021). Identifying altered metabolites in feces can be performed in two ways: targeted, analyzing only the specific metabolites of interest, and untargeted, comparing changes in the complete metabolomic profiles (Karnovsky and Li, 2020; Melnik et al., 2017). Most stress-related fecal metabolome analyses have focused on comparing fecal glucocorticoid or cortisol content as an indicator (Ataallahi et al., 2022; Josefson and Skibiel, 2021; Keay et al., 2006), and the effects of stress and psychobiotics using untargeted global metabolomic analyses of fecal metabolites have not been fully investigated. Therefore, this study was conducted to investigate the effects of probiotics on behavioral changes and the intestinal metabolome in mice subjected to unpredictable chronic mild stress (UCMS).

Materials and Methods

Animal and probiotics

Male C57BL/6 mice were purchased from Central Lab. Animal (Seoul, Korea) at five weeks old. *L. rhamnosus* IDCC 3201

(L3201) was provided by Ildong Bioscience (Pyeongtaek, Korea). L3201 were suspended in saline before use for fresh administration. Mice were divided into three groups: control group (CTL group), stress control group (S. CTL group), and stress probiotics (L3201 group). All groups were orally administered saline or L3201 (1.0×10^9 CFU/mouse/day) from 6 weeks old. We determined the consumption of L3201 based on the results of previous studies (Lee et al., 2016). They were exposed to UCMS from 8 weeks old until sacrifice. Behavioral tests were started at 8 weeks old. Body weight was checked weekly. Mice had *ad libitum* access to food and water under standard conditions: 21°C–23°C, 45%–55% humidity, and 12 h light/dark cycle. All animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Sejong University (SJ-20230110-01).

Unpredictable chronic mild stress (UCMS)

UCMS was slightly modified (Mineur et al., 2006; Oh et al., 2020). Stress groups were exposed to stressors such as tube restraint, tail tie-up, food deprivation, water deprivation, illumination, cold object, tilted cage, damp sawdust, sleep cycle change, foreign cage, and cold water bath. Two stressors were used on a randomized schedule in a day.

Elevated plus maze test (EPM)

The EPM was used to measure anxiety-like behavior (Komada et al., 2008). The apparatus is 40 cm above the floor and has a center zone (5 cm×5 cm), two closed arms with walls (30 cm×5 cm×16 cm), and two open arms (30 cm×5 cm×0.5 cm). Mice were placed at the center facing the closed arm and freely explored for 5 min. The tests were recorded and analyzed by Any-maze software version 6.0 (Stoelting, Wood Dale, IL, USA).

Forced swimming test (FST)

The forced swim test was used to measure despair-like behaviors (Porsolt et al., 1977). The apparatus was filled with water (25±1°C) to a depth of 16 cm in a glass beaker (Ø 19×26 cm). Mice were placed in water and forced to swim for 6 min, and the last four minutes of the test were analyzed. All tests were recorded with a camera and analyzed by Any-maze software version 6.0 (Stoelting).

Fecal metabolomic analysis

Fresh fecal samples were collected and stored at −80°C prior to metabolic analysis. Gas chromatography–mass spectrometry (GC–MS) analysis was conducted as reported (Thompson et al., 2020; Yoo et al., 2022) with slight modification. Briefly, 40 mg of fecal samples were homogenized in 1 mL of ice-cold methanol by vortexing and centrifuged at 15,000×g for 5 min at 4°C. The supernatant was filtered and thoroughly vacuum-dried. Methoxyamine hydrochloride in pyridine and *N,O*-bis(trimethylsilyl)trifluoroacetamide were used as derivatization agents. GC–MS analysis was conducted with a Trace 1310 Gas Chromatograph including the ISQ LT single quadrupole Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The detected metabolites were identified using AMDIS and the NIST Mass database (version 2.0, Gaithersburg, MD, USA). Statistical analysis was performed using Metaboanalyst 5.0 (Eom et al., 2022).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Duncan's post hoc test was conducted using SPSS statistics 23

(IBM, Armonk, NY, USA) to analyze the statistical significance between groups. Different superscript letters indicate significant differences. All data are presented as the mean±SEM.

Results and Discussion

Oral administration of the *Lacticaseibacillus rhamnosus* IDCC 3201 (L3201) strain relieved anxiety and depression-like behaviors under unpredictable chronic mild stress (UCMS)

A schematic diagram for the behavioral experiment is illustrated in Fig. 1A. The body weight of the S.CTL group decreased compared to the CTL group during the UCMS period, but the L3201 group did not differ from that of the CTL group (Fig. 1B). In addition, the S.CTL group showed a decrease in weight gain (%), but the L3201 group showed rescued body weight (Fig. 1C). The EPM was conducted to confirm the anti-anxiety effect of oral administration of the L3201 strain (Figs. 1D, E, F, G, H, and I). The EPM showed no difference in the total distance traveled in all groups (Fig. 1D). The S.CTL group spent significantly more time in the closed arms than the CTL group (Fig. 1E). On the other hand, the time spent in the closed arms of the L3201 group was similar to that of the CTL group (Fig. 1E). Additionally, the S.CTL group had a significantly decreased time spent in the open arms compared to the CTL group (Fig. 1F). However, there was no difference in the time spent in the open arms between the CTL and L3201 groups (Fig. 1F). The FST was performed to confirm the antidepressant effect of oral administration of the L3201 strain (Figs. 1H and I). The immobility time of the S.CTL group was significantly increased compared to that of the CTL group (Fig. 1I). The L3201 group tended to have an increased immobility time compared to the CTL group but a decreased immobility time compared to the S.CTL group (Fig. 1I). These results suggest that the administration of L3201 relieves stress-induced anxiety- and despair-like behaviors.

Chronic stress induces anxiety- and despair-like behavior in mice (Westfall et al., 2021). EPM is the most frequently performed test in rodents using open time as an anxiety index to identify anxiety-like behavior (Komada et al., 2008). Corticosterone- or UCMS-induced depressed mice spent less time in the open arms than normal mice in EPM (Peng et al., 2021; Zhu et al., 2020). The S.CTL group showed a decrease in open time, which is an anxiety-like behavior caused by stress. Stenman et al. showed the reduction of anxiety-like behaviors in EPM by administering probiotics in a depression model (Stenman et al., 2020). Consistent with previous anxiolytic results, the L3201 group showed a reversal in the reduced open time caused by UCMS, indicating a decrease in anxiety-like behavior.

Increased immobility time in the FST is used to measure a despair indicator (Porsolt et al., 1977). Previous research has shown that immobility is increased in mouse models of depression caused by corticosterone administration or UCMS (Badr et al., 2020; Mineur et al., 2006; Strekalova et al., 2005). Consistent with previous results, the S.CTL group showed increased immobility time, which indicated despair-like behavior caused by UCMS. Murray et al. (2019) and Stenman et al. (2020) showed the effect of reducing despair-like behaviors by reducing the immobility time of FST in a depression model through probiotics administration. Similar to previous studies, in the L3201 group, the increased immobility time induced by UCMS was alleviated, suggesting a stress-relieving effect of L3201 administration.

Oral administration of *Lacticaseibacillus rhamnosus* IDCC 3201 (L3201) causes changes in fecal metabolites of unpredictable chronic mild stress (UCMS) mice

GC–MS analysis was performed to confirm the effect of oral administration of the L3201 strain (Figs. 2A, B, and C) on fecal metabolomics. As a result, a total of 53 metabolites, including amino acids, fatty acids, organooxygen compounds, and

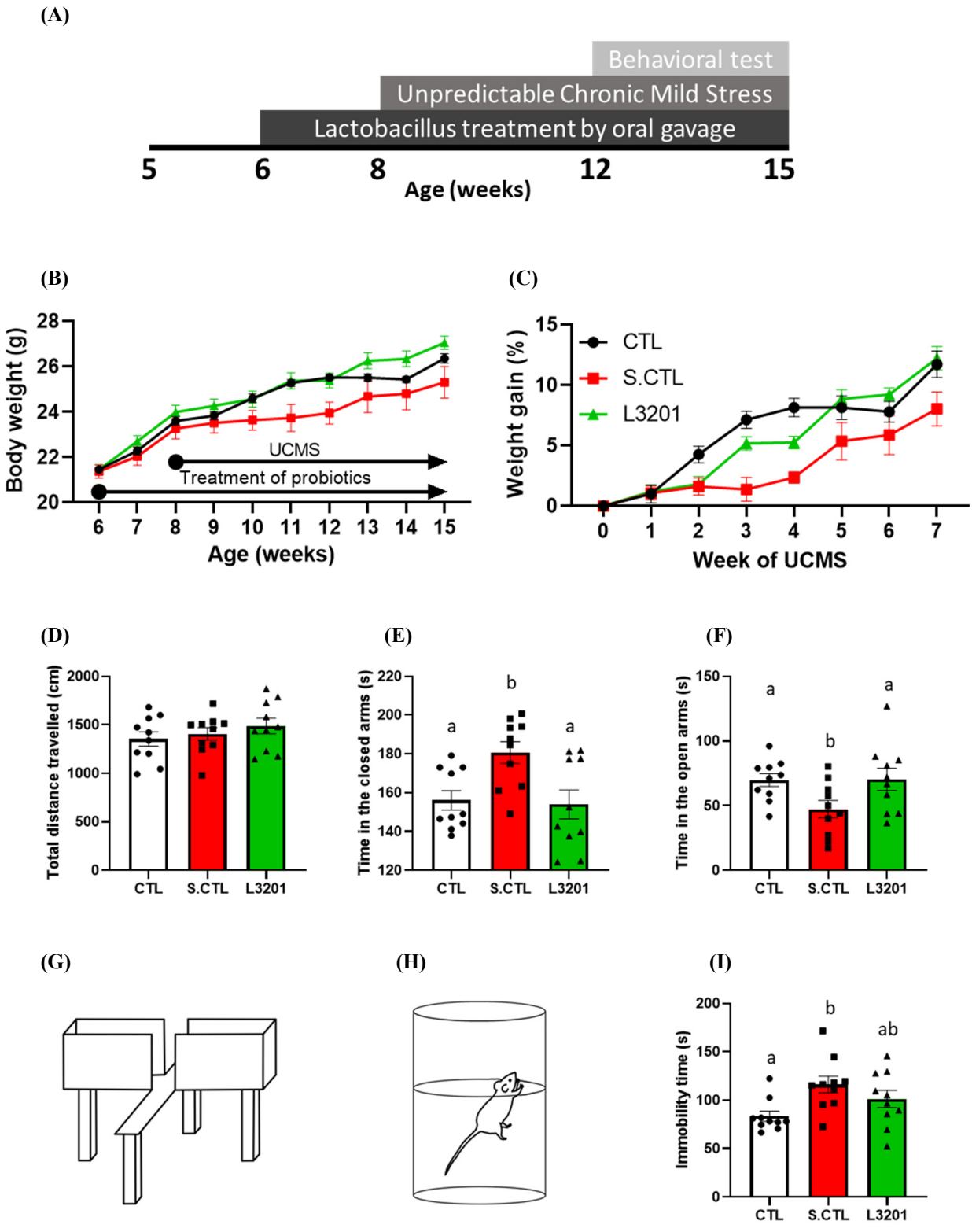

Fig. 1. Oral administration of L3201 relieved anxiety and depression-like behaviors under UCMS. (A) Schematic overview of the *in vivo* experiment, (B) variations in body weight, (C) percentage of weight gain during UCMS, (D) total distance travelled in the EPM, (E) time in the closed arms in the EPM, (F) time in the open arms in the EPM, (G) the elevated plus maze test apparatus, (H) the forced swimming test apparatus, (I) immobility time in the FST. All data are presented as mean±SEM. [a,b] Different superscript letters indicate statistical difference. UCMS, unpredictable chronic mild stress; CTL, control; S. CTL, stress control; L3201, *Lacticaseibacillus rhamnosus* IDCC 3201; EPM, elevated plus maze test; FST, forced swimming test.

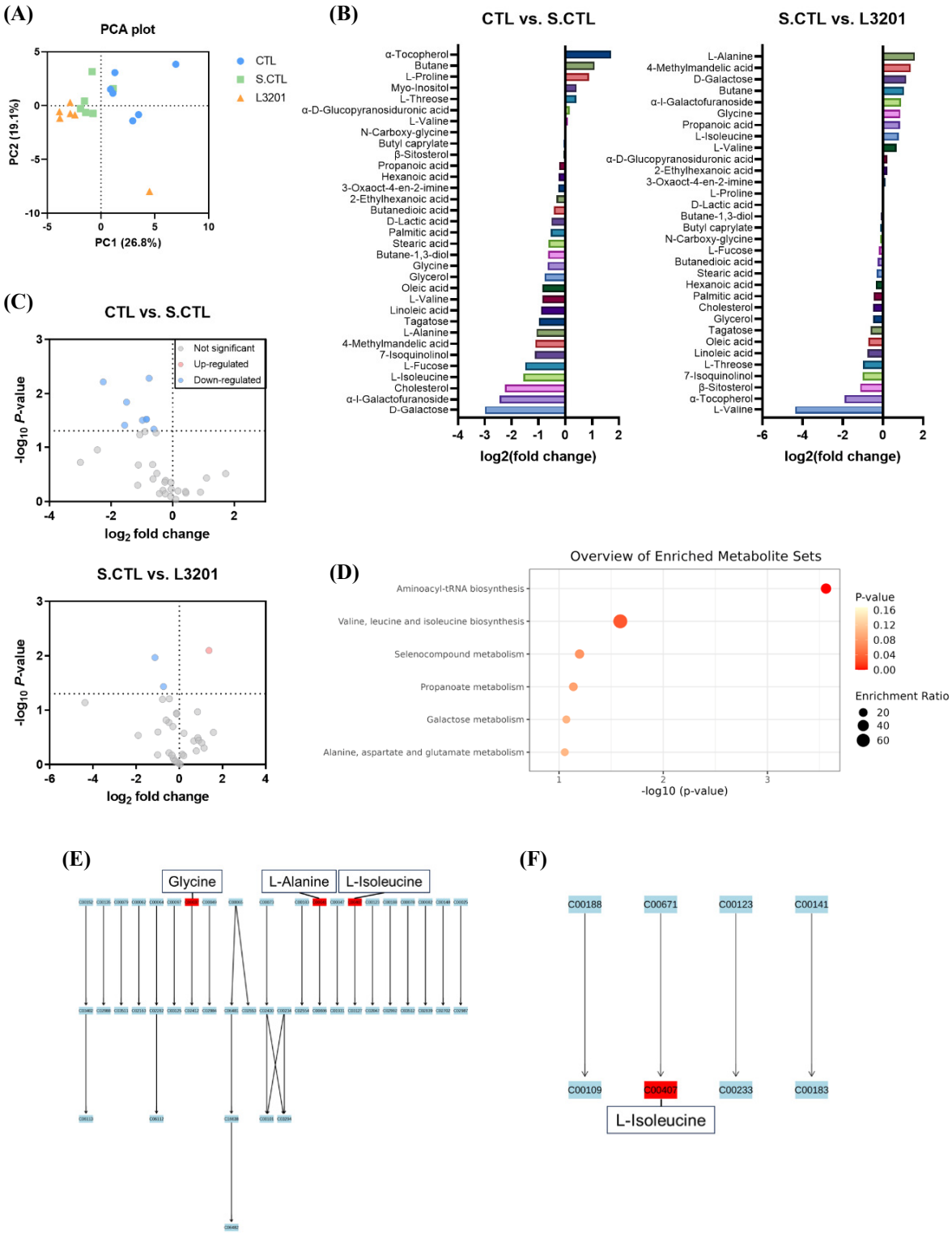

Fig. 2. Oral administration of L3201 causes changes in fecal metabolites of chronic stress-exposed mice. (A) Principal component analysis plot of fecal metabolomics of the three groups. (B) Log 2-fold change in fecal metabolites in CTL vs. S.CTL group (left) and S.CTL vs L3201 group (right). (C) A volcano plot for the fecal metabolites in CTL vs. S.CTL group (left) and S.CTL vs. L3201 group (right). (D) Enrichment analysis of the metabolites that were downregulated by UCMS and reinstated upon L3201 treatment. (E, F) Significantly altered metabolic pathways: aminoacyl-tRNA biosynthesis and valine (E), leucine and isoleucine biosynthesis (F) and significantly effected metabolites (red box). PCA, principal component analysis; CTL, control; S.CTL, stress control; L3201, *Lacticaseibacillus rhamnosus* IDCC 3201; UCMS, unpredictable chronic mild stress.

steroids, were detected; among them, 34 metabolites were identified. Multivariate analysis showed different metabolic profiles among the three groups (Fig. 2A). Within the identified metabolites, an upregulation was observed in the expression of 7 metabolites, while a decrease was noted in 26 metabolites, including L-alanine, L-isoleucine, and cholesterol, in the UCMS model compared to the CTL group (Fig. 2B). Conversely, administration of L3201 in the UCMS-induced model increased 13 metabolites, including L-alanine, L-isoleucine, and propanoic acid, while 19 metabolites exhibited a decrease in their levels. Furthermore, among the metabolites downregulated by UCMS induction, D-galactose, α-L-galactofuranoside, L-isoleucine, 4-methylmandelic acid, L-alanine, glycine, 2-ethylhexanoic acid, 3-oxaoct-4-en-2-imine, and propanoic acid were upregulated upon oral administration of L3201. In contrast, L-threose and α-tocopherol, which displayed upregulation in the S.CTL group compared to the CTL group, exhibited a downregulation in response to L3201 treatment.

The volcano plot visualized the significantly altered metabolic compounds between the two groups (Fig. 2C). L-valine, glycerol, tagatose, oleic acid, stearic acid, cholesterol, L-isoleucine, and L-fucose were significantly reduced in the S.CTL group compared to the CTL group, and oleic acid and β-sitosterol were significantly reduced in the L3201 group compared to the S.CTL group. Interestingly, 4-methylmandelic acid was significantly increased in the L3201 group compared to the S.CTL group. KEGG pathway enrichment analysis showed that metabolites increased upon L3201 treatment under stress are related to aminoacyl-tRNA biosynthesis and valine, leucine, and isoleucine biosynthesis (Figs. 2D, E, and F). These results confirm that the induction of UCMS disrupts gut metabolism and that the administration of L3201 can ameliorate this imbalance.

Recent studies have shown that the gut metabolome is affected by psychological anxiety. A previous study showed that 11 specific metabolic pathways, including amino acid metabolism and lipid metabolism, were altered in the fecal metabolome of multiple depressed animal models under stress (Liu et al., 2022). It has been reported that glutamate, alanine, and L-serine in plasma could represent the extent of depression (Mitani et al., 2006). L-threonine, oxidized proline, serine, tyrosine, alanine, and isoleucine and related amino acid metabolism were found to be altered in the fecal metabolome of rats exposed to chronic stress (Jianguo et al., 2019). Furthermore, the hepatic metabolites of mice transplanted with feces from depressed patients showed significant changes in aminoacyl-tRNA biosynthesis (Li et al., 2018a). Consistent with previous findings, this study found that metabolites involved in aminoacyl-tRNA biosynthesis, such as L-alanine, L-isoleucine, and glycine, were reduced in the stress model compared to the control group.

Specific probiotics, called psychobiotics, have been reported to improve stress and depression. According to Ma et al. (2021), consumption of *L. plantarum* P-8 has been shown to reduce stress and anxiety in adults, and this has been attributed to an increase in gut microbial neuroactive metabolites upon probiotic intake. Treatment with *L. rhamnosus* HN001 (HN001) and *Bifidobacterium animalis* subsp. *lactis* HN019 (HN019), alone and in combination, improved anxiety-related behavioral indicators and significantly improved inflammatory markers in stress-induced mice (Huang et al., 2022). It was also found that consumption of *L. rhamnosus* Probio-M9 in stressed adults increased gut microbial diversity and altered intestinal metabolism, including arachidonic acid metabolism and amino acid metabolism (Zheng et al., 2021). Similar to previous studies, the current study showed that the metabolites related to aminoacyl-tRNA biosynthesis and valine, leucine and isoleucine biosynthesis, which were decreased by UCMS induction, were enhanced by the administration of L3201 (Figs. 2E and F). UCMS increases pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-12 like LPS (Reyes and Chandler, 2023; Zhao et al., 2019). In the previous study, IDCC3201 reduced TNF-a and IL-6 among the increased cytokines caused by LPS in cells (Chae et al., 2022). Although this study did not investigate a decrease in cytokine levels by L3201 administration, it is expected to reduce cytokines increased by stress. These results suggest that imbalances in amino acid metabolism play an important role in stress-related psychological disorders and that treatment with L3201 ameliorates these disruptions.

Conclusion

In this study, we induced UCMS in mice after oral administration of probiotics to reveal that probiotic administration was associated with improvements in depressive-like phenotypes and changes in the gut metabolome. Our results show that depressive-like behavioral patterns and perturbations in the gut metabolome of UCMS mice are ameliorated by supplementation with L3201. These findings suggest that probiotics play a key role in preventing stress-induced depression through the restoration of gut metabolic pathways.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET-321037-5) and by a National Research Foundation of Korea Grant funded by the Korean government (MEST) (NRF-2021R1A2C3011051) and “Cooperative Research Program for Agriculture Science & Technology Development (Project No. RS-2023-00225838)” Rural Development Administration, Republic of Korea.

Author Contributions

Conceptualization: Song JG, Mun D, Kim Y, Kim HW. Investigation: Song JG, Mun D, Lee B. Writing - original draft: Song JG, Mun D, Lee B, Song M, Oh S, Kim JM, Yang J, Kim Y, Kim HW. Writing - review & editing: Song JG, Mun D, Lee B, Song M, Oh S, Kim JM, Yang J, Kim Y, Kim HW.

Ethics Approval

All animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Sejong University (SJ-20230110-01).

References

Ataallahi M, Nejad JG, Park KH. 2022. Selection of appropriate biomatrices for studies of chronic stress in animals: A review. J Anim Sci Technol 64:621-639.

Badr AM, Attia HA, Al-Rasheed N. 2020. Oleuropein reverses repeated corticosterone-induced depressive-like behavior in mice: Evidence of modulating effect on biogenic amines. Sci Rep 10:3336.

Cai T, Zheng S, Shi X, Yuan L, Hu H, Zhou B, Xiao S, Wang F. 2022. Therapeutic effect of fecal microbiota transplantation on chronic unpredictable mild stress-induced depression. Front Cell Infect Microbiol 12:900652.

Chae SA, Ramakrishnan SR, Kim T, Kim SR, Bang WY, Jeong CR, Yang J, Kim SJ. 2022. Anti-inflammatory and anti-pathogenic potential of *Lacticaseibacillus rhamnosus* IDCC 3201 isolated from feces of breast-fed infants. Microb Pathog 173:105857.

Chen Y, Xu J, Chen Y. 2021. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients 13:2099.

Cryan JF, Dinan TG. 2012. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 13:701-712.

Eom JS, Lee SJ, Kim HS, Choi Y, Jo SU, Lee SS, Kim ET, Lee SS. 2022. Metabolic profiling of serum and urine in lactating dairy cows affected by subclinical ketosis using proton nuclear magnetic resonance spectroscopy. J Anim Sci Technol 64:247-261.

Huang L, Lv X, Ze X, Ma Z, Zhang X, He R, Fan J, Zhang M, Sun B, Wang F, Liu H. 2022. Combined probiotics attenuate chronic unpredictable mild stress-induced depressive-like and anxiety-like behaviors in rats. Front Psychiatry 13:990465.

Jeong Y, Choi Y, Kim D, Min Y, Cho E, Kim J. 2021. Effects of cooling systems on physiological responses and intestinal microflora in early gestating sows exposed to high-temperature stress. J Anim Sci Technol 63:904-918.

Jianguo L, Xueyang J, Cui W, Changxin W, Xuemei Q. 2019. Altered gut metabolome contributes to depression-like behaviors in rats exposed to chronic unpredictable mild stress. Transl Psychiatry 9:40.

Josefson CC, Skibiel AL. 2021. Changes in maternal fecal corticosterone metabolites across lactation and in response to chronic stress. Gen Comp Endocrinol 314:113916.

Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, Soares JW. 2018. Effects of psychological, environmental and physical stressors on the gut microbiota. Front Microbiol 9:2013.

Karnovsky A, Li S. 2020. Pathway analysis for targeted and untargeted metabolomics. In Computational methods and data analysis for metabolomics. Li S (ed). Humana Press, New York, NY, USA.

Keay JM, Singh J, Gaunt MC, Kaur T. 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review. J Zoo Wildl Med 37:234-244.

Komada M, Takao K, Miyakawa T. 2008. Elevated plus maze for mice. J Vis Exp 22:e1088.

Konjevod M, Nikolac Perkovic M, Sáiz J, Svob Strac D, Barbas C, Rojo D. 2021. Metabolomics analysis of microbiota-gut-brain axis in neurodegenerative and psychiatric diseases. J Pharm Biomed Anal 194:113681.

Lee D, Goh TW, Kang MG, Choi HJ, Yeo SY, Yang J, Huh CS, Kim YY, Kim Y. 2022. Perspectives and advances in probiotics and the gut microbiome in companion animals. J Anim Sci Technol 64:197-217.

Lee SH, Yoon JM, Kim YH, Jeong DG, Park S, Kang DJ. 2016. Therapeutic effect of tyndallized *Lactobacillus rhamnosus* IDCC 3201 on atopic dermatitis mediated by down-regulation of immunoglobulin E in NC/Nga mice. Microbiol Immunol 60:468-476.

Li B, Guo K, Zeng L, Zeng B, Huo R, Luo Y, Wang H, Dong M, Zheng P, Zhou C, Chen J, Liu Y, Liu Z, Fang L, Wei H, Xie P. 2018a. Metabolite identification in fecal microbiota transplantation mouse livers and combined proteomics with chronic unpredictive mild stress mouse livers. Transl Psychiatry 8:34.

Li N, Wang Q, Wang Y, Sun A, Lin Y, Jin Y, Li X. 2018b. Oral probiotics ameliorate the behavioral deficits induced by chronic mild stress in mice via the gut microbiota-inflammation axis. Front Behav Neurosci 12:266.

Li N, Wang Q, Wang Y, Sun A, Lin Y, Jin Y, Li X. 2019. Fecal microbiota transplantation from chronic unpredictable mild stress mice donors affects anxiety-like and depression-like behavior in recipient mice via the gut microbiota-inflammation-brain axis. Stress 22:592-602.

Liu X, Li X, Teng T, Jiang Y, Xiang Y, Fan L, Yu Y, Zhou X, Xie P. 2022. Comparative analysis of gut microbiota and fecal metabolome features among multiple depressive animal models. J Affect Disord 314:103-111.

Ma T, Jin H, Kwok LY, Sun Z, Liong MT, Zhang H. 2021. Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota. Neurobiol Stress 14:100294.

Melnik AV, da Silva RR, Hyde ER, Aksenov AA, Vargas F, Bouslimani A, Protsyuk I, Jarmusch AK, Tripathi A, Alexandrov T, Knight R, Dorrestein PC. 2017. Coupling targeted and untargeted mass spectrometry for metabolome-microbiome-wide association studies of human fecal samples. Anal Chem 89:7549-7559.

Mineur YS, Belzung C, Crusio WE. 2006. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behav Brain Res 175:43-50.

Mitani H, Shirayama Y, Yamada T, Maeda K, Ashby CR Jr, Kawahara R. 2006. Correlation between plasma levels of glutamate, alanine and serine with severity of depression. Prog Neuropsychopharmacol Biol Psychiatry 30:1155-1158.

Murray E, Sharma R, Smith KB, Mar KD, Barve R, Lukasik M, Pirwani AF, Malette-Guyon E, Lamba S, Thomas BJ, Sadeghi-Emamchaie H, Liang J, Mallet JF, Matar C, Ismail N. 2019. Probiotic consumption during puberty mitigates lps-induced immune responses and protects against stress-induced depression- and anxiety-like behaviors in adulthood in a sex-specific manner. Brain Behav Immun 81:198-212.

Oh NS, Joung JY, Lee JY, Song JG, Oh S, Kim Y, Kim HW, Kim SH. 2020. Glycated milk protein fermented with *Lactobacillus rhamnosus* ameliorates the cognitive health of mice under mild-stress condition. Gut Microbes 11:1643-1661.

Patra AK, Kar I. 2021. Heat stress on microbiota composition, barrier integrity, and nutrient transport in gut, production performance, and its amelioration in farm animals. J Anim Sci Technol 63:211-247.

Peng B, Xu Q, Liu J, Guo S, Borgland SL, Liu S. 2021. Corticosterone attenuates reward-seeking behavior and increases anxiety via D2 receptor signaling in ventral tegmental area dopamine neurons. J Neurosci 41:1566-1581.

Porsolt RD, Bertin A, Jalfre M. 1977. Behavioral despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327-336.

Reyes AAA, Chandler DJ. 2023. Convergence of pro-stress and pro-inflammatory signaling in the central noradrenergic system: Implications for mood and anxiety disorders. Neuroglia 4:87-101.

Stenman LK, Patterson E, Meunier J, Roman FJ, Lehtinen MJ. 2020. Strain specific stress-modulating effects of candidate probiotics: A systematic screening in a mouse model of chronic restraint stress. Behav Brain Res 379:112376.

Strekalova T, Spanagel R, Dolgov O, Bartsch D. 2005. Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice. Behav Pharmacol 16:171-180.

Thompson RS, Vargas F, Dorrestein PC, Chichlowski M, Berg BM, Fleshner M. 2020. Dietary prebiotics alter novel microbial dependent fecal metabolites that improve sleep. Sci Rep 10:3848.

Vasquez R, Oh JK, Song JH, Kang DK. 2022. Gut microbiome-produced metabolites in pigs: A review on their biological functions and the influence of probiotics. J Anim Sci Technol 64:671-695.

Westfall S, Caracci F, Estill M, Frolinger T, Shen L, Pasinetti GM. 2021. Chronic stress-induced depression and anxiety priming modulated by gut-brain-axis immunity. Front Immunol 12:670500.

Yoo J, Lee J, Zhang M, Mun D, Kang M, Yun B, Kim YA, Kim S, Oh S. 2022. Enhanced γ-aminobutyric acid and sialic acid in fermented deer antler velvet and immune promoting effects. J Anim Sci Technol 64:166-182.

Zareie M, Johnson-Henry K, Jury J, Yang PC, Ngan BY, Mckay DM, Soderholm JD, Perdue MH, Sherman PM. 2006. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. Gut 55:1553-1560.

Zhang Y, Zhang J, Wu J, Zhu Q, Chen C, Li Y. 2023. Implications of gut microbiota dysbiosis and fecal metabolite changes in psychologically stressed mice. Front Microbiol 14:1124454.

Zhao X, Cao F, Liu Q, Li X, Xu G, Liu G, Zhang Y, Yang X, Yi S, Xu F, Fan K, Ma J. 2019. Behavioral, inflammatory and neurochemical disturbances in LPS and UCMS-induced mouse models of depression. Behav Brain Res 364:494-502.

Zheng Y, Yu Z, Zhang W, Sun T. 2021. *Lactobacillus rhamnosus* Probio-M9 improves the quality of life in stressed adults by gut microbiota. Foods 10:2384.

Zhu H, Tao Y, Wang T, Zhou J, Yang Y, Cheng L, Zhu H, Zhang W, Huang F, Wu X. 2020. Long-term stability and characteristics of behavioral, biochemical, and molecular markers of three different rodent models for depression. Brain Behav 10:e01508.

Zierer J, Jackson MA, Kastenmuller G, Mangino M, Long T, Telenti A, Mohney RP, Small KS, Bell JT, Steves CJ, Valdes AM, Spector TD, Menni C. 2018. The fecal metabolome as a functional readout of the gut microbiome. Nat Genet 50:790-795.