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A randomized, double-blind pilot study of a multi-strain probiotic formulation on depressive symptoms in adults

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Abstract

The gut–brain axis represents a bidirectional communication pathway influencing mood through microbiota-related mechanisms. This randomized, double-blind, active-controlled pilot trial evaluated the effects of a multi-strain *Lactobacillus* spp. probiotic formulation added to an active control on depressive symptoms in adults with mild-to-moderate depressive symptoms. Sixty participants were randomly assigned to receive either a daily combination containing *L. rhamnosus* HN001, *L. rhamnosus* SP1, *L. acidophilus* LA1, and *L. reuteri* LR92 (3×10^9 CFU total) plus L-theanine and *Eschscholtzia californica* (probiotic group) or an identical active control containing L-theanine and *E. californica* alone for six weeks. Depressive symptoms were assessed using the Patient Health Questionnaire-9 (PHQ-9) and the Beck Depression Inventory-II at baseline, week 3, week 6, and after a three-week washout. Significant within-group reductions in depressive symptom scores were

observed in the probiotic group, while changes in the active control group were smaller and not statistically significant. However, between-group comparisons did not reach statistical significance at any time point. These findings suggest that the probiotic-containing formulation was associated with improvements in depressive symptoms when administered on top of an active control. Due to the study design, effects cannot be attributed specifically to the probiotic component alone. Larger trials incorporating inert placebo controls and microbiome analyses are warranted.

Introduction

Depression represents a major public health challenge, with mood disorders affecting approximately 30-40% of individuals seeking psychological support globally.[1,2] The growing prevalence of depressive symptoms, particularly in young adult populations, underscores the urgent need for innovative therapeutic approaches that target underlying pathophysiological mechanisms.[3,4]

Recent advances in neuroscience have identified the gut-brain axis as a critical bidirectional communication pathway linking gastrointestinal microbiota composition with central nervous system function.[5,6] This sophisticated network involves multiple interconnected systems including neural, endocrine, and immune pathways that collectively influence mood regulation and emotional processing.[7,8]

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts,[9] have emerged as promising therapeutic tools for mental health applications. Preclinical studies have provided compelling evidence for probiotic efficacy in mood regulation, with multiple rodent studies showing beneficial effects of specific probiotic strains on anxiety and depression-like behaviors.[10,11] Limited human clinical trials have yielded encouraging preliminary results, though findings remain inconsistent.[12,13]

Complementary to probiotic research, other natural compounds have shown promise for mood regulation. L-theanine, an amino acid found primarily in tea leaves, has demonstrated anxiolytic properties and stress-reducing effects in both preclinical and clinical studies.[14,15] *Eschscholtzia californica* (California poppy) has been traditionally used for its sedative and anxiolytic properties, with emerging evidence supporting its potential benefits for sleep and anxiety disorders.[16]

Despite the individual promise of these compounds, few studies have investigated their combined effects. The rationale for combination approaches lies in the potential for synergistic mechanisms targeting different aspects of mood regulation through the gut-brain axis, stress response pathways, and neurotransmitter systems.[17-19]

The present randomized, double-blind, controlled trial was designed to investigate the effects of a novel combination containing multi-strain *Lactobacillus* spp. probiotics, L-theanine, and *Eschscholtzia californica* on depressive symptoms in adults with mild-to-moderate depression. Our study employed well-validated assessment instruments to comprehensively evaluate mood changes over a 6-week intervention period with subsequent washout assessment.

Materials and Methods

Study design

This randomized, double-blind, active control-controlled study investigated the effects of probiotic combination supplementation on depressive symptoms over a 9-week period. The intervention consisted of 6 weeks of active treatment followed by a 3-week washout period. Sixty adults with mild-to-moderate depressive symptoms were randomly assigned to either treatment or control conditions using stratified randomization. The treatment group received daily supplementation with a multi-strain *Lactobacillus* spp. formulation combined with L-theanine and *Eschscholtzia californica*, while the active control group received L-theanine and *Eschscholtzia californica* without probiotics. Both groups received indistinguishable capsules to ensure proper blinding, though the use of an active control rather than an inert placebo represents a fundamental design limitation that prevents isolation of probiotic-specific effects. Depressive symptoms were assessed at four time points: baseline (T0), week 3 (T1), week 6 (T2), and after a 3-week washout period (T3).

The study investigated a probiotic combination supplement consisting of *Lactobacillus rhamnosus* HN001 (1×10^9 CFU), *L. rhamnosus* SP1 ($<10^9$ CFU), *L. acidophilus* LA1 ($<10^9$ CFU), and *L. reuteri* LR92 ($<10^9$ CFU) (PSICOBRAIN®, Bromatech, Milano, Italy). The multi-strain formulation was selected based on existing literature supporting the individual strains' neurotropic properties. *L. rhamnosus* HN001 has demonstrated efficacy in reducing anxiety and depression scores in previous clinical trials. *L. rhamnosus SP1* and *L. acidophilus LA1* have shown GABA-producing capabilities *in vitro*, potentially contributing to anxiolytic effects through the gut-brain axis. *L. reuteri* LR92 has been associated with modulation of the hypothalamic-pituitary-adrenal axis and stress response pathways. The combination approach was designed to target multiple mechanisms

involved in mood regulation, including neurotransmitter production, immune modulation, and stress hormone regulation. The total CFU count of 3×10^9 was selected to ensure adequate bacterial viability and colonization potential based on previous probiotic intervention studies. Available in 0.31 g capsules as lyophilized powder, each capsule delivered 3×10^9 colony-forming units (CFU). The formulation also included microcrystalline cellulose, gelatin (capsule ingredient), L-theanine, and *Eschscholtzia californica* Cham. *Eschscholtzia californica* was administered as a standardized powdered extract containing the active alkaloid compounds, not as crude plant material.

The complete formulation included specific strains, microcrystalline cellulose, gelatin (capsule), L-theanine, and *Eschscholtzia californica* Cham. (aqueous extract, aerial part). The active control capsules contained the same excipients (microcrystalline cellulose, gelatin capsule, L-theanine, and *Eschscholtzia californica* extract) but without probiotic strains. Thus, the active control group matched the active product in non-bacterial components and sensory characteristics. To maintain study blindness, participants received standardized instructions for capsule consumption. If a dose was missed, participants were instructed to skip it and continue with the next scheduled dose.

Participants

From 71 screened individuals, 9 were excluded due to inclusion/exclusion criteria or unwillingness to participate. Of 62 randomized participants, 1 from the probiotic group (treatment discontinuation) and 1 from the comparator group (antibiotic intervention) were excluded. Sixty participants completed the study (Figure 1). The study enrolled 60 healthy adults (35 women and 25 men) aged 18–65 years, with a mean age of 22.00±3.02 years and mean Body Mass Index (BMI) of 23.1±2.8 kg/m² (range: 18.5–28.7); the majority of participants were aged between 19 and 29 years. All were university students or staff, with 85% holding a bachelor's degree or higher. Eligible participants were adults who smoked fewer than 10 cigarettes daily. Exclusion criteria included psychiatric or neurological disorders, celiac disease, lactose intolerance, allergies, chronic conditions (irritable bowel syndrome, diabetes, ulcerative colitis), and antibiotic treatment within three months prior to the study.

Two investigators (FD, FB) carried out structured interviews aimed at gathering demographic data and evaluating participants' levels of physical activity. Female participants were additionally screened for possible premenstrual syndrome in order to consider cyclical monthly variations.

Group assignment was performed through stratified randomization based on computer-generated sequences, allowing comparable distribution of sample size and physical activity across study arms. The researchers involved in participant recruitment, preparation of the randomization lists, and allocation of the intervention were kept unaware of group assignment, and the same blinding applied to participants.

The research took place from June to September 2024 at the Ecotekne campus of the University of Salento and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all individuals prior to participation, and they were informed of their right to withdraw from the study at any time without justification.

Questionnaires measuring depressive symptoms

Two validated instruments were utilized to assess depressive symptoms: the Patient Health Questionnaire-9 (PHQ-9)[20,21] and the Beck Depression Inventory-II (BDI-II),[22] both well-established tools for measuring depressive symptomatology. The PHQ-9 consists of nine items assessing the frequency of depressive symptoms, with responses ranging from 0 (no occurrence) to 3 (near-daily occurrence). Based on total PHQ-9 scores, participants were categorized into three groups: scores 0 to <5 indicated minimal or no depression; scores 5 to <10 indicated mild depression; and scores ≥10 suggested moderate to severe depression.

The BDI-II assessed depression presence and severity through 21 questions, each with four possible responses ranging from 0 (least severe) to 3 (strongest symptom expression).[22] Total scores (0-63) were calculated, with classifications ranging from "No depression" (0-8 points) to "Minimal

depression" (9-13 points), "Mild depression" (14-19 points), "Moderate depression" (20-28 points), and "Severe depression" (29-63 points).

Statistical analysis

Sample size computation was performed using G-Power 3.1,[23] considering F-test within-between interaction with two groups and four evaluation sessions. Assuming a priori medium effect size of 0.25, α error probability of 0.05, and power of 0.90, the resulting total sample size was 30. Additional participants were recruited to prevent statistical power reduction due to potential dropouts. Data are presented as mean ± standard deviation (SD).

The Shapiro-Wilk test assessed normality of questionnaire responses. Given non-normal data distribution, non-parametric statistical methods were employed. Friedman tests evaluated changes in questionnaire scores across time intervals (T0, T1, T2, T3) separately for experimental and active control groups. Subsequent post-hoc analyses employed Wilcoxon signed-rank tests with Bonferroni correction, establishing an adjusted significance threshold of $p<0.017$ for multiple comparisons. Between-group questionnaire score comparisons utilized Mann-Whitney U-tests, with statistical significance set at $p<0.05$.

Results

No significant differences ($p>0.05$) in clinical or demographic characteristics existed between groups at baseline. Groups did not differ by age [t-test = 1.062, $p = 0.78$] or gender distribution [$\chi^2 = 0.12$, $p = 0.83$]. Baseline demographic and clinical characteristics are summarized in Table 1.

Measuring depressive symptoms

A significant improvement was observed within the probiotic group after treatment ($p < 0.01$, Wilcoxon test), while no significant change was detected in the active control group. Significant time effects on PHQ-9 and BDI-II scores in the probiotic combination group were found [$\chi^2(3) = 12.1$, $p = 0.007$; $\chi^2(3) = 12.0$, $p = 0.006$, respectively] (Figure 2).

Post-hoc comparisons with Wilcoxon signed-rank tests (after Bonferroni correction, $p<0.017$) showed lower PHQ-9 scores after 6 weeks of treatment (T2) (mean±SD, 5.2±2.26) compared to baseline (T0) (mean±SD, 12.00±3.1) ($Z = -2.6$, $p = 0.009$, effect size = −0.425). The effect was

maintained after 3 weeks of washout, as revealed by significant differences between T3 (mean±SD, 5.2±3.1) and T0 ($Z = -2.52$, $p = 0.024$, effect size = −0.41).
Similar results were obtained with BDI-II scale scores. Post-hoc comparisons showed lower scores after 6 weeks of probiotic combination intake (T2) (mean±SD, 10.6±3.6) compared to baseline (T0) (mean±SD, 18.00±3.1) ($Z = -2.6$, $p = 0.009$, effect size = −0.425). The effects were maintained after 3 weeks of washout, as revealed by significant differences between T3 (mean±SD, 10.2±3.1) and T0 ($Z = -2.42$, $p = 0.034$, effect size = −0.41). Between-group comparisons using Mann–Whitney U tests did not reveal statistically significant differences between the probiotic and active control groups at any time point. Effect sizes for between-group differences were small, consistent with the pilot nature of the study and limited statistical power (Cohen's $d < 0.3$ for all comparisons). These results demonstrate that the probiotic combination group experienced significant within-group reductions in depressive symptoms that remained stable after a 3-week washout period. However, the active control group showed similar, though non-significant, trends in symptom improvement, and the study design prevents attribution of improvements specifically to the probiotic component, resulting in no significant between-group differences.

Discussion

This randomized, double-blind pilot study showed that a probiotic-containing formulation administered on top of an active control was associated with significant within-group improvements in depressive symptoms in adults with mild-to-moderate depressive symptoms. However, no statistically significant differences were observed between the probiotic and active control groups, indicating that the present findings cannot be attributed specifically to the probiotic component alone.

The persistence of benefits through the washout period suggests durable effects on mood regulation, possibly related to modulation of the gut–brain axis. The selected *Lactobacillus* strains (*L. rhamnosus* HN001, *L. rhamnosus* SP1, *L. acidophilus* LA1, and *L. reuteri* LR92) are known to influence neurotransmitter pathways, immune responses, and stress regulation.[24-26] These mechanisms could explain the observed symptom reduction. However, strain-level characterizations such as purity, viability, and resistance profiles were not performed, representing a technical limitation.[27-29]

The combination approach may act through multiple complementary mechanisms, including modulation of neurotransmission and hypothalamic–pituitary–adrenal axis activity.[30-33] These findings are consistent with previous evidence supporting the psychobiotic potential of specific *Lactobacillus* spp. strains in modulating depressive and anxiety symptoms. Sleep-related measures were included as secondary outcomes given the bidirectional relationship between sleep disturbances and depressive symptoms. Although no between-group differences emerged, baseline impairment and within-group trends suggest that future adequately powered studies should further investigate sleep as a potential mediator of mood-related effects.

The main limitation of this study is the absence of microbiome analysis. The inclusion of 16S rRNA sequencing or metabolomic profiling would have enabled direct correlation between microbial changes and mood improvement. Identifying which bacterial taxa are altered in association with symptom reduction will be crucial to confirm the causal relationship between dysbiosis and depression through the gut–brain axis.

Despite these constraints, this work provides promising evidence that targeted probiotic supplementation may improve depressive symptoms and support emotional well-being. Future studies should include microbiome profiling, objective biomarkers, and larger, more diverse populations to confirm these findings and elucidate the microbial species most critically involved in the gut–brain connection underlying mood regulation.

Conclusions

This pilot study suggests that a multi-strain probiotic formulation administered in combination with an active control was associated with improvements in depressive symptoms over time. Given the absence of significant between-group differences and the use of an active control, causal attribution to the probiotic component alone is not possible.

Future randomized controlled trials employing inert placebo controls, larger sample sizes, and integrated microbiome and metabolomic analyses will be essential to clarify the specific role of probiotics in modulating depressive symptoms through the gut–brain axis.

References

1. Kit A, Conway K, Makarowski S, et al. Can the gut-brain axis provide insight into psilocybin's therapeutic value in reducing stress? Neurobiol Stress 2025;36:100732.
2. Khan MT, Zohair M, Khan A, et al. From gut to brain: the roles of intestinal microbiota, immune system, and hormones in intestinal physiology and gut-brain-axis. Mol Cell Endocrinol 2025;112599.
3. Bankah AZ, Tagoe TA, Darko E, et al. Combined administration of *Lactobacillus* or *Bifidobacterium* offers enhanced antidepressant and anxiolytic activity in a dose dependent manner. Brain Behav 2025;15:e70564.
4. Mehta I, Juneja K, Nimmakayala T, et al. Gut microbiota and mental health: a comprehensive review of gut-brain interactions in mood disorders. Cureus 2025;17:e81447.
5. Rathore K, Shukla N, Naik S, et al. The bidirectional relationship between the gut microbiome and mental health: a comprehensive review. Cureus 2025;17:e80810.
6. Eastwood J, van Hemert S, Stolaki M, et al. Exploring the acute and chronic effects of a multistrain probiotic supplement on cognitive function and mood in healthy older adults: a randomized controlled trial. Am J Clin Nutr 2025;121:1268-80.
7. Dacaya P, Sarapis K, Moschonis G. The role and mechanisms of probiotic supplementation on depressive symptoms: a narrative review. Curr Nutr Rep 2025;14:53.
8. Guan Y, Zhu R, Zhao W, et al. Effects of *Lacticaseibacillus paracasei* K56 on perceived stress among pregraduate students: a double-blind, randomized, placebo-controlled trial. Front Nutr 2025;12:1544713.
9. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. Nat Rev Neurosci 2011;12:453-66.
10. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. Physiol Rev 2019;99:1877-2013.

11. Foster JA, Rinaman L, Cryan JF. Gut-brain axis: how the microbiome influences anxiety and depression. Nat Rev Neurosci 2017;18:374.
12. Strandwitz P. Neurotransmitter modulation by the gut microbiota. Brain Res 2018;1693:128-33.
13. Liu RT. The microbiome as a novel paradigm in studying stress and mental health. Am Psychol 2017;72:655.
14. Hidese S, Ogawa S, Ota M, et al. Effects of L-theanine administration on stress-related symptoms and cognitive functions in healthy adults: a randomized controlled trial. Nutrients 2019;11:2362.
15. Hidese S, Ota M, Wakabayashi C, et al. Effects of chronic L-theanine administration in patients with major depressive disorder: an open-label study. Acta Neuropsychiatr 2017;29:72-9.
16. Fedurco M, Gregorová J, Šebrlová K, et al. Modulatory effects of *Eschscholzia californica* alkaloids on recombinant GABAA receptors. Biochem Res Int 2015;2015:617620.
17. Vogt NM, Kerby RL, Dill-McFarland KA, et al. The gut microbiota-brain axis in health and disease. Gastroenterol Clin North Am 2017;46:77-89.
18. Bonaz B, Bazin T, Pellissier S. The vagus nerve at the interface of the microbiota-gut-brain axis. Front Neurosci 2018;12:49.
19. Hill C, Guarner F, Reid G, et al. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506-14.
20. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med 2001;16:606-13.
21. Cho HS, Choi JH, Park KH, et al. Standardization of the Korean version of Patient Health Questionnaire-9 as a screening instrument for major depressive disorder. Clin Psychopharmacol Neurosci 2021;19:104-11.
22. Beck A, Steer R, Brown G. *Manual for Beck Depression Inventory II (BDI-II).* San Antonio, TX: Psychology Corporation; 1996.

23. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.
24. Shen F, Zhu H, Bao Z, et al. *Lactiplantibacillus plantarum* T34 alleviates constipation by enhancing intestinal barrier and modulating gut homeostasis. Food Biosci 2025;66:106195.
25. Zhong H, Xu J, Yang M, et al. Protective effect of anthocyanins against neurodegenerative diseases through the microbial-intestinal-brain axis: a critical review. Nutrients 2023;15:496.
26. Verma A, Inslicht SS, Bhargava A. Gut-brain axis: role of microbiome, metabolomics, hormones, and stress in mental health disorders. Cells 2024;13:1436.
27. Steenbergen L, Sellaro R, van Hemert S, et al. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun 2015;48:258-64.
28. Reis DJ, Ilardi SS, Punt SEW. The anxiolytic effect of probiotics: a systematic review and meta-analysis of the clinical and preclinical literature. PLoS One 2018;13(6):e0199041.
29. Li Y, Zhong X, Cheng G, et al. Probiotics for depression and anxiety: a systematic review and meta-analysis. J Affect Disord 2023;324:646-60.
30. Liu Y, Zhang W. Recent advances in understanding the gut-brain axis. Nat Rev Neurosci 2023;24:156-71.
31. Cryan JF, O'Riordan KJ. The gut microbiome in neurological disorders. Lancet Neurol 2020;19:179-94.
32. Valles-Colomer M, Falony G, Darzi Y, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 2019;4:623-32.
33. Mayer EA, Tillisch K, Gupta A. Gut microbiota: a player in depression and anxiety. Nat Rev Gastroenterol Hepatol 2023;20:3-15.

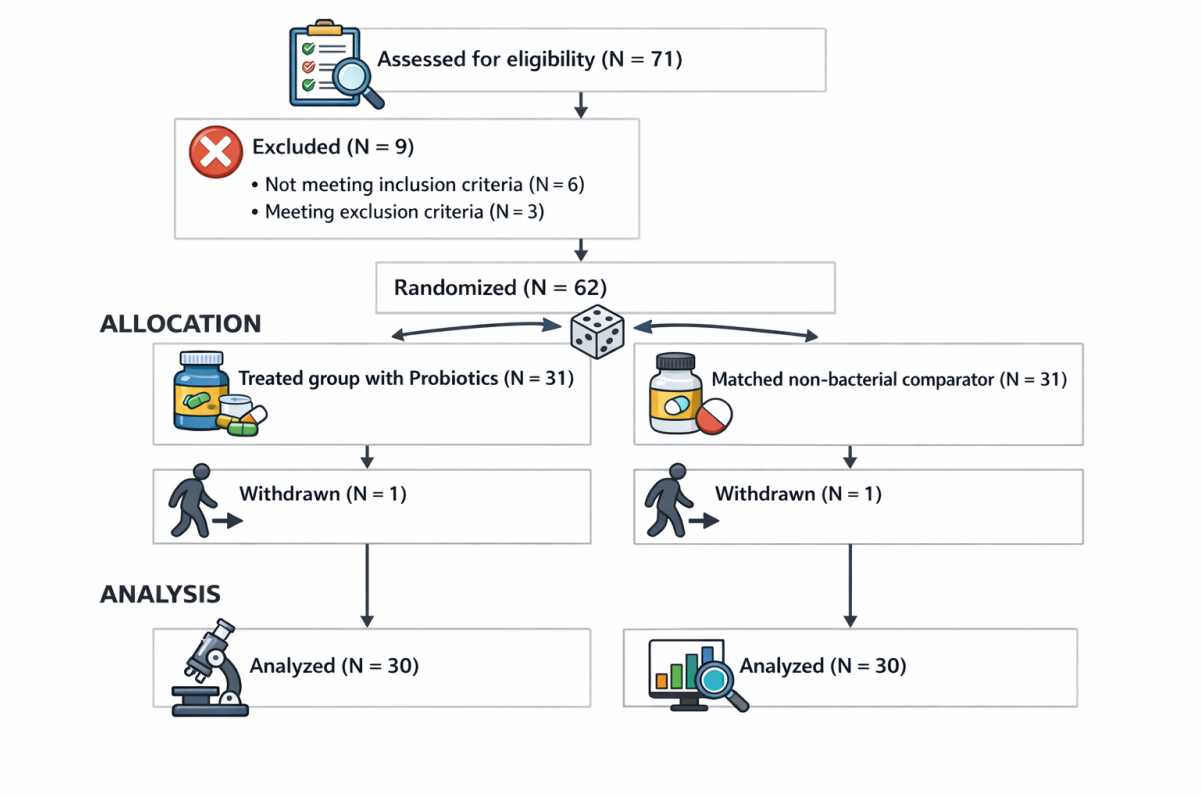

Figure 1. Flow diagram of participant enrolment, randomization, and analysis in the clinical trial evaluating the efficacy of a multi-strain *Lactobacillus* spp. probiotic on effects on depressive symptoms.

Table 1. Baseline demographic and clinical characteristics of participants at study entry (T0). No significant differences were observed between probiotic and active control groups. Values are presented as mean ± Standard Deviation (SD) or n (%).

Characteristic	**Probiotic (n=30)**	**Active control group (n=30)**	**p value**
Age, mean (SD)	22.1±3.0	21.9±3.1	0.78
Women, n (%)	18 (60%)	17 (57%)	0.83
Men, n (%)	12 (40%)	13 (43%)	0.81
PHQ-9 score, mean ± SD	12.2 ±3.1	11.9 (3.3)	0.72
BDI-II score, mean ± SD	18.02± 2.41	18.11 ± 2.22	0.68

Body Mass Index (BMI); Patient Health Questionnaire-9 (PHQ-9); Beck Depression Inventory-II (BDI-II)

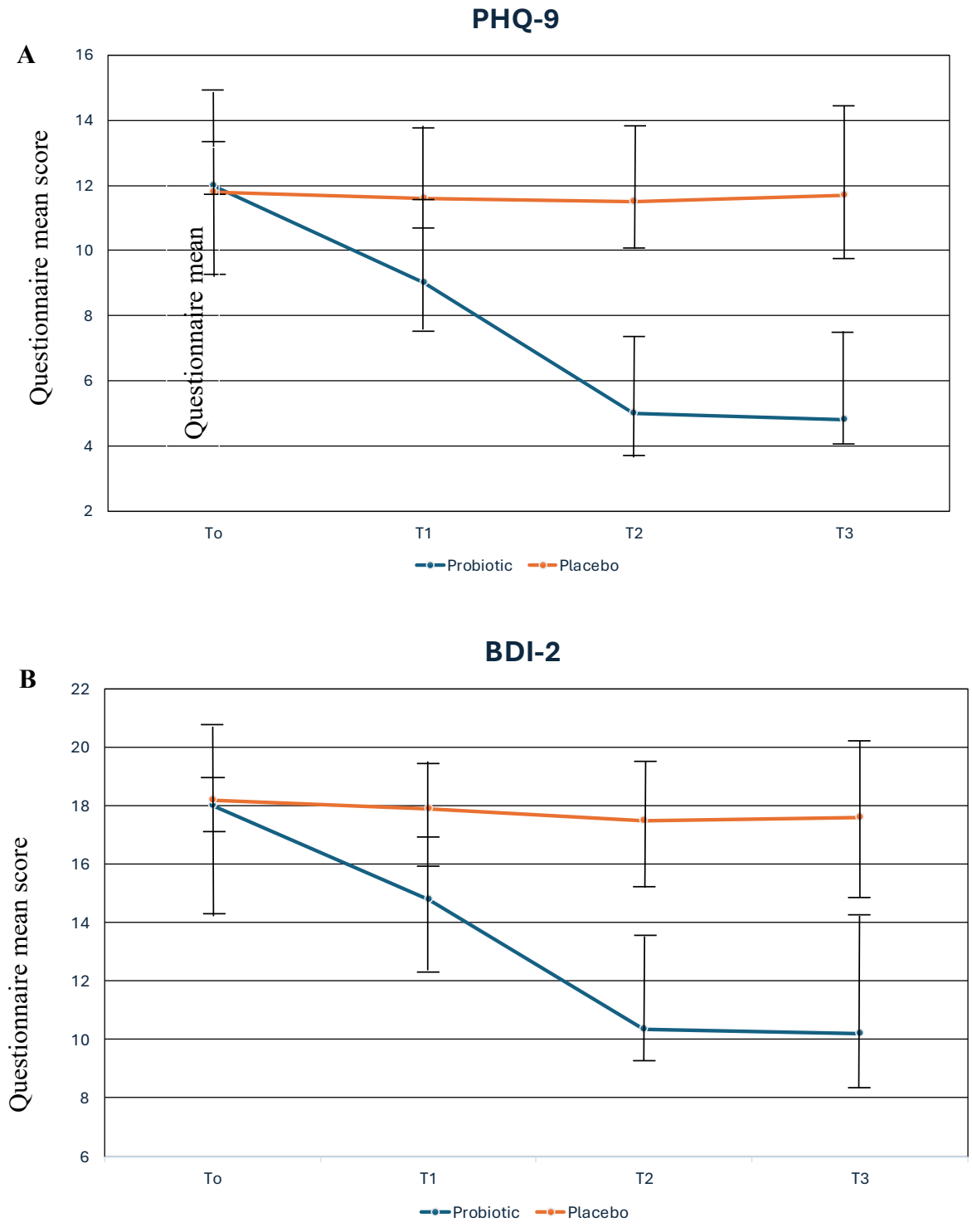

Figure 2. Changes in subjective with depressive symptoms during the study period across timepoints (T0: baseline, T1: 3 weeks, T2: 6 weeks, T3: washout) in probiotic and active control groups. Data are presented as mean ± SD for (A) Patient Health Questionnaire-9 (PHQ-9); and (B) Beck Depression Inventory-II (BDI-II)

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Conflicts of interest: the authors declare no conflicts of interest.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Ethics approval: the protocol was approved by institutional Ethics Committee of Palermo 1, Italy (Protocol no. 8 14/09/2022, Identifier NCT05565651).

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