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Assessment of optimal combinations of therapeutic probiotics for depression, anxiety, and stress

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Abstract

Background. Accumulating data show that probiotics may be beneficial for reducing depressive, anxiety, and stress symptoms. However, the best combinations and species of probiotics have not been identified. The objective of our study was to assess the most effective combinations and components of different probiotics through network meta-analysis.

Method. A systematic search of four databases, PubMed, Web of Science, Cochrane, and Embase, was conducted from inception to 11 January 2024. The GRADE framework was used to assess the quality of evidence contributing to each network estimate.

Results. We deemed 45 trials eligible, these included 4053 participants and 10 types of interventions. The quality of evidence was rated as high or moderate. The NMA revealed that *Bifidobacterium* exhibited a greater probability of being the optimal probiotic species for improving anxiety symptoms (SMD = −0.80; 95% CI −1.49 to −0.11), followed by *Lactobacillus* (SMD = −0.49; 95% CI −0.85 to −0.12). In addition, for multiple strains, compared with the other interventions, *Lactobacillus* + *Bifidobacterium* (SMD = −0.41; 95% CI −0.73 to −0.10) had a positive effect on depression.

Conclusion. The NMA revealed that *Lactobacillus* and *Bifidobacterium* had prominent efficacy in the treatment of individuals with anxiety, depression, and combination of *Lactobacillus* + *Bifidobacterium* had a similar effect. With few direct comparisons available between probiotic species, this NMA may be instrumental in shaping the guidelines for probiotic treatment of psychological disorders.

Introduction

The prevalence and severity of mental health disorders such as depression, anxiety, and stress are increasing. According to the World Health Organization, approximately 1 billion people worldwide were estimated to have a mental health disorder in 2019. In 2020, the significant increase in the prevalence of anxiety and depressive disorders was attributed to the COVID-19 pandemic (World Health Organization, 2022). The significant physical, psychological, and socioeconomic consequences of mental disorders warrant the development of innovative treatment strategies, which have attracted considerable attention in recent years. Long used to treat depression and anxiety, antidepressants and antianxiety medications have a variety of side effects, including altered weight and an increased risk of suicide, which raises questions regarding the effectiveness, safety, and tolerance of these medications (Jakobsen et al., 2017; Khin, Chen, Yang, Yang, & Laughren, 2011). New antidepressant compounds and nonpharmacological treatments are still needed. Due to their numerous therapeutic applications and advantageous effects on a range of clinical conditions, probiotics have recently gained much attention.

Probiotics have been proven effective at treating a variety of conditions, including acute diarrhea, allergic disease, and inflammatory diseases (Plaza-Diaz, Ruiz-Ojeda, Gil-Campos, & Gil, 2019; Rhoads et al., 2018). Associated with these cases is the concept of gut microbiota disorder, a disruption in the structure and number of gut flora due to chronic inflammation, which can be observed in individuals with depression, anxiety, and stress (Molina-Torres, Rodriguez-Arrastia, Roman, Sanchez-Labraca, & Cardona, 2019; Simpson et al., 2021). The literature suggests that the hypothalamic–pituitary–adrenal axis, which coordinates the body's response to adaptive stress, may play a role in the growth and operation of the gut microbiota (Foster, Rinaman, & Cryan, 2017; Sudo et al., 2004). A meta-analysis of randomized controlled trials (RCTs) assessing the efficacy of probiotics via the use of relevant meta-analysis indicated that probiotic preparations do have a psychological benefit in reducing depression symptoms significantly (ES = − 1.41; 95% CI −2.53, to −0.30) (Musazadeh et al., 2023). Unfortunately, *Musazadeh*'s research did not further determine which probiotics had the best effect on

improving depression, nor did it focus on other neuropsychiatric symptoms such as anxiety and stress. To the best of our knowledge, there is no research comparing which probiotics are optimal for treating neuropsychiatric symptoms.

Probiotics may represent a paradigm shift in the management of mental health disorders, either as a supplement to conventional therapy or as a stand-alone therapy (Chen et al., 2019; Desbonnet et al., 2010; Naseribafrouei et al., 2014). There has been an increase in clinical trials examining the use of probiotics for treating mental health conditions, including depression, anxiety, and stress. Several probiotics, including *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Clostridium*, and *Saccharomycete*, are the most studied probiotics for treating mental illness (Vaghef-Mehrabany, Maleki, Behrooz, Ranjbar, & Ebrahimi-Mameghani, 2020). However, there are no direct clinical outcome trials on the optimal combination of the above strains, and probiotic treatment regimens for depression, anxiety, and stress are uncertain due to this inconsistency and potential negative cost effects.

We hypothesize that there are optimized probiotics for improving symptoms of depression, anxiety, and stress. The effectiveness of several interventions can be compared and examined concurrently across a network of trials by employing a network meta-analysis (NMA) (Cipriani, Higgins, Geddes, & Salanti, 2013). Hence, an NMA was conducted to compare the effects of probiotics to identify the best interventions.

Method

This review was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for NMA (Hutton et al., 2015).

Literature information sources and search strategy

The four databases, PubMed, Web of Science, Cochrane, and Embase, were searched from the date of their inception to 11 January 2024, utilizing combinations of the following search terms: ('probiotic' OR 'probiotics' OR 'symbiotic' OR '*Lactobacillus*' OR '*Bifidobacterium*' OR '*Enterococcus*' OR '*Streptococci*' OR '*Bacillus*' OR '*Clostridium*' OR '*Saccharomycete*'), along with ('anxiety' OR 'depression' OR 'stress' OR 'mood' OR 'mental health' OR 'psychological stress'). The search strategy is reported in Appendix Table S1. An additional search of the grey literature was performed using Google Scholar, OpenGrey, and Clinical trials.gov on the same day. The search was limited to human studies (clinical trials) written in the English language. We manually searched and screened the reference lists from reviews and meta-analyses to identify any missing literature.

Study eligibility and selection

The eligibility criteria were established using five PICOS dimensions: (I) participants, (II) interventions, (III) comparators, (IV) outcomes, and (V) study design.

(I) Participants (P): Participants were adults (⩾18 years) of both sexes who suffered from anxiety, depression, or perceived stress. The study scope was not limited to the general population or to populations with clinical symptoms if measurements of depression, anxiety, and perceived stress could be completed.

(II) Interventions (I): Any type and form of probiotic (e.g. capsule, sachet, yogurt) were regarded as eligible interventions, for which detailed information about the probiotic strains and dosages was available. A three-week minimum treatment period was needed. Studies investigating prebiotics, synbiotics, or antibiotics illicit drugs; certain prescription medications; vitamin or antioxidant supplements; high caffeine intake; or dietary intake of these substances were excluded.

(III) Comparison (C): Studies were eligible if a blinded placebo control group was included.

(IV) Outcomes (O): The primary outcome was to determine whether probiotics had any impact on depression, anxiety, or stress symptoms using a validated measure and the presence of these moods was ascertained with the use of a validated scale. To reduce the possibility of concealed reporting bias caused by differences in baseline depression severity, the mean differences in psychological test scores were chosen as continuous outcomes rather than endpoint values.

(V) Study design (S): Randomized, blinded, placebo-controlled trials. Single-blinded trials were excluded.

Study quality assessment

The Cochrane Handbook for Systematic Reviews of Interventions' risk of bias criteria were used to assess the quality of all relevant studies (Cumpston et al., 2019); these criteria include seven indicators: (1) condition allocation through random sequence generation (selection bias); (2) concealment of condition allocation (allocation bias); (3) concealment of participants and study (implementation bias); (4) blinding of outcome assessment (measurement bias); (5) completeness of outcome (follow-up bias); (6) selective outcome reporting of results for depression, stress, or anxiety (reporting bias); and (7) other bias (other sources of bias).

Data extraction

The titles and abstracts were assessed by two independent reviewers (Y.F.Y. and M.W.) for the initial screening; then the full text of eligible articles was retrieved and assessed. A third evaluator (C.Y.) was requested to discuss the articles of debate in cases of controversy. For study characteristics, we extracted data including primary author, publication year, country, sample size, age, gender, race, ethnicity, education, income, and treatment details (types of probiotics, dosages, duration of treatment, and psychological measures). Data extracted from the eligible studies are summarized in Table 1.

Statistical analysis

For all direct comparisons, conventional pairwise meta-analysis using a DerSimonian and Laird random effects model was performed (DerSimonian, 1996). We evaluated heterogeneity in direct comparisons using the I^2 statistic and visual inspection of the forest plots. We then used Stata MP 16 to perform a frequentist random effects NMA. Effect estimates were reported with a 95% confidence interval (CI) as weighted mean differences for continuous outcomes. Comparing the direct and indirect comparison estimations allowed us to check the NMA's consistency. To indirectly compare intervention effects, a network meta-analysis was applied with a consistency or inconsistency

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Table 1. The main characteristics of the randomized controlled trials were included in the network meta-analysis.

Reference	Country	Sample size (control/ intervention1/ intervention2)	Gender (%)	Age (years)	Mean age (years)	Race or ethnicity (%)	Education	Income	Type of bacteria	Dose (CFU/day)	Duration (weeks)	Psychological symptoms	Outcome measures
Akhgarjand et al. (2022)	Iran	30/30/30	M: 53.33 F: 46.67	50–90	PRO1: 67.93 ± 7.80 PRO2: 67.90 ± 7.90 PLA: 67.77 ± 7.90	NR	Illiterate (n = 73) Educated (n = 17)	NR	*Lactobacillus*, *Bifidobacterium*	2.00×10^{15}	12	Anxiety	GAD-7
Akkasheh et al. (2016)	Iran	20/20	M: NR F: NR	20–55	PRO: 38.3 ± 12.1 PLA: 36.2 ± 8.2	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	6.00×10^{9}	8	Depression	BDI
Baião et al. (2022)	UK	36/35	M: 36.60 F: 63.40	18–55	PRO: 27.94 ± 6.99 PLA: 29.64 ± 10.52	Caucasian (81.70) Black (1.40) Hispanic (1.40) Asian (14.10) Mixed (1.40)	Secondary (n = 22) Undergraduate (n = 16) Postgraduate (n = 33)	NR	*Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus*	8.00×10^{9}	4	Anxiety	STAI
Barthow et al. (2022)	New Zealand	39/38	M: 45.75 F: 53.25	18–80	PRO: 60 (52.1, 66.5) PLA: 59.9 (55.3, 67)	NR	NR	NR	*Lactobacillus*	6.00×10^{9}	24	Anxiety, Depression, Stress	DASS
Chahwan et al. (2019)	Australia	37/34	M: 30.98 F: 69.02	⩾18	PRO: 36.65 ± 11.75 PLA: 35.49 ± 12.34	Caucasian (67.60) Non-Caucasian (32.40)	NR	NR	*Bifidobacterium*, *Lactobacillus*, *Lactococcus*	2.50×10^{9}	48	Depression, Anxiety, Stress	BDI, DASS, BAI
Dawe et al. (2020)	New Zealand	76/88	M: NR F: NR	NR	T: 29.75 ± 5.45	European (21.34) Pasifika (47.56) Asian (8.54) Latin American/ African (2.44) Māori (20.12)	Did not complete high school (n = 46) Completed high school (n = 25) Tertiary education (n = 60) Other qualification (n = 33)	NR	*Lactobacillus*, *Bifidobacterium*	6.50×10^{9}	22	Depression, Anxiety	EPDS, STAI
Eskandarzadeh et al. (2019)	Iran	24/24	M: 18.75 F: 81.25	18–65	PRO: 34.17 ± 6.14 PLA: 33.67 ± 6.56	NR	NR	NR	*Bifidobacterium*, *Lactobacillus*	1.80×10^{10}	8	Anxiety	HAMA, STAI, BAI
Freijy et al. (2023)	Australia	27/29	M: 3.57 F: 96.43	18–65	PRO: 30.5 ± 13.7 PLA: 32.6 ± 13.9	Caucasian (82.14)	Secondary (n = 7) College or trade certificate (n = 5) Tertiary (n = 47)	\$0–\$39999 (n = 9) \$40000–\$79999 (n = 11) \$80000–\$99999 (n = 13) \$100000+ (n = 26)	*Bifidobacterium*, *Lactobacillus*	2.40×10^{10}	8	Anxiety, Depression, Stress	BAI, BDI, PSS

(*Continued*)

Table 1. (*Continued.*)

Reference	Country	Sample size (control/ intervention1/ intervention2)	Gender (%)	Age (years)	Mean age (years)	Race or ethnicity (%)	Education	Income	Type of bacteria	Dose (CFU/day)	Duration (weeks)	Psychological symptoms	Outcome measures
Gawlik-Kotelnicka et al. (2023)	Poland	22/26	M: 85.42 F: 14.58	⩾18	PRO: NR PLA: NR	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	3.00×10^9	8	Anxiety, Depression, Stress	MADRS, DASS
Ghorbani et al. (2018)	Iran	20/20	M: 30.00 F: 70.00	18–55	PRO: 34.45 ± 3.95 PLA: 35.5 ± 5.27	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*, *Streptococcus*	8.60×10^9	6	Depression	HAMD
Haghighat et al. (2021)	Iran	19/23	M: NR F: NR	30–65	PRO: NR PLA: NR	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	1.40×10^8	12	Depression, Anxiety	BDI, BAI
Hulkkonen et al. (2021)	Finland	134/128	M: NR F: NR	NR	T: 30.6 ± 4.6	NR	College or university education ($n = 239$) other ($n = 23$)	NR	*Lactobacillus*, *Bifidobacterium*	1.00×10^{10}	48	Depression, Anxiety	EPDS, SCL-90
Kazemi et al. (2019)	Iran	36/38	M: 31.08 F: 68.92	18–50	PRO: 36.15 ± 7.85 PLA: 36 ± 8.47	NR	No high school certificate ($n = 5$) Completed high school ($n = 20$) Undergraduate degree ($n = 33$) Postgraduate degree ($n = 16$)	NR	*Lactobacillus*, *Bifidobacterium*	1.00×10^{10}	8	Depression	BDI
Kim et al. (2021)	Korea	26/27	M: 49.06 F: 50.94	⩾65	PRO: 71.11 ± 5.02 PLA: 72 ± 3.36	NR	Elementary or less ($n = 9$) Junior-high school ($n = 12$) High school ($n = 14$) College or more ($n = 18$)	NR	*Bifidobacterium*	1.00×10^9	12	Depression, Stress	GDS-K
Kreuzer et al. (2022)	Austria	29/28	M: 21.05 F: 78.95	⩾18	PRO: 44.63 ± 15.12 PLA: 40.38 ± 11.30	NR	NR	NR	*Bifidobacterium*, *Lactobacillus*	7.50×10^9	4	Depression	BDI, HAMD
Lee et al. (2021)	Korea	28/34	M: 62.90 F: 37.10	⩾20	PRO: 23.44 ± 2.88 PLA: 23.75 ± 3.42	NR	NR	NR	*Weissella cibaria*	1.00×10^8	8	Depression	Depression Scale
Lee et al. (2021a)	Korea	59/63	M: 31.97 F: 68.03	19–65	PRO: 38.86 ± 10.89 PLA: 37.63 ± 11.04	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	2.50×10^9	8	Depression, Anxiety, Stress	BDI, BAI, SRI

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Mahboobi et al. (2022)	Iran	35/39	M: 21.62 F: 78.38	18–50	PRO: 38.94 ± 7.19 PLA: 35.90 ± 8.64	NR	⩽6 years of official education (*n* = 9) 6–10 years of official education (*n* = 35) B.Sc. Degree (*n* = 24) M.Sc. degree and above (*n* = 6)	NR	*Lactobacillus*, *Bifidobacterium*	1.80×10^{10}	9	Depression	BDI
Majeed et al. (2018)	India	20/20	M: 15.00 F: 85.00	20–65	PRO: 40.36 ± 10.28 PLA: 43.88 ± 9.85	Asian (100.00)	NR	NR	*Bacillus*	2.00×10^{9}	12	Depression	HAMD, MADRS
Marotta et al. (2019)	Italy	15/18	M: 63.64 F: 36.36	19–33	PRO: 21.61 ± 2.22 PLA: 21.67 ± 2.19	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	4.00×10^{9}	9	Depression, Anxiety	BDI, STAI
Meng et al. (2022)	China	100/100	M: 40.50 F: 59.50	⩾18	PRO: 44.52 ± 7.57 PLA: 44 ± 6.80	NR	NR	NR	*Lactobacillus*	6.00×10^{10}	12	Anxiety	HAMA, SAS
Moludi et al. (2022)	Iran	24/24	M: 64.58 F: 35.42	18–85	PRO: 51.25 ± 12.66 PLA: 51.82 ± 12.22	NR	Illiterate (*n* = 3) Diploma and lower (*n* = 41) Bachelors and higher (*n* = 4)	NR	*Lactobacillus*	1.90×10^{9}	8	Depression, Anxiety	BDI, STAI
Mutoh et al. (2023)	Japan	28/29	M: 17.54 F: 82.46	20–64	PRO: 20.7 ± 0.4 PLA: 20.9 ± 0.5	NR	NR	NR	*Lactobacillus*	5.00×10^{9}	6	Anxiety	STAI
Patterson et al. (2020)	Germany	58/55	M: NR F: NR	⩾18	PRO: 23.73 ± 4.27 PLA: 23.25 ± 4.20	NR	NR	NR	*Lacticaseibacillus*	1.75×10^{10}	5	Anxiety, Stress, Depression	STAI, VAS, DASS
Pinto-Sanchez et al. (2017)	Canada	22/22	M: 45.45 F: 54.55	⩾18	PRO: 40.0 (26, 57) PLA: 46.5 (30, 58)	Caucasian (90.91) other (9.09)	NR	NR	*Bifidobacterium*	1.00×10^{10}	6	Depression, Anxiety	HAD, STAI
Reininghaus et al. (2020)	Austria	33/28	M: 22.95 F: 77.05	18–75	PRO: 43 ± 14.31 PLA: 40.11 ± 11.45	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	7.50×10^{10}	4	Depression	HAMD, BDI
Salleh et al. (2021)	Italy	15/15	M: NR F: NR	18–30	PRO: 19.5 ± 1.0 PLA: 19.9 ± 1.3	NR	NR	NR	*Lactobacillus*	3.00×10^{10}	6	Anxiety, stress	CSAI-2R, PSS
Sawada et al. (2019)	Japan	25/24	M: NR F: NR	18–22	PRO: 19.8 ± 1.4 PLA: 20.1 ± 1.1	NR	NR	NR	*Lactobacillus*	1.00×10^{10}	12	Depression, Anxiety	HADS, STAI

(*Continued*)

Table 1. (*Continued.*)

Reference	Country	Sample size (control/ intervention1/ intervention2)	Gender (%)	Age (years)	Mean age (years)	Race or ethnicity (%)	Education	Income	Type of bacteria	Dose (CFU/day)	Duration (weeks)	Psychological symptoms	Outcome measures
Shafie et al. (2022)	Iran	33/33	M: NR	45–55	PRO: 51.80 ± 2.33	NR	Illiterate (n = 4)	NR	*Lactobacillus*, *Bifidobacterium*, *Streptococcus*	1.00×10^8	6	Anxiety, Depression, Stress	DASS
							Primary school (n = 9)						
							Secondary school (n = 10)						
			F: NR		PLA: 52.36 ± 2.43		High school (n = 14)						
							Diploma (n = 22)						
							University (n = 7)						
Slykerman et al. (2022)	New Zealand	300/300	M: 3.17	18–70	PRO: NR	European (74.33)	NR	NR	*Lactobacillus*	6.00×10^9	12	Anxiety, Stress	STAI, PSS,
						Māori (7.00)							
			F: 96.83		PLA: NR	Pacific (1.50)							
						Asian (6.33)							
						other (10.84)							
Slykerman et al. (2022a)	New Zealand	242/241	M: 75.16	⩾18	PRO: NR	European (44.72)	NR	NR	*Lactobacillus*	6.00×10^9	10	Anxiety, Stress	STAI, PSS,
			F: 24.22			Māori (7.87)							
					PLA: NR	Pacific (4.14)							
			Unspecified: 0.62			Asian (36.02)							
						other (7.25)							
Ullah et al. (2022)	Italy	32/33	M: 41.54	18–65	PRO: 37.75 ± 14.06	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	3.00×10^9	48	Depression	HAMD
			F: 58.46		PLA: 38.60 ± 15.80								
Zhang et al. (2021)	China	31/38	M: 36.23	18–60	PRO: 45.8 ± 12.3	NR	NR	NR	*Lacticaseibacillus*	1.00×10^{10}	8	Depression	HAMD, BDI
			F: 63.74		PLA: 49.7 ± 9.6								
Boehme et al. (2023)	Switzerland	21/24	M: 57.78	25–65	PRO: 37.5 ± 10	NR	NR	NR	*Bifidobacterium*	1.00×10^{10}	6	Anxiety, Depression, Stress	HADS, PSS
			F: 42.22		PLA: 40.7 ± 9.0								
Zhu et al. (2023)	China	30/30	M: 50.00	⩾18	PRO: 22.30 ± 0.25	NR	NR	NR	*Lactobacillus*	3.00×10^{10}	3	Anxiety, Depression	HAMA, HDRS
			F: 50.00		PLA: 22.5 ± 0.25								
Nikolova et al. (2023)	United Kingdom	25/24	M: 20.41	18–55	PRO: 32.5 (24.3, 39.0)	Asian (14.28)	NR	NR	*Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*	8.00×10^{10}	8	Anxiety, Depression	HAMD, HAMA
						Multiracial (12.24)							
			F: 79.59		PLA: 27.0 (23.0, 41.0)	White (67.35)							
						Another race (6.13)							

Ustaoğlu et al. (2023)	Turkey	26/26	M: 0.00 F: 100.00	20–55	PRO: NR PLA: NR	NR	NR	NR	*Lactobacillus*	6.00×10^{10}	6	Anxiety, Depression	HADS
Walden et al. (2023)	United States	35/35	M: 50.00 F: 50.00	31.0 ± 9.5	PRO: 29.7 ± 9.0 PLA: 32.3 ± 10.0	NR	NR	NR	*Limosilactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Bifidobacterium*	4.00×10^{9}	6	Anxiety, Depression	BDI, STAI
Heidarzadeh et al. (2020)	Turkey	26/28	M: 64.81 F: 35.19	20–50	PRO: 37.8 ± 7.9 PLA: 36 ± 8.5	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	1.0×10^{10}	8	Depression	BDI
Reiter et al. (2020)	Austria	33/28	M: 22.95 F: 77.05	18–75	PRO: 43 ± 14.31 PLA: 40.11 ± 11.45	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	7.5×10^{9}	4	Depression	BDI, HAMD
Raygan et al. (2019)	Iran	27/27	M: 38.89 F: 61.11	45–85	PRO: 64.8 ± 8.3 PLA: 62.4 ± 13.1	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*, *Streptococcus*	8.0×10^{9}	12	Anxiety, Depression	BDI, BAI
Raygan et al. (2018)	Iran	30/30	M: 50.00 F: 50.00	45–85	PRO: 71.5 ± 10.9 PLA: 67.3 ± 11	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*, *Streptococcus*	8.0×10^{9}	12	Anxiety, Depression	BDI, BAI
Salami et al. (2019)	Iran	24/24	M: 25.00 F: 75.00	20–60	PRO: 34.79 ± 1.06 PLA: 36.54 ± 1.44	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	2.0×10^{9}	16	Depression	BDI
Ostadmohammadi et al. (2019)	Iran	30/30	M: NR F: NR	18–40	PRO: 24.4 ± 4.7 PLA: 25.4 ± 5.1	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	8.0×10^{9}	12	Depression	BDI
Roman et al. (2018)	Spain	15/16	M: 9.68 F: 90.32	NR	PRO: 55 ± 2.09 PLA: 50.27 ± 2.03	NR	NR	NR	*Lactobacillus*, *Bifidobacterium*	6.0×10^{6}	8	Anxiety, Depression	BDI

BAI, Beck Anxiety Index; BDI, Beck Depression Inventory; CSAI-2R, Competitive State Anxiety Inventory-2; DASS, Depression Anxiety Stress Scale; EPDS, Edinburgh Postnatal Depression Scale; F, Female; GAD, Generalized Anxiety Disorder; GDS, Geriatric Depression Scale; HADS, Hamilton Depression Rating Scale; Hospital Anxiety and Depression Scale; HAMA, Hamilton Rating Scale for anxiety; HAMD, Hamilton rating scale for depression; HDRS, Hamilton Depression Rating Scale; M, Male; MADRS, Montgomery-Asberg Depression Rating Scale; NR, Not reported; PLA, Placebo; PRO, Probiotic; PSS, Perceived Stress Scale; SAS, Self-Rating Anxiety Scale; SCL-90, Symptoms Checklist; SRI, Stress Response Inventory; STAI, State Trait Anxiety Inventory; T, Total; VAS, Visual Analog Scale.

model, where appropriate. The node-splitting method was conducted to evaluate the inconsistency of the model, which divided the data on a specific comparison into direct and indirect evidence (Dias, Welton, Caldwell, & Ades, 2010; Veroniki, Vasiliadis, Higgins, & Salanti, 2013). We estimated the surface under the cumulative ranking curve (SUCRA) probabilities between all treatments for the results to rank the efficient interventions (Salanti, Ades, & Ioannidis, 2011). We utilize the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework which was specifically developed for concluding a network meta-analysis to appraise the certainty of the evidence (Brignardello-Petersen et al., 2020).

To ascertain whether study characteristics had an impact on the results, we conducted a subgroup analysis to explore whether the duration of intervention and treatment dosage was associated with the efficacy of probiotics in patients with stress, anxiety, or depression. To ascertain whether a single study could have had an impact on the outcomes, a sensitivity analysis was carried out by deleting one study at a time. Publication bias was evaluated through funnel-plot asymmetry, Begg's and Egger's tests, and the trim and fill method. If the above analysis showed conflicting results from publication bias, the trim and fill method was the first choice (Chaimani, Higgins, Mavridis, Spyridonos, & Salanti, 2013; Macaskill, Walter, & Irwig, 2001).

Regarding the collection of questionnaire data, the State-Trait Anxiety Inventory (STAI) was prioritized for anxiety, the Beck Depression Inventory (BDI) for depression, and the Depression-Anxiety-Stress Scale (DASS) for stress. If the scale mentioned above was not available, we considered utilizing the one from the article.

Result

Literature search and screening

Figure 1 depicts the procedure for extracting data. Using a planned search technique, the last electronic database search completed on 11 January 2024, yielded a total of 10 968 articles. Finally, the qualitative synthesis includes 45 double-blind, randomized, placebo-controlled trials.

Baseline characteristics of included studies

A summary of studies included in the quantitative review and their results are presented in Table 1. The included studies are comprised of 4053 participants, and there are 1184 males and 2040 females in the reported literature. The included RCTs were performed in 18 countries, in which 13 studies were conducted in Iran, four in New Zealand, three in Korea, three in Italy, three in China, two in Japan, two in Australia, three in Austria, two in the UK, one in Poland, one in Finland, one in India, one in Canada, one in Germany, one in Spain, one in Switzerland, two in Turkey, and one in the United States, with the sample size ranging from 30 to 600 participants. There are 10 different races and ethnicities in the reported study population, of which Europeans account for the largest proportion (44.45%), followed by Asians (18.05%) and Caucasians (12.24%). The age of participants ranged from 18 to 90 years. Furthermore, based on published data on educational attainment, the majority of individuals hold a college degree or higher ($n = 563$), followed by secondary school ($n = 191$), uneducated people ($n = 80$), and primary school ($n = 78$). The income level of the study population was only given in one study. The study duration varied from 4 weeks to 48 weeks.

Among the included trials, the following probiotic genera were mainly focused on including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Weissella*, *Lacticaseibacillus*, *Limosilactobacillus*, *Lactiplantibacillus* and *Lactococcus*. The types of probiotics administered to participants were based on the following combinations or single strains: *Lactobacillus* + *Bifidobacterium* (18 trials), *Lactobacillus* (11 trials), *Bifidobacterium* (4 trials), *Lactobacillus* + *Bifidobacterium* + *Streptococcus* (4 trials), *Bacillus* + *Lactobacillus* + *Bifidobacterium* + *Streptococcus* (2 trial), *Lactobacillus* + *Bifidobacterium* + *Lactococcus* (1 trial), *Weissella* (1 trial), *Bacillus* (1 trial), *Lacticaseibacillus* (2 trial) and *Limosilactobacillus* + *Lacticaseibacillus* + *Lactiplantibacillus* + *Bifidobacterium* (1 trial).

Risk of bias assessment

The risk of bias in the trials that were included according to the Cochrane Collaboration tool is shown in Appendix 1 Fig. S1. Among the 45 studies, 73.33% (33/45) reported adequate random sequence generation but were considered high risk in 11 studies, while the risk was unclear in the remaining study. The risk of bias in allocation concealment was 77.78%, and the risk was high in seven trials and unclear in three studies. The outcome assessment was double- or triple-blinded in 91.11% of the trials and was unclear in four trials. Whereas most of the trials had a low risk of bias due to the blinding of participants and key researchers, two trials had an unclear risk of bias. Additionally, a low risk of bias was shown in most of the trials based on incomplete outcome data and selective outcome reporting but was unclear in one study.

Effectiveness of probiotics for anxiety

Thirty RCTs ($n = 2960$) were included in the assessment, and the results of the global and local inconsistency tests are presented in Appendix 2 Figs S1 and Table S1. Since neither of the tests revealed any substantial contradiction between direct and indirect comparisons, the consistency model was applied. The NMA showed that *Lactobacillus* (SMD = −0.49; 95% CI −0.85 to −0.12), and *Bifidobacterium* (SMD = −0.80; 95% CI −1.49, to −0.11) were among the most effective treatments. The net graphs are shown in Fig. 2a. The SUCRA analysis (Appendix 2 Table S2 and Fig. S3) and league table (Table 2) showed that *Bifidobacterium* had the best rank among all the interventions; moreover, *Lactobacillus* was the second most common bacteria. There was a high level of evidence indicating anxiety based on the GRADE method (Appendix 1 Table S2).

Evidence of loop-specific heterogeneity was found in Appendix 2 Fig. S2. Direct and indirect evidence did not appear to be inconsistent when the results from network meta-analysis and conventional pairwise meta-analysis were compared (Fig. 3a). For the study outcome, we observed obvious heterogeneity across all treatment contrasts ($I^2 = 69.50\%$). Direct pairwise evidence showed that probiotic supplements could improve anxiety syndrome (SMD = −0.43; 95% CI −0.60 to −0.26). We conducted a subgroup analysis based on intervention time and dosage. NMA suggested that *Bifidobacterium* (12w) had a beneficial effect on participants (SMD = −1.82; 95% CI −3.29 to −0.34) and on participants supplemented with *Lactobacillus* (12w) (SMD = −0.88; 95% CI −1.68 to −0.08). Based on the SUCRA analysis, *Bifidobacterium* (12w) had the highest rank, followed

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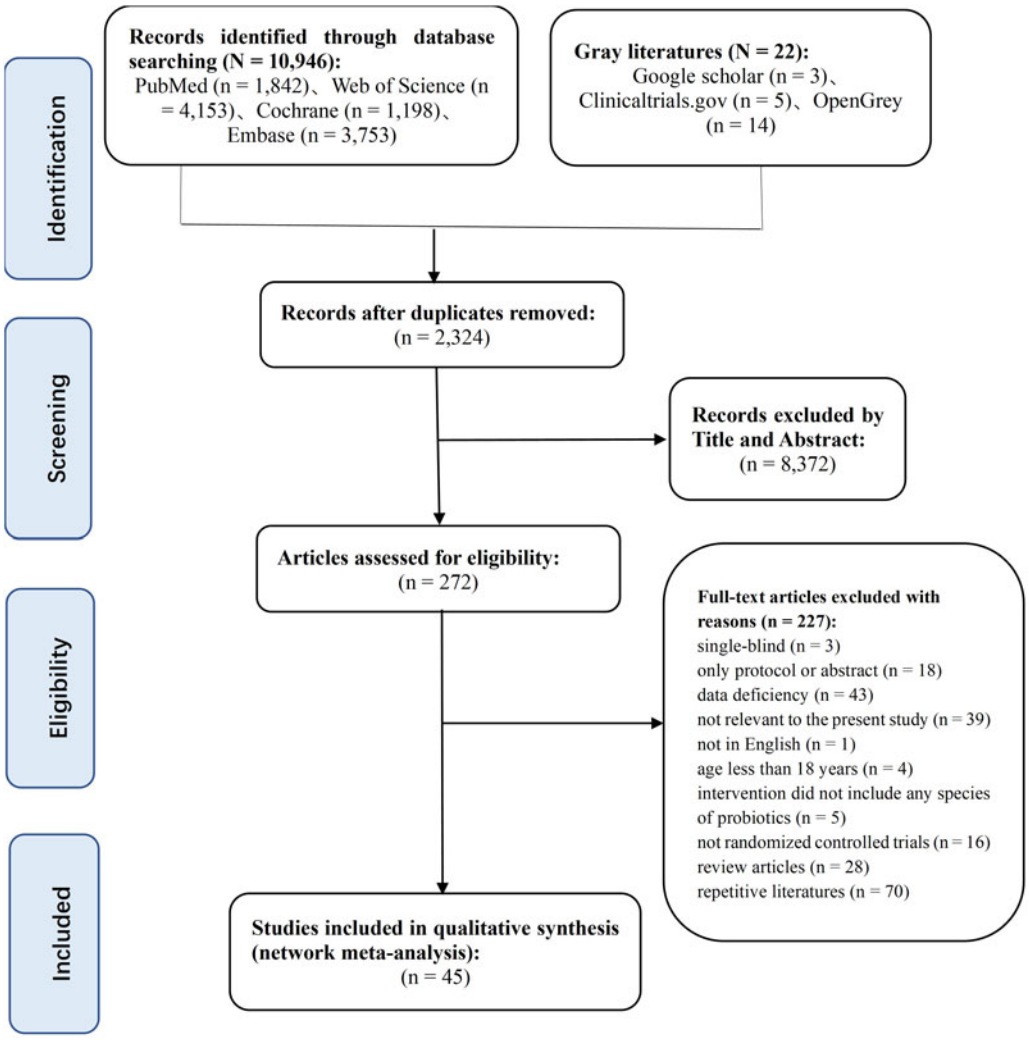

Figure 1. Flow diagram of assessment of studies.

by *Lactobacillus* (12w) (Appendix 3 Table S1). In addition, *Lactobacillus* (SMD = −3.02; 95% CI −3.79 to −2.26) and *Bifidobacterium* (SMD = −2.96; 95% CI −3.72 to −2.21) had positive effects on anxiety when the dosage was greater than 10^{11} CFU/day (Appendix 3 Table S3). According to Begg's test ($p < 0.01$) and Egger's regression test ($p < 0.01$), there was publication bias (Appendix 2 Fig. S4). As a result, trim-and-fill analysis was used. The heterogeneity test and iterative technique were used to determine the number of missing studies. After seven iterations, the results demonstrated that the pooled effect size estimates did not significantly change (SMD = −0.52, 95% CI −0.67 to −0.36; $p < 0.01$), which indicated that publication bias had little effect and that the results were relatively stable.

Effectiveness of probiotics for depression

A total of 37 RCTs ($n = 2467$) reported the effect of probiotics on subjects with depression, and the network plot is shown in Fig. 2b. The consistency model was selected because neither the global inconsistency test nor the node-splitting assessment revealed any appreciable inconsistency between direct and indirect comparisons (Appendix 2 Fig. S5 and Table S3). The NMA results (Table 3) revealed significant improvement in individuals with depression who received *Lactobacillus* + *Bifidobacterium* (SMD = −0.41; 95% CI −0.73 to −0.10) compared with those who received placebo. The SUCRA analysis (Appendix 2 Table S4 and Fig. S6) demonstrated that *Bifidobacterium* and *Lactobacillus* + *Bifidobacterium* were the most common genera for improving depression symptoms, while *Bifidobacterium* was not a significant factor. Appendix 1 Table S2 shows that the quality of evidence (calculated by the GRADE method) for depression was moderate.

There was significant heterogeneity across all intervention contrasts ($I^2 = 77.40\%$). A pairwise meta-analysis for each type of probiotic compared with the placebo is presented in Fig. 3b, and the effectiveness of probiotics for depression was assessed (SMD: −0.33 95% CI −0.50 to −0.15). Moreover, we conducted a subgroup analysis based on treatment duration and intervention dose. NMA significantly improved depression in individuals who received *Lactobacillus* + *Bifidobacterium* (16w) and at a dosage of 10^9–10^{11} CFU/day (Appendix 3 Table S2 and Table S4). Begg's test ($p < 0.01$) and Egger's test ($p = 0.01$) revealed publication bias (Appendix 3 Fig. S7). The trim-and-fill analysis suggested that seven iterations of the iterative technique did not significantly change the pooled effect size estimates (SMD = −0.48, 95% CI

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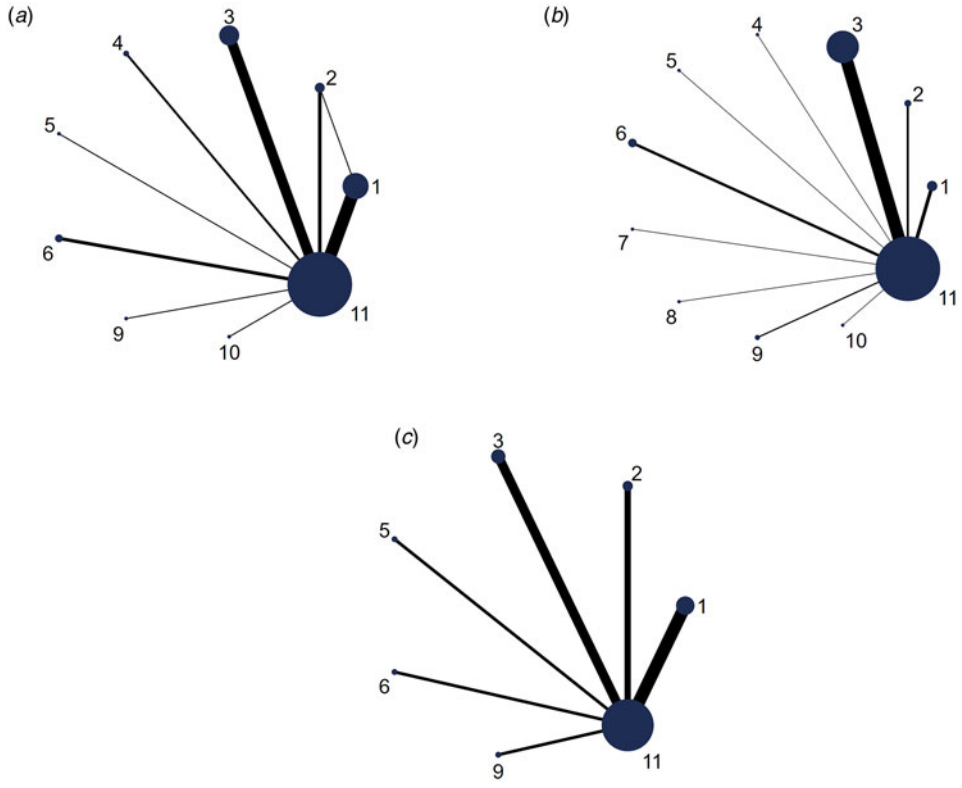

Figure 2. Network plot of intervention comparisons for mental health disorders. The width of the lines reflects the quantity of trials comparing each treatment pair. Each circle's size varies according to the number of individuals that were chosen at random (i.e., sample size). (a) anxiety; (b) depression; (c) stress. 1 = Lactobacillus 2 = Bifidobacterium 3 = Lactobacillus + Bifidobacterium 4 = Bacillus + Bifidobacterium + Lactobacillus + Streptococcus 5 = Bifidobacterium + Lactobacillus + Lactococcus 6 = Lactobacillus + Bifidobacterium + Streptococcus 7 = Weissella 8 = Bacillus 9 = Lacticaseibacillus 10 = Placebo.

−0.67 to −0.28), which indicates that the results are generally stable and that publication bias has little impact.

Effectiveness of probiotics for stress

The effect of probiotics on subjects with stress was reported in 12 RCTs. The network plot is shown in Fig. 2c. The consistency model was utilized owing to the lack of inconsistent resources (Appendix 2 Fig. S8 and Table S5). The NMA results revealed that there are no treatment interventions better than a placebo for improving stress (Table 4). The SUCRA analysis and league table are available in Appendix 2 Fig. S9 and Table S6. There was a moderate level of evidence for stress due to inconsistency based on the GRADE method (Appendix 1 Table S2). Additionally, we observed significant heterogeneity ($I^2 = 57.80\%$) across all studies in this outcome. Direct pairwise evidence indicated that probiotics had a positive effect (SMD: −0.23 95% CI −0.41 to−0.05), although there was no discernible difference between the intervention groups (Appendix 2 Fig. S11). Egger's test ($p = 0.01$) revealed publication bias (Appendix 2 Fig. S10). The trim-and-fill analysis suggested that three iterations of the iterative technique did not significantly change pooled effect size estimates (SMD = −0.29, 95% CI −0.48 to −0.10), which indicates that the results are generally stable and that publication bias has little impact.

Discussion

In this study, a thorough literature search was performed to gather information about the use of probiotics with the aim of providing high-quality evidence for the effectiveness of probiotics. The NMA results demonstrated that *Lactobacillus*, *Bifidobacterium*, and *Lactobacillus* + *Bifidobacterium* had beneficial effects on improving anxiety and depression compared to the placebo.

We found that *Bifidobacterium* was effective at improving anxiety. The potential antianxiety effects of probiotics can be explained by a variety of mechanisms. First, probiotics and the brain may interact in important ways that are accounted for by the hypothalamic–pituitary–adrenal (HPA) axis and inflammatory pathways (Ait-Belgnaoui et al., 2014; Liu et al., 2016). In individuals with anxiety, corticosterone, IL-6, and TNF-α were shown to be notably expressed (Amitai et al., 2016; Guo, Ren, & Zhang, 2018; Rudzki et al., 2019). *Jiang* et al. (Jang, Lee, & Kim, 2019) reported that *Bifidobacterium* species reduce IL-6 and corticosterone levels in the blood of stressed mice by decreasing the number of Iba1 + and LPS + /CD11b + cells (activated microglia) in the hippocampus and inhibiting the activation of the HPA axis, thereby alleviating anxiety-like behavior. Recent findings indicate that *Bifidobacterium* not only affects neurons through cytokine control but also modulates intestinal metabolic toxicity, which is one of the major mechanisms involved in the treatment of anxiety (Zhang et al., 2023). Anxiety is caused by excessive exposure to lipopolysaccharides (LPS), which causes the brain to express TNF-α and inhibit brain-derived neurotrophic factor (BDNF) (Campos et al., 2016; Jang, Lee, Jang, Han, & Kim, 2018a; Jang et al., 2018b). Several studies have shown that *Bifidobacterium* can dramatically decrease the amount of LPS in the blood by suppressing gut bacterial LPS production and/or intestinal permeability. Furthermore, research has shown that

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Table 2. Network estimated standardized mean difference (95% confidence intervals) of interventions on anxiety.

A									
0.31 (−0.43 to 1.05)	B								
−0.13 (−0.67 to 0.42)	−0.44 (−1.24 to 0.36)	C							
−0.50 (−1.45 to 0.44)	−0.81 (−1.92 to 0.30)	−0.38 (−1.34 to 0.59)	D						
0.03 (−1.23 to 1.28)	−0.28 (−1.67 to 1.10)	0.15 (−1.12 to 1.42)	0.53 (−0.96 to 2.01)	E					
−0.17 (−0.96 to 0.62)	−0.48 (−1.46 to 0.50)	−0.04 (−0.86 to 0.77)	0.33 (−0.79 to 1.45)	−0.20 (−1.59 to 1.20)	F				
−0.17 (−1.39 to 1.05)	−0.48 (−1.84 to 0.87)	−0.05 (−1.28 to 1.19)	0.33 (−1.13 to 1.79)	−0.20 (−1.87 to 1.48)	−0.00 (−1.36 to 1.36)	G			
−0.22 (−1.47 to 1.04)	−0.53 (−1.91 to 0.86)	−0.09 (−1.36 to 1.18)	0.28 (−1.20 to 1.77)	−0.25 (−1.94 to 1.45)	−0.05 (−1.44 to 1.34)	−0.05 (−1.72 to 1.63)	H		
−0.49 (−0.85 to −0.12)	**−0.80 (−1.49 to −0.11)**	−0.36 (−0.77 to 0.05)	0.01 (−0.86 to 0.89)	−0.51 (−1.72 to 0.69)	−0.32 (−1.02 to 0.39)	−0.32 (−1.48 to 0.85)	−0.27 (−1.47 to 0.93)	I	

The data in bold indicates that the effect size is statistically significant ($p < 0.05$).
A = *Lactobacillus* **B** = *Bifidobacterium* **C** = *Lactobacillus* + *Bifidobacterium* **D** = *Bacillus* + *Bifidobacterium* + *Lactobacillus* + *Streptococcus* **E** = *Bifidobacterium* + *Lactobacillus* + *Lactococcus* **F** = *Lactobacillus* + *Bifidobacterium* + *Streptococcus* **G** = *Lacticaseibacillus* **H** = *Limosilactobacillus* + *Lacticaseibacillus* + *Lactiplantibacillus* + *Bifidobacterium* **I** = Placebo.

Lactobacillus could also lower corticosterone levels and suppress the HPA axis, indicating that *Lactobacillus* in the central nervous system have important physiological effects (Bravo et al., 2011). Although *Bifidobacterium* and *Lactobacillus* both assist in alleviating anxiety symptoms, *Bifidobacterium* performed slightly better than *Lactobacillus*. The differences in development and reproduction patterns between the two strains could also be one of the causes. *Bifidobacterium* are strict anaerobes that operate under anaerobic conditions (Cukrowska, Bierła, Zakrzewska, Klukowski, & Maciorkowska, 2020). *Lactobacillus* is a facultative anaerobic bacterium that can lower the pH of the intestine by consuming any leftover oxygen that enters the colon and generating lactic acid (Nishiyama, Sugiyama, & Mukai, 2016). *Lactobacillus* creates an environment that is favor for *Bifidobacterium* spp. though synergistic effects (Turroni et al., 2014). The presence of distinct metabolites could be another explanation. Short-chain fatty acids (SCFAs), which *Bifidobacterium* produces, can regulate the production of 5-HT and elevated levels of BDNF, which has a positive impact on behaviors connected to mood (Dalile, Van Oudenhove, Vervliet, & Verbeke, 2019; Tsukuda et al., 2021). However, the secondary metabolites of *Lactobacillus* are primarily lactic acid and are not directly involved in the production of short-chain fatty acids (LeBlanc et al., 2017).

Interestingly, although *Bifidobacterium* and *Lactobacillus* have received the most attention in probiotic trials, they have no effect on depression when considered alone. Conversely, we discovered that the combination of *Lactobacillus* + *Bifidobacterium* had a favorable effect on depression incidence. Depression and anxiety disorders are the two most common mental health conditions. It has been discovered that anxious symptoms often precede depressive symptoms. According to the World Mental Health Survey, 68% of individuals with anxious depression initially exhibit symptoms of anxiety, followed by depression (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Compared to individuals with anxiety disorders, people who develop depression are more amenable to treat, more severely depressed, and longer treatment (Thase, Weisler, Manning, & Trivedi, 2017). Consistent with the results of the subgroup analysis, the duration of probiotic supplementation was longer for depressed patients than for anxious patients. The involvement of inflammation in depressive syndrome is well-known (Maes, 2001). Patients with depression frequently have increased levels of inflammatory substances, and the combination of *Lactobacillus* + *Bifidobacterium* could reduce the proinflammatory cytokines IL-1α, IL-6, interferon-γ, and TNF-α (Bisson, Hidalgo, Rozan, & Messaoudi, 2010). Some studies have shown that the consumption of probiotic preparations (*Lactobacillus* + *Bifidobacterium*) is negatively correlated with the response of the human HPA axis, and improves brain plasticity abnormalities, neurogenesis, and HPA axis hyperactivity in chronic stress-induced depression model mice (Messaoudi et al., 2011). Therefore, we speculate that *Lactobacillus* and *Bifidobacterium* may improve mental health by synergistically regulating the activation of the HPA axis and the inflammatory response caused by anxiety / depression. However, additional experiments are needed to confirm these results.

Probiotics must colonize in the intestine through two stages to play a role. The first stage is the combination of nonspecific physical contact (including spatial recognition and hydrophobic recognition) with the mucosa to establish a reversible, weak physical binding. In the second stage, stable binding with mucus or

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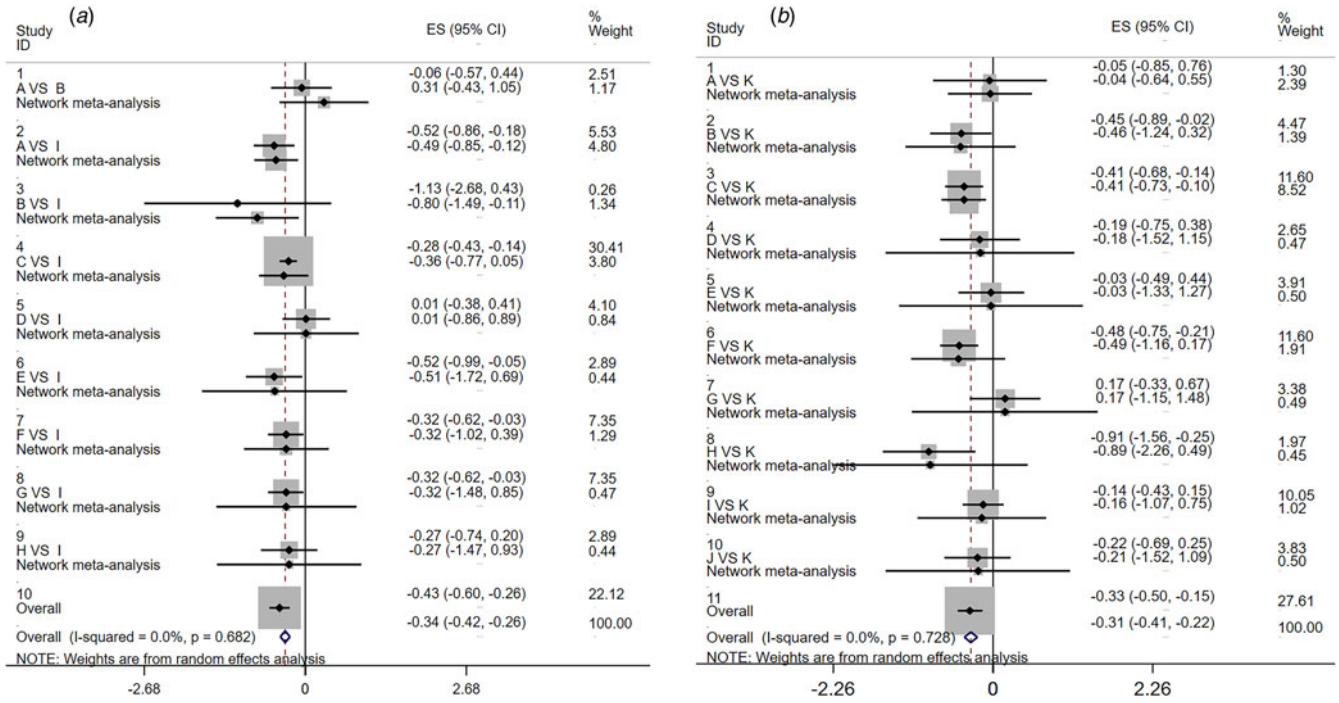

Figure 3. Pooled effect size (ES) and confidence interval (CI) for stress by network meta-analysis and traditional meta-analysis. (A: Anxiety; A = *Lactobacillus* B = *Bifidobacterium* C = *Lactobacillus* + *Bifidobacterium* D = *Bacillus* + *Bifidobacterium* + *Lactobacillus* + *Streptococcus* E = *Bifidobacterium* + *Lactobacillus* + *Lactococcus* F = *Lactobacillus* + *Bifidobacterium* + *Streptococcus* G = *Lacticaseibacillus* H = *Limosilactobacillus* + *Lacticaseibacillus* + *Lactiplantibacillus* + *Bifidobacterium* I = Placebo; B: Depression; A = *Lactobacillus* B = *Bifidobacterium* C = *Lactobacillus* + *Bifidobacterium* D = *Bacillus* + *Bifidobacterium* + *Lactobacillus* + *Streptococcus* E = *Bifidobacterium* + *Lactobacillus* + *Lactococcus* F = *Lactobacillus* + *Bifidobacterium* + *Streptococcus* G = *Weissella* H = *Bacillus* I = *Lacticaseibacillus* J = *Limosilactobacillus* + *Lacticaseibacillus* + *Lactiplantibacillus* + *Bifidobacterium* K = Placebo).

intestinal epithelial cells is established through specific interactions between adhesin and complementary receptors, so as to successfully colonize and play a role in the intestine (Han et al., 2021; Zmora et al., 2018). Our findings confirmed the significant efficacy of *Lactobacillus* and *Bifidobacterium* in treating anxiety, particularly after at least 12 weeks of intervention, as confirmed by our study. Similarly, *Lactobacillus* + *Bifidobacterium* had a better effect on depression after more than 12 weeks. Thus, we speculate that a 12-week probiotic supplement may be an option for probiotics to attach steadily to gut mucus or epithelial cells. Another common issue is the number of probiotics to use. The International Scientific Association for Probiotics and Prebiotics proposed that the recommended number of probiotics be 10^8–10^{11} CFU/day in the application guidelines for probiotics (Binda et al., 2020). Regarding the International Dairy Federation (IDF) suggestion, the recommended daily intake of each probiotic strain is estimated to be approximately 10^9 CFU/day. This network meta-analysis revealed that *Lactobacillus* + *Bifidobacterium* at 10^9–10^{10} CFU/day had a beneficial effect on depression and that *Lactobacillus* or *Bifidobacterium* at $\geqslant 10^{11}$ CFU/day could successfully alleviate anxiety. Our results showed that the combination strain may be more effective than the single strains. The following explanations could explain why multiple strains exhibit better health outcomes. First, multi-strain compound probiotics can break down and change more nutrients, including a greater variety of digestive enzymes, and improve the micro-ecological conditions in the human gut (anaerobic, appropriate pH) (Kwoji, Aiyegoro, Okpeku, & Adeleke, 2021). Furthermore, cross-feeding has synergistic effects on multiple strains (Boger, Lammerts van Bueren, & Dijkhuizen, 2018); its possible physiological regulatory mechanism is enhanced by the combination strain. Multiple strains can boost the intestinal adherence of different target strains, hence enhancing the interaction between strains and host cells, according to studies based on VSL # 3 microecological preparation (Douillard, Mora, Eijlander, Wels, & de Vos, 2018). Considering aspects such as the health status, age, and sex of different populations and the diversity of probiotics, it is challenging to determine which combination of probiotics is most effective for treating mental health disorders. Therefore, multi-center clinical trials with large sample sizes are still needed.

Limitations

This study has several limitations. First, the lack of consistency in sample size and overall distribution of the included studies may affect the validity and generalizability of the results. The methods of the included RCTs differed in terms of different diagnoses, different microbiota, different measurement times, and outcome measures, which may have influenced the results. We attempted to reduce diagnostic heterogeneity by further grouping the duration of intervention, the dose of probiotics taken, and the type of probiotic, and we used sensitivity analysis and meta-regression to confirm the results. Second, to the best of our knowledge, anxiety and depression are more common in women than in men (Altemus, Sarvaiya, & Neill Epperson, 2014; Kessler et al., 2012). More than half of the population in the present study was female, which may have led to a deviation in the findings. Although we hoped to conduct subgroup analysis by gender stratification, the research subjects included in the original

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Table 3. Network estimated standardized mean difference (95% confidence intervals) of interventions on depression

A										
0.42 (−0.56 to 1.40)	B									
0.37 (−0.30 to 1.04)	−0.05 (−0.89 to 0.79)	C								
0.14 (−1.32 to 1.61)	−0.28 (−1.82 to 1.27)	−0.23 (−1.60 to 1.15)	D							
−0.01 (−1.44 to 1.41)	−0.43 (−1.95 to 1.08)	−0.38 (−1.72 to 0.95)	−0.16 (−2.02 to 1.71)	E						
0.45 (−0.44 to 1.35)	0.04 (−0.99 to 1.06)	0.08 (−0.65 to 0.82)	0.31 (−1.18 to 1.80)	0.47 (−0.99 to 1.93)	F					
−0.21 (−1.65 to 1.23)	−0.63 (−2.15 to 0.90)	−0.58 (−1.93 to 0.77)	−0.35 (−2.22 to 1.52)	−0.19 (−2.04 to 1.65)	−0.66 (−2.13 to 0.81)	G				
0.84 (−0.66 to 2.35)	0.43 (−1.15 to 2.01)	0.47 (−0.94 to 1.89)	0.70 (−1.22 to 2.62)	0.86 (−1.03 to 2.75)	0.39 (−1.14 to 1.92)	1.05 (−0.85 to 2.96)	H			
0.12 (−0.97 to 1.20)	−0.30 (−1.50 to 0.90)	−0.25 (−1.21 to 0.71)	−0.02 (−1.64 to 1.59)	0.13 (−1.45 to 1.72)	−0.34 (−1.46 to 0.79)	0.33 (−1.27 to 1.92)	−0.73 (−2.38 to 0.92)	I		
0.17 (−1.26 to 1.60)	−0.24 (−1.76 to 1.27)	−0.20 (−1.53 to 1.14)	0.03 (−1.83 to 1.90)	0.19 (−1.65 to 2.03)	−0.28 (−1.74 to 1.18)	0.38 (−1.47 to 2.23)	−0.67 (−2.57 to 1.22)	0.06 (−1.53 to 1.64)	J	
−0.04 (−0.64 to 0.55)	−0.46 (−1.24 to 0.32)	**−0.41 (−0.73 to −0.10)**	−0.18 (−1.52 to 1.15)	−0.03 (−1.33 to 1.27)	−0.49 (−1.16 to 0.17)	0.17 (−1.15 to 1.48)	−0.89 (−2.26 to 0.49)	−0.16 (−1.07 to 0.75)	−0.21 (−1.52 to 1.09)	K

The data in bold indicates that the effect size is statistically significant ($p < 0.05$).
A = *Lactobacillus* **B** = *Bifidobacterium* **C** = *Lactobacillus* + *Bifidobacterium* **D** = *Bacillus* + *Bifidobacterium* + *Lactobacillus* + *Streptococcus* **E** = *Bifidobacterium* + *Lactobacillus* + *Lactococcus* **F** = *Lactobacillus* + *Bifidobacterium* + *Streptococcus* **G** = *Weissella* **H** = *Bacillus* **I** = *Lacticaseibacillus* **J** = *Limosilactobacillus* + *Lacticaseibacillus* + *Lactiplantibacillus* + *Bifidobacterium* **K** = Placebo.

Table 4. Network estimated standardized mean difference (95% confidence intervals) of interventions on stress

A						
0.07 (−0.90 to 1.04)	B					
−0.22 (−1.06 to 0.62)	−0.29 (−1.29 to 0.71)	C				
−0.37 (−1.57 to 0.83)	−0.44 (−1.75 to 0.88)	−0.15 (−1.37 to 1.07)	D			
−0.11 (−1.32 to 1.10)	−0.18 (−1.50 to 1.14)	0.11 (−1.12 to 1.34)	0.26 (−1.24 to 1.76)	E		
−0.11 (−1.27 to 1.06)	−0.17 (−1.46 to 1.11)	0.11 (−1.08 to 1.30)	0.26 (−1.20 to 1.73)	0.00 (−1.47 to 1.48)	F	
−0.39 (−0.96 to 0.19)	−0.46 (−1.24 to 0.33)	−0.17 (−0.79 to 0.45)	−0.02 (−1.07 to 1.04)	−0.28 (−1.34 to 0.79)	−0.28 (−1.30 to 0.74)	G

A = *Lactobacillus* **B** = *Bifidobacterium* **C** = *Lactobacillus* + *Bifidobacterium* **D** = *Bifidobacterium* + *Lactobacillus* + *Lactococcus* **E** = *Lactobacillus* + *Bifidobacterium* + *Streptococcus* **F** = *Lacticaseibacillus* **G** = Placebo.

literature were a mixed population (including both males and females), or the studies did not include data on gender/sex of the participants. In addition, other risk factors associated with mental health, such as ethnicity, education, and income level, were included, but very little information was collected. Further research may examine the connection between psychiatric symptoms of stress, anxiety, and depression and socioeconomic characteristics such as gender, race, and educational attainment. Third, the study-level effects included in the present study were based on measures of depression, anxiety, and stress taken after the completion of probiotic therapy. As a result, we cannot assess the extent of the potential psychopharmacological effects of these treatment regimens that persist after cessation of treatment. Finally, in accordance with the results of the present study, participants opted to rate their depression risk using a self-rating questionnaire. The results can be affected by the variations in the information gathered between the standardized scale and the self-rated questionnaire. Thus, this paper was omitted, and the outcome was unchanged.

Conclusion

In summary, the findings of our NMA suggest that *Lactobacillus*, *Bifidobacterium,* and *Lactobacillus* + *Bifidobacterium* were particularly effective at improving anxiety and depression, but not in individuals with stress. The results of a few studies of patients who experienced stress are preliminary. Considering the variety of probiotic species and strains used in clinical trials, the effectiveness of other components should be further validated in further studies with larger sample sizes.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291724000679.

Author contributions. Conceptualization: Yafang Yang; Methodology: Yafang Yang; Software: Min Wan and Da Pan; Formal Analysis: Yafang Yang and Ligang Yang; Writing – Original Draft Preparation: Yafang Yang and Chao Yang; Writing – Review & Editing: Min Wan and Ligang Yang; Project Administration: Chao Yang; Funding Acquisition: Chao Yang and Ligang Yang.

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Competing interests. The author(s) declare none.

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