



Psychobiotic food systems: fermentation-derived microbes and metabolites modulating the microbiota–gut–brain axis



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Psychobiotics are emerging microbiota-targeted interventions with potential relevance for mood, stress resilience, anxiety, sleep, cognition, and neuroimmune regulation. Although initially viewed mainly as probiotic strains acting through the microbiota–gut–brain axis, the concept is now expanding toward food-associated systems integrating live microorganisms, fermented matrices, prebiotic substrates, postbiotic fractions, and neuroactive metabolites. This review examines psychobiotic foods as integrated strain–matrix–metabolite–host systems, emphasizing fermentation as both a source of candidate microorganisms and a biotechnological process generating gut–brain-relevant bioactives. Fermented dairy products, kefir, sourdough, cereals, legumes, vegetables, fruits, tea, cocoa, coffee, and plant-based products may provide strains and metabolites such as γ -aminobutyric acid, short-chain fatty acids, tryptophan derivatives, bioactive peptides, exopolysaccharides, organic acids, vitamins, and phenolic metabolites. These compounds may influence gut–brain communication through microbial ecology, vagal signaling, HPA-axis regulation, immune modulation, intestinal and blood–brain barrier integrity, oxidative-stress control, and enteroendocrine pathways. However, evidence remains heterogeneous, with stronger support for depressive symptoms than for anxiety, sleep, cognition, or other psychiatric outcomes. Future development requires strain authentication, genome-based safety screening, controlled fermentation, metabolomics, simulated digestion, undesirable metabolite monitoring, and well-powered human trials linking food composition to validated mental-health-related endpoints.

Mental health is increasingly understood as a systemic condition shaped not only by neurochemical imbalance, but also by diet, gut microbial ecology, intestinal barrier function, immune tone, microbial metabolism, and neuroendocrine signaling. The microbiota–gut–brain axis links intestinal microorganisms to brain function through vagal communication, hypothalamic–pituitary–adrenal (HPA) axis regulation, inflammation, short-chain fatty acids (SCFAs), tryptophan metabolism, gut permeability, and blood–brain barrier modulation^{1,2}. Psychobiotic effects should not be attributed solely to microbial taxonomy because outcomes also depend on

strain identity, matrix composition, processing, metabolite production, gastrointestinal survival, and host-specific microbiome configuration.

Terminological precision is essential because probiotic, psychobiotic, prebiotic, synbiotic, postbiotic, paraprobiotic, and fermented food are related but non-equivalent concepts. Probiotics are live microorganisms that confer a health benefit when administered in adequate amounts³. Psychobiotics were initially defined as live microorganisms conferring mental-health-related benefits, although some authors have extended the term to other microbiota-directed interventions^{1,4}. Prebiotics are substrates selectively utilized by host microorganisms to confer a health benefit⁵,

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whereas synbiotics are mixtures of live microorganisms and selectively utilized substrates that confer a health benefit⁶. Postbiotics are preparations of inanimate microorganisms and/or their components with demonstrated health benefits⁷, while paraprobiotics generally denote non-viable microbial cells or cell fractions with potential activity⁸. Fermented foods result from desired microbial growth and enzymatic conversion of food components but are not automatically probiotic or psychobiotic products⁹. Here, a “psychobiotic food system” denotes a food-based intervention supported by product-specific mechanistic evidence and, where a health benefit is claimed, appropriate human clinical evidence.

Fermented foods represent a relevant reservoir for isolating candidate psychobiotic strains because fermentation functions as both a microbial selection process and a neuroactive metabolite-biomanufacturing system. Fermentation selects microorganisms with stress tolerance, metabolic plasticity, and technological persistence^{10,11}. Bentahar et al.¹², for example, identified *Lactobacillus* strains with promising probiotic properties from Dhan, a traditional Algerian goat-milk butter, although such in vitro characteristics do not establish psychobiotic functionality. Acid and bile tolerance, adhesion, antimicrobial activity, and storage stability are preliminary screening attributes requiring genome-based safety assessment, mechanistic validation, and appropriate human studies.

Beyond microbial delivery, fermentation can transform raw food substrates into biologically active matrices enriched in γ -aminobutyric acid (GABA), organic acids, tryptophan-derived indoles, bioactive peptides, exopolysaccharides, B-group vitamins, transformed phenolics, microbial enzymes, and inanimate-cell components^{13,14}. These constituents may influence inflammatory, barrier, enteroendocrine, oxidative, and microbial metabolic pathways. Nevertheless, detecting a neuroactive compound does not establish physiological relevance; concentration, digestive stability, bioaccessibility, biologically relevant exposure, and host-response biomarkers must also be considered. SCFA-related claims require caution, as acetate, propionate, and butyrate are often generated after ingestion through colonic fermentation rather than delivered directly at functionally relevant concentrations by the product¹⁵.

Despite growing interest, the translational value of psychobiotics remains constrained by heterogeneity and incomplete characterization. A high-fermented-food diet increased gut-microbial diversity and reduced inflammatory markers in healthy adults, although mental-health outcomes were not primary endpoints¹⁶. A prebiotic- and fermented-food-rich diet reduced perceived stress within the intervention group, but the between-group difference was not significant and microbiome changes were limited¹⁷. At the strain level, *Bifidobacterium breve* CCFM1025 was associated with improved depressive symptoms and altered microbial tryptophan metabolism in major depressive disorder¹⁸, whereas *Lactocaseibacillus rhamnosus* JB-1 did not significantly improve stress, mood, sleep, or cognition in healthy adults¹⁹. These findings show that responses are strain-, formulation-, population-, and endpoint-specific. Psychological improvement alone does not establish microbiota-mediated causality; stronger evidence requires alignment among product composition, microbiome function, metabolite profiles, mechanistic biomarkers, and clinically meaningful outcomes. Psychobiotics should therefore be positioned as potential adjunctive strategies rather than replacements for established psychiatric treatments.

Recent reviews have addressed conceptual and precision-oriented psychobiotic frameworks^{1,2}, strain survival in fermented foods¹⁰, fermented-food mechanisms¹³, clinical interventions²⁰, neuroactive-metabolite quantification²¹, product standardization²², and postbiotic and paraprobiotic applications⁸. However, a major limitation remains the insufficient integration of microbial screening, matrix and fermentation chemistry, gastrointestinal functionality, metabolite profiling, safety assessment, mechanistic biomarkers, and clinical validation within a single food-development pathway. Accordingly, this review proposes a strain-matrix-process-metabolite-host framework linking food biochemistry and fermentation science with microbiome function and human outcomes. It aims to distinguish validated psychobiotic food systems from

conventional fermented or probiotic-containing products and define the evidence required for responsible development.

Concepts and food-relevant classification of psychobiotics

From probiotics to psychobiotics

Psychobiotics should be considered a specialized class of microbiota-targeted interventions rather than a synonym for probiotics. A probiotic strain may improve intestinal, immune, or metabolic health without necessarily exerting measurable effects on mood, stress, cognition, sleep, or neuroimmune regulation. By contrast, psychobiotic classification requires evidence of a mental-health-relevant effect mediated, at least partly, through the microbiota-gut-brain axis. Recent reviews describe psychobiotics as microorganisms or microbiota-directed interventions capable of influencing brain-related functions through neural, immune, endocrine, barrier-related, and metabolic pathways¹².

This distinction is essential because psychobiotic activity is not a generic species-level property. The same microbial species may contain strains with different capacities for acid and bile tolerance, adhesion, neurotransmitter-related metabolism, anti-inflammatory signaling, epithelial barrier protection, and metabolite production. However, technological characteristics such as gastrointestinal tolerance, processing stability, and food-matrix compatibility determine delivery feasibility but do not independently demonstrate psychobiotic efficacy. Therefore, psychobiotic classification must be based on strain-level evidence, functional phenotype, safety profile, food-matrix compatibility, and host response, rather than on the simple presence of genera such as *Lactobacillus*, *Lactocaseibacillus*, *Lactiplantibacillus*, *Bifidobacterium*, or *Pediococcus*^{2,10}.

Recent work has also expanded the concept from “psychobiotic strains” to psychobiotic systems. In food applications, this broader operational concept recognizes that the overall response may involve viable microorganisms, inanimate microbial preparations, selectively utilized substrates, and fermentation-derived compounds acting within a specific matrix. This is particularly relevant for fermented foods, where live microorganisms, peptides, organic acids, phenolic derivatives, exopolysaccharides, and matrix-derived bioactives coexist and may act through complementary mechanisms^{8,13}. Nevertheless, their presence alone is insufficient to justify a psychobiotic claim without product-specific mechanistic or appropriate human evidence.

Psychobiotics, prebiotics, synbiotics, postbiotics, and paraprobiotics

A rigorous classification is necessary because the literature often uses the terms probiotic, psychobiotic, prebiotic, synbiotic, postbiotic, paraprobiotic, and fermented food interchangeably (Table 1). In a food-relevant context, live microorganisms should be classified as psychobiotics only when strain-specific evidence supports a gut-brain-related health effect. Prebiotics are selectively utilized substrates that confer a health benefit, whereas synbiotics are mixtures comprising live microorganisms and substrates selectively utilized by host microorganisms^{1,6}. Prebiotic or synbiotic formulations may therefore have psychobiotic potential when their effects are linked to emotional, cognitive, stress-related, or neuroimmune outcomes, but these terms should not be treated as automatically equivalent to psychobiotics.

The synbiotic concept is particularly important for product development because the food matrix can be engineered to support both microbial viability and functional metabolite output. For example, a GABA-producing lactic acid bacterium may require sufficient glutamate availability, controlled pH, and appropriate fermentation time to maximize glutamate decarboxylase activity. Similarly, fiber-rich cereal, legume, fruit, or vegetable matrices may support short-chain fatty acid production after colonic fermentation, while polyphenol-rich matrices may generate microbial phenolic metabolites with antioxidant and anti-inflammatory relevance¹³.

Postbiotics and paraprobiotics are also important for psychobiotic food design, especially when live-cell stability is difficult to guarantee. Postbiotics are preparations of inanimate microorganisms and/or their components

Table 1 | Conceptual classification and validation requirements for psychobiotic food systems

Category	Food-relevant definition	Representative food/ application case	Dominant gut-brain mechanism	Minimum analytical/ technological validation	Main limitation or risk of overclaiming	References
Probiotic psychobiotic	Live microbial strain with demonstrated mental-health-relevant effects through the microbiota-gut-brain axis.	<i>Bifidobacterium longum</i> , <i>Lactiplantibacillus plantarum</i> , <i>Lactocaseibacillus rhamnosus</i> , or <i>Limosilactobacillus reuteri</i> incorporated into fermented milk, capsules, or functional beverages.	Modulation of gut microbiota, inflammatory tone, HPA-axis activity, intestinal barrier integrity, neurotransmitter-related metabolism, and stress-response pathways.	Strain-level identification, viable count, acid/bile tolerance, simulated gastrointestinal survival, safety screening, and human or biomarker-based evidence.	Calling any probiotic strain "psychobiotic" without evidence on mood, stress, sleep, cognition, anxiety, or depression-related endpoints.	1,2,21
Prebiotic psychobiotic	Selectively utilized substrate that promotes gut microbial activities linked to mental-health-relevant outcomes.	Inulin, fructo-oligosaccharides, galacto-oligosaccharides, resistant starch, cereal β -glucans, legume fibers, or fruit/vegetable fibers.	Enhancement of saccharolytic fermentation, SCFA production, gut barrier support, anti-inflammatory signaling, and microbial cross-feeding.	Demonstration of selective microbial utilization, SCFA profiling, microbiome shift, dose-response evaluation, and host biomarker response.	Assuming that all dietary fibers are psychobiotic, even when no gut-brain biomarker or psychological endpoint is measured.	20,65,72
Synbiotic psychobiotic	Combination of a live microorganism and a substrate designed to enhance survival, colonization, or neuroactive metabolite production.	GABA-producing LAB combined with glutamate-rich substrates; <i>Bifidobacterium</i> with GOS; LAB with cereal, legume, or fruit fibers.	Improved microbial survival, increased SCFA production, enhanced GABA generation, modulation of tryptophan metabolism, and improved host-microbe metabolic interaction.	Compatibility between strain and substrate, metabolite output, gastrointestinal survival, stability during storage, and evidence of additive or synergistic action.	Mixing a probiotic and a fiber without proving functional synergy or improved gut-brain-related outcome.	13,65,72
Postbiotic psychobiotic	Inanimate microbial preparation or microbial component with demonstrated gut-brain-relevant biological activity.	Heat-treated lactic acid bacteria, inactivated bifidobacteria, fermented-cell fractions, microbial cell-wall components, or extracellular fractions.	Immune modulation, epithelial barrier support, reduction of inflammatory signaling, and interaction with host pattern-recognition receptors.	Defined microbial origin, controlled inactivation, reproducible composition, safety confirmation, and biological activity after inactivation.	Calling purified metabolites alone "postbiotics" without a defined microbial preparation or demonstrated biological effect.	21,22,73
Paraprobiotic	Non-viable microbial cells that may retain biological effects despite loss of viability.	Heat-killed LAB in beverages, pasteurized fermented products, or non-viable microbial biomass in functional foods.	Interaction of microbial cell structures with immune and epithelial pathways; possible modulation of inflammatory and barrier-related responses.	Confirmation of non-viability, cell integrity, safety, reproducible dose, and immune/barrier-related functional testing.	Assuming that dead cells are functional simply because they originate from probiotic species.	22,73
Fermented food with psychobiotic potential	Fermented food containing microbial communities and metabolites that may influence microbiota-gut-brain signaling, but not yet clinically validated as psychobiotic.	Yogurt, kefir, fermented milk, sourdough, fermented cereals, kimchi, sauerkraut, fermented legumes, kombucha, cocoa, coffee, and fermented vegetables.	Delivery of live microbes, microbial enzymes, organic acids, peptides, GABA, phenolic metabolites, vitamins, SCFAs or SCFA precursors, and microbial cross-feeding substrates.	Microbial profiling, metabolite profiling, serving-size standardization, safety assessment, viable-cell quantification, and matrix-specific stability.	Assuming all fermented foods are psychobiotic without strain-level, metabolite-level, or clinical evidence.	13,22,74,75
Validated psychobiotic food	A food product supported by strain identity, safety, metabolite profile, gastrointestinal robustness, and human evidence on gut-brain-related outcomes.	Clinically tested fermented milk, kefir-like product, cereal beverage, or plant-based fermented food with defined strains and quantified neuroactive metabolites.	Integrated action through strain survival, metabolite production, microbiome modulation, immune regulation, barrier function, HPA-axis modulation, and psychological outcome improvement.	Complete validation pipeline: WGS/MALDI-TOF/qPCR, CFU stability, LC-MS/MS or GC-MS metabolomics, GABA/SCFA/tryptophan metabolite quantification, digestion model, safety, and clinical endpoints.	Rare category; many products are marketed before full mechanistic and clinical validation.	2,20,21,65
Fermentation-derived neuroactive metabolite system	Fermented matrix enriched in metabolites with potential gut-brain relevance, even when live-cell delivery is not the main mechanism.	GABA-enriched fermented milk or legumes; peptide-rich fermented dairy; phenolic-metabolite-rich fermented tea/cocoa/vegetables.	Direct or indirect modulation of oxidative stress, inflammation, endoendocrine signaling, microbial cross-feeding, tryptophan metabolism, and barrier function.	Targeted metabolite quantification, bioaccessibility testing, in vitro digestion, colonic fermentation, dose relevance, and safety monitoring for undesirable metabolites.	Attributing psychobiotic effects to metabolites without demonstrating bioavailability or host response.	13,21,74
Precision psychobiotic system	Personalized or stratified psychobiotic intervention designed according to host microbiome, diet, phenotype, and biomarker response.	Tailored synbiotic or fermented-food intervention for individuals with stress, depressive symptoms, sleep disturbance, or gut dysbiosis.	Host-specific modulation of microbiota, metabolites, inflammatory status, stress biomarkers, and psychological outcomes.	Baseline microbiome profiling, metabolomics, host stratification, biomarker monitoring, controlled intervention design, and validated mental-health scales.	Premature personalization without enough mechanistic or clinical evidence.	2,65,70

that confer a demonstrated health benefit⁷. Paraprobiotics generally refer to non-viable microbial cells or cell fractions with potential biological activity, although no universally harmonized definition has yet been adopted⁸. These approaches are technologically attractive because they may offer improved stability during heat processing, oxygen exposure, acidic storage, and long shelf life.

Fermented foods as natural psychobiotic platforms

Fermented foods occupy a distinct position because they are not automatically probiotics, prebiotics, synbiotics, or postbiotics, but they may contain elements of these categories. A fermented food may deliver live microorganisms, microbial enzymes, non-viable cells, bioactive peptides, organic acids, vitamins, and transformed phytochemicals. Caffrey et al.²² therefore recommended evaluating fermented foods according to their microbial and metabolite composition, safety, serving size, health relevance, and regulatory status.

From a psychobiotic perspective, fermented foods combine microbial diversity with biochemical complexity. Dairy products, kefir, cheese, sourdough, fermented cereals, legumes, vegetables, kombucha, cocoa, coffee, and other plant-based products may provide candidate strains and generate γ -aminobutyric acid (GABA), tryptophan-derived indoles, bioactive peptides, exopolysaccharides, B-group vitamins, and phenolic metabolites. These components may influence gut–brain communication through immune modulation, epithelial-barrier support, enteroendocrine signaling, microbial cross-feeding, and neuroactive metabolism^{13,23}. However, short-chain fatty acids are generally produced after ingestion through colonic fermentation rather than delivered directly at physiologically relevant concentrations. Primary studies illustrate different levels of evidence. Liu et al.²⁴ isolated a GABA-producing *Levilactobacillus brevis* strain from kimchi, while Bentahar et al.¹² identified food-adapted *Lactobacillus* strains with probiotic properties in traditional Dhan butter. In humans, a high-fermented-food diet increased microbial diversity and reduced inflammatory markers, although mental-health outcomes were not primary endpoints¹⁶.

Criteria required to classify a food-derived strain or product as psychobiotic

A food-derived microorganism should be classified as psychobiotic only after passing a multi-level validation framework. The first level is taxonomic and genomic authentication. Strain identity should be established primarily by whole-genome sequencing (WGS), supported where appropriate by 16S rRNA sequencing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), or strain-specific quantitative polymerase chain reaction (qPCR). However, 16S rRNA sequencing or MALDI-TOF MS alone should not be considered sufficient for strain-level authentication. The strain code, genome accession, identification workflow, and traceable biological deposit should be reported²⁵. This step is essential because psychobiotic effects are strain-dependent, and inaccurate identification can compromise reproducibility, safety assessment, and regulatory credibility.

The second level is safety qualification. Candidate strains should be screened for hemolytic activity, transferable antimicrobial resistance genes, virulence determinants, toxin production, mucin degradation, harmful enzymatic traits, excessive D-lactate production, and biogenic amine formation. A candidate should pass this level only when no clinically relevant transferable resistance, virulence, or toxigenic determinant is detected and any intrinsic resistance is experimentally characterized as non-transferable. Traditional use or qualified taxonomic status should support, but not replace, strain-specific safety assessment²⁵.

The third level is functional gut–brain relevance. A candidate psychobiotic should show at least one mechanistically plausible activity, such as γ -aminobutyric acid production, short-chain-fatty-acid modulation, tryptophan–kynurenine pathway interaction, indole production, anti-inflammatory signaling, epithelial-barrier protection, or stress-response modulation. The selected activity must be quantified using a validated

analytical method, appropriate positive and negative controls, dose–response testing, and reproducible independent preparations. Detection of a neuroactive compound alone is insufficient. Because no universally accepted effective GABA, short-chain-fatty-acid, or indole concentration exists, the product must deliver an exposure that matches the dose associated with the reported mechanistic or biological effect².

The fourth level is technological suitability. A psychobiotic strain intended for food application must survive fermentation, processing, packaging, storage, and simulated gastrointestinal digestion or, in the case of an inactivated product, retain reproducible biological activity after processing. Viable-cell counts should be reported at manufacture and at the end of shelf life. Rather than applying a generic colony-forming-unit threshold, the minimum acceptable count per serving should equal or exceed the dose used in the supporting efficacy study. Product specifications should also define serving size, matrix composition, pH, water activity, storage conditions, batch variability, and metabolite retention. For postbiotic or paraprobiotic preparations, inactivation must be confirmed and the composition and biological activity of the final preparation standardized⁷.

The fifth and strongest level is biological and clinical validation. Pre-clinical studies should include justified sample sizes, randomization, blinding, appropriate controls, dose–response assessment, and reporting consistent with ARRIVE 2.0²⁶. Human studies should be preregistered, adequately powered through an a priori sample-size calculation, and preferably randomized and placebo-controlled. They should specify one validated primary psychological or cognitive endpoint, characterize the administered product and dose, assess adherence and adverse events, and include microbiome, metabolomic, immune, barrier, neuroendocrine, sleep, or cognitive measures where mechanistically relevant. No single sample size is appropriate for all psychobiotic trials; the required number must be calculated from the prespecified primary outcome, expected effect size, variability, power, and anticipated attrition²⁷. Accordingly, strains or products satisfying Levels 1–4 should be described as candidate psychobiotics or products with psychobiotic potential. An unqualified psychobiotic food claim should be reserved for a fully characterized product that also demonstrates reproducible, product-specific benefit in an appropriately designed human study.

Fermentation as a source and selective platform for psychobiotic isolation

Fermented foods as ecological reservoirs of psychobiotic candidates

Fermented foods represent highly relevant reservoirs for discovering candidate psychobiotic microorganisms because they contain diverse food-adapted communities with long-term associations with edible matrices. Fermented dairy products, kefir, sourdough, fermented cereals, legumes, vegetables, cocoa, coffee, kombucha, and traditional fermented foods may therefore provide strains with acidification capacity, enzymatic plasticity, and survival during processing and storage^{10,11}.

Their value at this stage is primarily ecological and bioprospecting-related: fermented foods provide candidate isolates but not automatically validated psychobiotics. Landis et al.²⁸ characterized the bacterial and fungal communities of 500 sourdough starters and demonstrated substantial taxonomic diversity, microbial interactions, and associations with fermentation-related functional properties. Bentahar et al.¹² similarly isolated *Lactobacillus* strains with probiotic and technological properties from Dhan, a traditional Algerian goat-milk butter. These studies demonstrate the potential of fermented foods as reservoirs of matrix-adapted microorganisms, although strain safety, gut–brain-related mechanisms, and appropriate human evidence remain necessary before psychobiotic classification.

Fermentation as a microbial selection pressure

Fermentation should be considered a selective ecological process, not merely a preservation method. During fermentation, pH decline, organic acid accumulation, salt concentration, oxygen limitation, reduced water activity,

bacteriocin production, nutrient competition, and storage temperature progressively reshape the microbial community. These pressures can enrich microorganisms capable of tolerating stress and maintaining metabolic activity under adverse conditions. However, fermentation-associated fitness should not be equated with psychobiotic functionality. Acid tolerance, technological stability, and gastrointestinal survival support strain delivery but do not independently demonstrate a gut–brain-related health effect. Such candidates must additionally satisfy strain-level safety, mechanistic, and biological validation criteria. This selection effect is strongly matrix-dependent. Dairy matrices favor lactose utilization, proteolysis, peptide release, and buffering tolerance, whereas cereal and legume fermentations select microorganisms adapted to complex carbohydrates, fibers, phenolic compounds, antinutritional factors, and plant proteins. Vegetable fermentations often select acid- and salt-tolerant lactic acid bacteria, while kombucha fermentations involve interactions among yeasts, acetic acid bacteria, and lactic acid bacteria.

In sourdough, microbial community composition and interspecies interactions were associated with differences in aroma and dough-rise performance²⁸. In kombucha, Liao et al.²⁹ demonstrated temporal microbial succession accompanied by coordinated changes in the metabolite profile during back-slopping fermentation. These findings show that fermentation stage and process conditions influence both the microorganisms recovered and their functional phenotypes. Therefore, psychobiotic isolation should not aim only to recover “beneficial LAB”, but to identify matrix-adapted strains with stable technological performance and measurable gut–brain-relevant functions. Isolation studies should report the source matrix, fermentation stage, pH, temperature, salt or substrate concentration, oxygen conditions, storage history, and isolation procedure. Preserving this ecological and processing context is essential for reproducing the strain phenotype and distinguishing technological adaptation from genuine psychobiotic potential.

Isolation workflow for food-derived psychobiotic strains

A robust isolation pipeline should begin with fermented foods representing microbial diversity, traditional relevance, and technological potential. Samples may include fermented milk, kefir grains, cheese, sourdough, fermented cereals, legumes, vegetables, fruit fermentations, kombucha, cocoa fermentations, and regional fermented foods. Sampling should cover independent production batches and, where relevant, different fermentation stages to capture temporal and ecological variability. Culture-independent microbial profiling may also guide the choice of selective media, atmosphere, temperature, and incubation time, thereby reducing culture-dependent recovery bias¹¹.

Classical isolation on selective media, followed by purification, Gram staining, catalase testing, acidification assessment, and isolate preservation, remains useful. After preliminary phenotyping, isolates should be dereplicated, cryopreserved, assigned traceable strain codes, and authenticated. Although 16S rRNA sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry can support preliminary identification, whole-genome sequencing is preferred for final strain-level assignment and genome-based safety assessment. Detailed safety, mechanistic, technological, and clinical criteria should then be evaluated through the validation framework described in Section “Criteria required to classify a food-derived strain or product as psychobiotic” rather than repeated here². Original studies illustrate this staged approach. Bentahar et al.¹² isolated and characterized food-adapted *Lactobacillus* strains from traditional Dhan butter, whereas Devi et al.³⁰ screened isolates from indigenous fermented foods for γ -aminobutyric acid (GABA) production. Such screening identifies promising candidates but does not alone establish psychobiotic efficacy.

Impact of fermentation substrate on psychobiotic traits

The fermentation substrate is not a passive carrier; it actively determines microbial metabolism, survival, and metabolite output. Milk provides lactose, caseins, whey proteins, minerals, and buffering capacity, which can

support acidification, proteolysis, peptide release, and the survival of lactic acid bacteria. Cereals and sourdough provide starch, resistant starch, arabinoxylans, β -glucans, phenolic acids, and fermentable carbohydrates, while legumes provide proteins, fibers, glutamate, phenolics, and antinutritional compounds that can be transformed during fermentation into more digestible and bioactive matrices¹³. Dietary fibers and fermented foods nevertheless act through partly distinct routes. Fibers primarily resist upper-gastrointestinal digestion and serve as substrates for colonic microorganisms; their effects depend on fiber structure, dose, microbial fermentation capacity, and host microbiome composition. Fermented foods may instead deliver live or inactivated microorganisms, microbial enzymes, organic acids, peptides, vitamins, and food components already transformed before ingestion. Plant-based fermented foods may combine both mechanisms because they contain residual fermentable fibers together with fermentation-derived microorganisms and metabolites¹⁵.

This distinction is supported by the controlled dietary intervention of Wastyk et al.¹⁶: high fermented-food intake increased microbiota diversity and reduced several inflammatory markers, whereas high fiber intake increased microbiome-encoded carbohydrate-degrading capacity without producing the same diversity response. These findings should not be generalized to all foods, but they demonstrate that fiber enrichment and fermentation are not biologically interchangeable. Substrate dependence also influences functional phenotype. A GABA-producing strain may require glutamate-rich substrates and appropriate acidic conditions to activate the glutamate decarboxylase pathway, whereas SCFA-related effects require fermentable substrates and cooperation with the recipient’s colonic microbiota. Phenolic-rich matrices may similarly favor microbial transformation of complex polyphenols into smaller and potentially more bioaccessible metabolites. Psychobiotic foods should therefore be validated as product-specific strain–substrate–metabolite systems rather than as isolated strains added to an interchangeable carrier.

Fermentation parameters controlling neuroactive metabolite production

Fermentation parameters strongly regulate the production of gut–brain-relevant metabolites. Temperature, fermentation time, pH, oxygen availability, inoculum size, starter composition, salt concentration, carbon-to-nitrogen ratio, substrate structure, glutamate availability, and storage conditions can shift both microbial growth and metabolite profiles. For GABA production, several lactic acid bacteria convert glutamate into GABA under acidic conditions through the glutamate decarboxylase pathway. The optimum pH, temperature, precursor concentration, and fermentation duration are strain- and matrix-specific and should therefore be experimentally established rather than assumed from species identity¹⁴. Primary studies demonstrate the importance of process optimization. Devi et al.³⁰ used response-surface methodology to optimize GABA production by fermented-food-derived strains, while Uriho et al.³¹ showed that strain selection and co-culture fermentation modified GABA enrichment, enzyme activity, and the functional properties of a Cili-fruit substrate. Co-cultures may enhance precursor release, acidification, proteolysis, phenolic transformation, exopolysaccharide formation, and GABA synthesis through cross-feeding. However, they may also suppress the target strain, alter sensory quality, or increase undesirable metabolite production.

Process acceptance criteria should therefore be defined before optimization and include the target strain count per serving at the end of shelf life, pH trajectory, product-borne metabolite yield, sensory acceptability, metabolite retention during storage, and batch-to-batch reproducibility. SCFAs should be quantified as direct product constituents only when they are demonstrably present; otherwise, SCFA-production potential should be assessed using validated colonic-fermentation models. The safety dimension is essential because conditions that enhance desirable metabolites may also promote undesirable compounds. Histamine, tyramine, putrescine, and cadaverine can accumulate through microbial amino acid decarboxylation. Their control requires characterization of starter cultures, raw-material quality, hygiene, temperature, pH, salt concentration, and storage.

Pawul-Gruba et al.³² demonstrated the occurrence of biogenic amines and their association with bacterial content in retail ripened cheeses, while Javaid et al.⁴³ highlighted the distribution of biogenic-amine-synthesis genes among food-associated lactic acid bacteria. Psychobiotic fermentation must therefore optimize benefit and safety simultaneously rather than maximizing a single target metabolite.

Fermented foods versus isolated psychobiotic strains

A major distinction should be made between fermented foods as complex microbial ecosystems and isolated psychobiotic strains as standardized biological agents. Whole fermented foods may act through microorganisms, microbial structures, enzymes, peptides, organic acids, exopolysaccharides, vitamins, phenolic metabolites, fibers, and matrix-derived compounds. This complexity may generate complementary effects but complicates dose standardization, causal interpretation, batch reproducibility, and regulatory claims. Isolated strains allow clearer taxonomic identification, viable-count control, safety screening, and clinical testing, but may not reproduce the biochemical context of the original fermented matrix. Neither approach is inherently superior. Isolated strains are more suitable for attributing effects to a defined biological agent, whereas whole fermented foods are more appropriate for testing integrated matrix-level responses. In both cases, the final formulations should constitute the unit of technological, mechanistic, and clinical validation. The strongest strategy is therefore to integrate both approaches. Fermented foods should be used as discovery ecosystems for food-adapted microorganisms and functional metabolites, while selected isolates should be evaluated in defined matrices under controlled fermentation, storage, digestion, safety, and human-intervention conditions. Figure 1 summarizes this progression from ecological discovery to strain authentication, product development, mechanistic testing, and product-specific human validation.

Microbiota–gut–brain axis mechanisms linking psychobiotic foods to mental health

Functional microbial ecology rather than simple taxonomic modulation

The microbiota–gut–brain axis is a bidirectional communication network linking intestinal microorganisms, epithelial cells, immune mediators, enteroendocrine signaling, the enteric nervous system, the vagus nerve, systemic metabolism, and the central nervous system. Psychobiotic foods may influence this network by modifying microbial community structure, but their most relevant effect is probably functional rather than purely taxonomic. Changes in short-chain fatty acids, tryptophan metabolites, GABA-related pathways, bile acid transformation, epithelial barrier function, and inflammatory tone are more informative than increases in broadly defined “beneficial bacteria”^{1,33}.

This distinction is important because taxonomically different communities may perform similar metabolic functions, whereas apparently similar communities may differ in functional genes and metabolite production. Wastyk et al.¹⁶, for example, showed that fermented-food and high-fiber diets produced distinct microbial and immune responses. Psychobiotic efficacy should therefore be evaluated by integrating microbiome, metabolome, host biomarkers, and validated psychological outcomes rather than relying on genus-level changes alone².

Neural signaling: vagus nerve, enteric nervous system, and enteroendocrine interfaces

The neural pathway is one of the fastest routes by which intestinal signals can influence brain function. The vagus nerve connects the gastrointestinal tract with brain regions involved in stress regulation, emotional processing, autonomic balance, and interoception. However, food-derived psychobiotic effects on vagal signaling should be interpreted cautiously because direct human evidence remains limited^{1,33}.

Psychobiotic foods may affect vagal communication indirectly by modifying enteroendocrine hormone release, epithelial signaling, mucosal immunity, and microbial metabolite availability. Fermentation-derived

organic acids, SCFAs, bioactive peptides, GABA-associated compounds, and tryptophan derivatives may interact with epithelial cells, immune cells, enteroendocrine cells, and enteric neurons^{13,33}. The presence of a neuroactive compound does not, however, demonstrate direct central nervous system activity. Food-derived, intestinal, circulating, and central GABA represent different biological exposures. Neural claims should therefore be supported by physiological endpoints such as autonomic markers, gut-hormone responses, stress reactivity, or mechanistic animal evidence Fig. 2.

Endocrine regulation: HPA axis and stress biology

The hypothalamic–pituitary–adrenal axis is a central endocrine pathway connecting psychological stress, intestinal physiology, immune activation, and microbial ecology. Stress can alter gut motility, mucus secretion, intestinal permeability, immune responses, microbial composition, and metabolite production, while microbial signals may influence cortisol release through immune, neural, and metabolic pathways^{33,34}.

Psychobiotic foods may support stress regulation by improving barrier integrity, reducing low-grade inflammation, and modifying microbial metabolites. However, HPA-axis modulation should not be inferred from mood or perceived-stress scores alone. Relevant studies should combine validated psychological scales with repeated cortisol measurements, inflammatory markers, microbiome profiles, and metabolomic data. Berding et al.¹⁷ reported reduced perceived stress following a psychobiotic dietary intervention, but biological stress markers showed less consistent changes. Psychological improvement and endocrine mediation should therefore be reported separately unless a linked pathway is demonstrated.

Immune modulation, cytokines, and neuroinflammatory signaling

The immune system is an important communication channel between the gut microbiota and the brain. Gut dysbiosis, impaired epithelial integrity, and microbial-product translocation may promote systemic low-grade inflammation, influencing neurotransmission, stress sensitivity, neuroinflammation, and affective behavior^{34,35}.

Psychobiotic foods may counteract these processes by supporting barrier function, increasing anti-inflammatory metabolites, and regulating innate and adaptive immune responses. Wastyk et al.¹⁶ reported that increased fermented-food intake was associated with greater microbial diversity and reductions in several inflammatory proteins. However, because psychological outcomes were not primary endpoints, this study supports a diet–microbiome–immune pathway rather than a proven mental-health mechanism. Inflammatory biomarkers may include IL-6, TNF- α , IL-1 β , IL-10, C-reactive protein, lipopolysaccharide-binding protein, and markers of epithelial injury. The biomarker panel should be selected according to a prespecified mechanistic hypothesis, and simultaneous changes in cytokines and psychological scores should not be interpreted as causality without temporal or mediation analysis.

Microbial metabolites as biochemical mediators

Microbial metabolites are central mediators of gut–brain communication. SCFAs, mainly acetate, propionate, and butyrate, are produced through microbial fermentation of dietary fibers and resistant starch. They may support epithelial integrity, immune regulation, enteroendocrine signaling, and microbial cross-feeding^{13,34}. SCFAs contained in food, produced during colonic fermentation, measured in feces, and detected systemically are not equivalent. SCFA-related claims should therefore specify the biological compartment and source of production.

Tryptophan can enter serotonin, kynurenine, and microbial indole pathways. Psychobiotic foods may affect these routes by modifying microbial metabolism, inflammatory activation of indoleamine 2,3-dioxygenase, and substrate availability^{36,37}. Stronger profiling should include tryptophan, kynurenine, the kynurenine-to-tryptophan ratio, kynurenin acid, quinolinic acid, and relevant indole derivatives.

GABA-producing lactic acid bacteria are also relevant, but peripheral, microbial, and central GABA are not automatically equivalent.

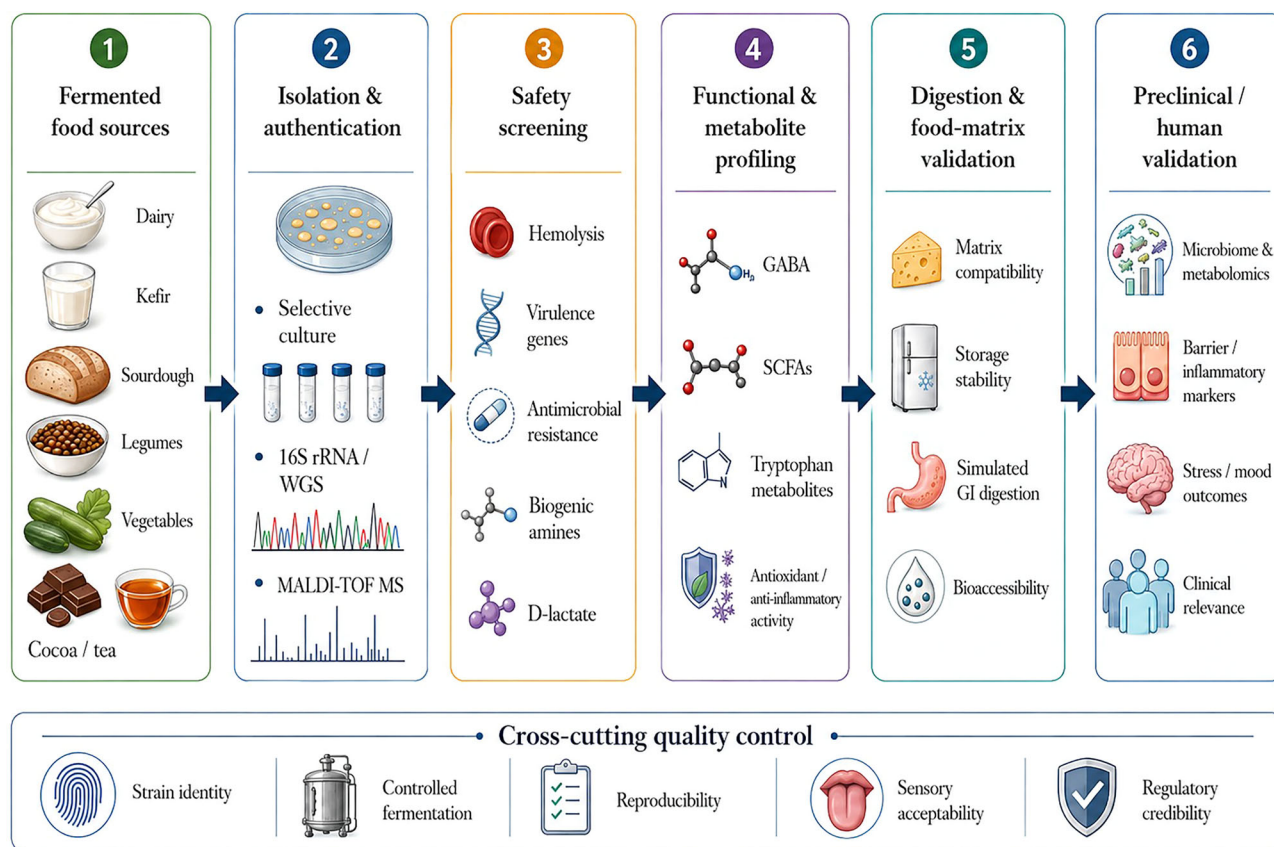


Fig. 1 | Fermentation-driven pipeline for psychobiotic discovery and validation.

GABA quantification should therefore be combined with serving-size exposure, storage stability, digestion, bioaccessibility, and host-response biomarkers¹⁴. Other fermentation-derived compounds, including peptides, exopolysaccharides, phenolic metabolites, and bile acid derivatives, may influence immune, oxidative, barrier, or enteroendocrine pathways. Their relevance must be demonstrated at physiologically meaningful concentrations rather than inferred from their detection in the food.

Intestinal barrier and blood–brain barrier integrity

The intestinal barrier is a critical control point because epithelial disruption can increase exposure to microbial products and amplify systemic inflammation. Tight-junction proteins, mucus integrity, epithelial oxidative stress, and permeability markers are therefore relevant endpoints^{1,35}. Psychobiotic strains and fermentation-derived metabolites may support barrier function through SCFA production, mucin regulation, anti-inflammatory signaling, and oxidative-stress reduction. Barrier restoration should not be inferred from one marker alone. Functional permeability tests should be combined with indicators such as intestinal fatty-acid-binding protein, lipopolysaccharide-binding protein, transepithelial electrical resistance, and tight-junction localization.

The blood–brain barrier represents a second interface through which systemic inflammation and microbial metabolites may influence the brain. However, evidence is substantially stronger in cellular and animal models than in human food interventions^{34,38}. Human studies should therefore describe blood–brain-barrier involvement as a plausible mechanism unless supported by validated neurological, endothelial, imaging, or permeability-related measurements.

Post-ingestion food-matrix effects on gut–brain mechanisms

Section “impact of fermentation substrate on psychobiotic traits” explains how substrate composition influences fermentation and metabolite production. In contrast, this section focuses on the effects of the final food

matrix after ingestion. Food matrix composition can influence microbial protection, gastric buffering, digestive-enzyme access, metabolite release, intestinal absorption, and delivery of fermentable substrates to the colon. Dairy proteins and lipids may protect microorganisms during gastric passage, while cereal and legume structures may regulate the accessibility of starch, proteins, fibers, and phenolic compounds. Processing, storage, heating, encapsulation, and food structure can further modify cell survival and metabolite bioaccessibility.

Consequently, the same strain delivered in a capsule, dairy product, sourdough, or plant-based beverage should not be assumed to produce equivalent exposure or biological effects. Validation should use the final formulation, serving size, processing history, and storage conditions. Matrix-matched controls may include an unfermented product, a strain-free fermented matrix, an inactivated-cell product, or the same strain in another carrier. Such comparisons help separate effects attributable to the microorganism, fermentation process, substrate, and their interaction (Table 2).

Host-specific response, medication interactions, and mechanistic validation

Interindividual variability remains one of the main reasons for inconsistent psychobiotic outcomes. Baseline microbiota, habitual diet, fiber intake, stress, medication exposure, sex, age, immune status, sleep, and metabolic health may all influence response². Baseline microbial functions determine whether substrates can be degraded, whether cross-feeding occurs, and which metabolites are produced. Wastyk et al.¹⁶ showed that baseline microbial diversity was associated with different immune responses to increased fiber intake, demonstrating that the initial microbiome can modify dietary response.

Age and sex may influence psychobiotic effects through gastrointestinal physiology, sex hormones, immune tone, epithelial integrity, microbial metabolism, and medication patterns. These variables should be

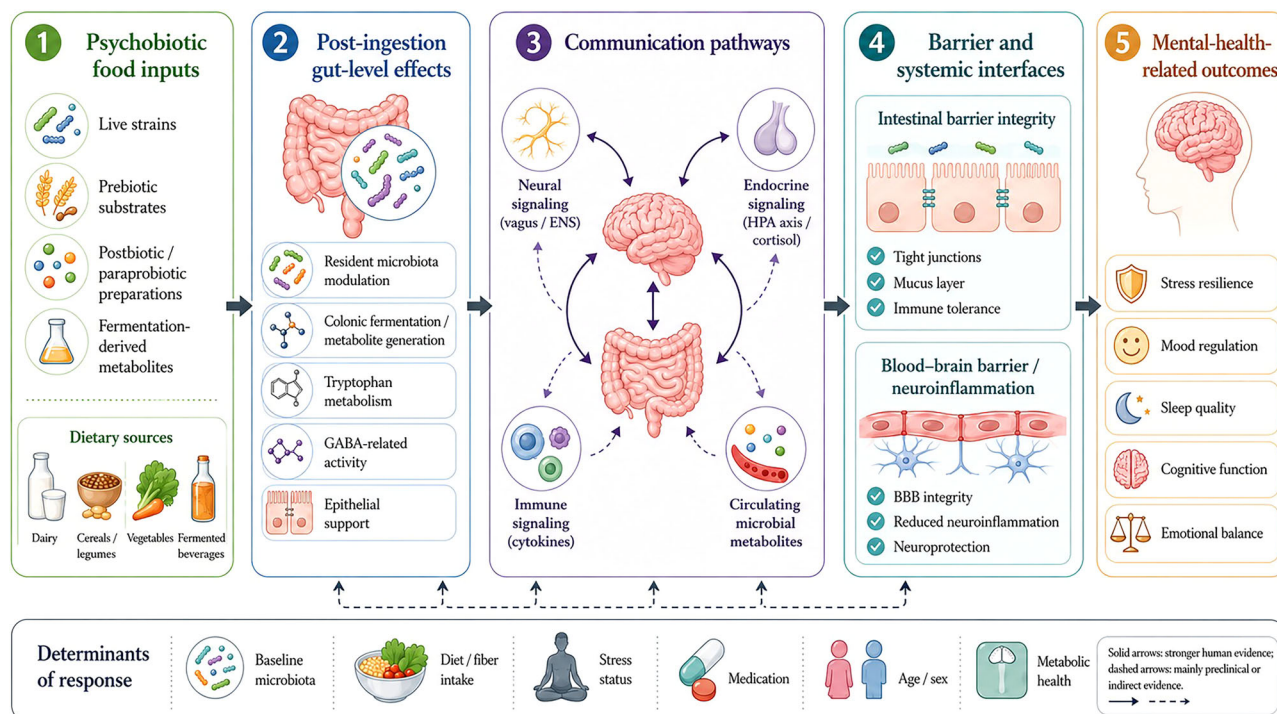


Fig. 2 | Integrated signal-flow model linking psychobiotic food inputs to microbiota-gut-brain communication pathways and mental-health-related outcomes.

prespecified as potential effect modifiers rather than examined only through underpowered post hoc analyses. Medication use is particularly important because antibiotics, proton-pump inhibitors, metformin, laxatives, antidepressants, and other psychotropic agents may alter microbial composition, intestinal transit, gastric pH, and microbial metabolism. Trials should document medication class, dose, duration, recent antibiotic exposure, and treatment changes. Randomization or statistical analysis should account for medication use when it is likely to modify the intervention response.

Interactions among diet, medication, and fermented foods remain poorly characterized. Claims of synergy or antagonism should therefore be restricted to directly tested combinations. Current responder-stratification models are also largely exploratory and require prespecified predictors, independent validation, and evidence that stratification improves clinical benefit. Psychobiotic foods may influence mental health through interconnected microbial, neural, endocrine, immune, barrier, and metabolic pathways. Current human evidence is stronger for changes in microbial functions, metabolites, inflammatory markers, and selected psychological outcomes than for direct vagal activation, HPA-axis mediation, or blood-brain-barrier restoration. Future studies must clearly distinguish association, statistical mediation, and experimentally demonstrated causality.

Psychobiotic microorganisms, food matrices, and fermentation-derived neuroactive metabolites

Major psychobiotic microbial candidates

Psychobiotic functionality is primarily strain-specific; therefore, candidate microorganisms should not be classified as psychobiotic only because they belong to traditionally probiotic genera. The most frequently investigated food-compatible candidates include *Lactiplantibacillus plantarum*, *Lactocaseibacillus rhamnosus*, *Limosilactobacillus reuteri*, *Lactobacillus helveticus*, and *Lactobacillus delbrueckii*, as well as *Bifidobacterium longum*, *B. breve*, *B. adolescentis*, and *B. bifidum*. However, these taxa are not supported by equivalent evidence, and psychobiotic status must be assigned to an authenticated strain rather than inferred from species identity. Table 3 therefore distinguishes food-derived candidates, mechanistically

characterized strains, strains supported by preclinical studies, and strains evaluated in humans.

Lactic acid bacteria remain central to psychobiotic food development because they occur widely in fermented dairy products, sourdough, vegetables, legumes, and plant-based beverages. Their technological relevance derives from acidification, proteolysis, exopolysaccharide formation, antimicrobial activity, stress tolerance, and production of compounds such as γ -aminobutyric acid (GABA), organic acids, and bioactive peptides^{10,14}. However, acid and bile tolerance, processing stability, and acidification capacity are delivery traits rather than direct gut-brain mechanisms. Mechanistic relevance requires evidence of neuroactive-metabolite production, immune or epithelial modulation, microbiota-dependent activity, or effects on validated psychological or neurophysiological endpoints.

Human findings illustrate why strain-level and outcome-specific interpretation is necessary. *Lactiplantibacillus plantarum* PS128 has been examined in human mood and sleep studies, but its effects have varied according to population and study design^{39,40}. In patients with major depressive disorder, *Bifidobacterium breve* CCFM1025 improved depression scores relative to placebo and was associated with changes in the gut microbiome and tryptophan metabolism¹⁸. By contrast, *B. longum* 1714 improved sleep quality and duration during examination stress but did not improve chronic stress, depression, or cognitive performance⁴¹. These contrasting results show that efficacy in one endpoint cannot be generalized to mood, anxiety, sleep, and cognition collectively.

Multi-strain formulations provide additional evidence but present an attribution problem. Walden et al.⁴² reported improvements in several self-reported mood and anxiety measures after administration of a formulation containing *Limosilactobacillus fermentum* LF16, *Lactocaseibacillus rhamnosus* LR06, *Lactiplantibacillus plantarum* LP01, and *Bifidobacterium longum* 04. However, the design could not determine whether the effect originated from one strain, additive activity, or strain-strain interactions. Such formulations should therefore be evaluated as complete products rather than used to validate each component strain independently.

Certain bifidobacterial species, particularly infant-associated lineages, are prominent early-life colonizers and may influence carbohydrate fermentation, immune development, and epithelial function. Nevertheless,

Table 2 | Mechanistic pathways, biomarkers, and references supporting psychobiotic food effects on microbiota–gut–brain outcomes

Pathway	Food/microbial mediator	Suggested biomarkers/endpoints	Potential mental-health relevance	References
Microbial ecology modulation	Live psychobiotic strains, fermented food microbiota, prebiotic substrates, synbiotic combinations	16S rRNA sequencing, shotgun metagenomics, WGS, α/β -diversity, strain persistence, microbial functional genes	Responder identification, microbiome resilience, dysbiosis correction, gut–brain communication	1,2,13
Neural signaling	SCFAs, GABA and related metabolites, enteroendocrine signals, bioactive peptides	Vagal tone, autonomic markers, gut peptides, enteric nervous system markers, animal vagotomy models	Stress regulation, emotional reactivity, autonomic balance, gut–brain communication	1,33,34
HPA-axis regulation	SCFAs, barrier-supporting strains, anti-inflammatory metabolites, fermented-food bioactives	Salivary/serum cortisol, corticosterone, perceived stress scales, sleep-quality indices	Stress resilience, anxiety-related response, sleep quality, emotional regulation	1,2,21
Immune modulation	SCFAs, exopolysaccharides, bioactive peptides, phenolic metabolites, postbiotic fractions	IL-6, TNF- α , IL-1 β , IL-10, CRP, LPS-binding protein, immune-cell profiles	Neuroinflammation modulation, depressive-symptom relevance, fatigue reduction, cognitive support	1,34,35
Tryptophan metabolism	Microbial indoles, kynurenine-pathway modulation, serotonin precursors, fermented protein substrates	Tryptophan, kynurenine, kynurenic acid, quinolinic acid, serotonin, IPA, IAA, indole derivatives	Mood regulation, neuroimmune balance, cognitive function, stress-related metabolic regulation	21,34,36
SCFA-mediated signaling	Fermentable fibers, resistant starch, cereal β -glucans, legume fibers, acetate-, propionate-, and butyrate-producing microbiota	Acetate, propionate, butyrate, fecal/serum SCFAs, colonic fermentation profiles	Barrier support, immune regulation, enteroendocrine signaling, microbiota-derived energy metabolism	13,15,21
GABA-related activity	GABA-producing LAB, glutamate-rich fermented matrices, fermented dairy, legumes, cereals, vegetables	GABA quantification, glutamate/GABA ratio, glutamate decarboxylase genes, bioaccessibility after digestion	Stress-response modulation, anxiety-related pathways, inhibitory signaling support	1,13,21
Barrier integrity	Butyrate, LAB strains, exopolysaccharides, organic acids, bioactive peptides	Zonulin, tight-junction proteins, occludin, claudins, I-FABP, LPS-binding protein, mucus-layer markers	Reduced endotoxemia, lower systemic inflammation, improved gut–brain stability	1,34,35
Blood–brain barrier and neuroinflammatory interface	SCFAs, tryptophan metabolites, anti-inflammatory microbial metabolites, phenolic metabolites	S100B, MMP-9, claudin-5, occludin, inflammatory cytokines, microglial activation markers	Neuroinflammation modulation, neuroprotection, cognitive and emotional regulation	34,35,38
Food-matrix effects	Dairy proteins, cereal fibers, legume proteins, vegetable fibers, plant polyphenols, fermented beverages	Bioaccessibility, peptide profile, phenolic metabolites, SCFA output, matrix-dependent survival, metabolomic fingerprints	Matrix-specific psychobiotic efficacy, product validation, personalized food response	13,15,21
Clinical-response determinants	Baseline microbiota, diet quality, fiber intake, stress status, medication exposure, age, sex, metabolic health	Baseline microbiome, dietary records, metabolomics, stress scores, medication profile, stratified responder analysis	Identification of responders/non-responders, precision psychobiotic nutrition, improved trial reproducibility	2,20,21

Table 3 | Psychobiotic microorganisms, food matrices, metabolites, technological value, safety concerns, and validation requirements

Microbial group/ candidate	Typical food reservoirs or delivery matrices	Main neuroactive/gut-brain-relevant metabolites or functions	Technological advantages	Main safety or formulation concerns	Required validation before psychobiotic claim	References
<i>Lactiplantibacillus plantarum</i>	Fermented vegetables, sourdough, fermented cereals, legumes, fruit/ vegetable beverages	GABA production, organic acids, antioxidant activity, phenolic transformation, epithelial barrier support, immune modulation	Highly adaptable to plant matrices, acid tolerant, good survival in fermented foods, suitable for vegan products	Strain-dependent biogenic amine risk, antimicrobial resistance screening required, variable metabolite production across matrices	WGS/MALDI-TOF, acid/bile survival, GABA and organic acid profiling, biogenic amine screening, digestion model, gut-brain biomarker testing	1,14,21
<i>Lactisacibacillus rhamnosus</i>	Fermented milk, yogurt, functional beverages, encapsulated foods	Immune modulation, epithelial barrier protection, HPA-axis regulation, stress- response modulation, possible GABA- related signaling	Strong probiotic history, good technological robustness, suitable for dairy and encapsulated delivery	Effects are highly strain-specific; not all <i>L. rhamnosus</i> strains are psychobiotic	Strain-level identification, viable count during storage, cortisol/stress biomarkers, cytokines, gut permeability markers, clinical endpoints	1,2,21
<i>Limosilactobacillus reuteri</i>	Fermented dairy, sourdough, infant-oriented probiotic foods, functional beverages	Immune regulation, epithelial interaction, antimicrobial activity, possible vagal and neuroimmune modulation	Can interact strongly with host mucosa; suitable for targeted probiotic formulation	Safety and efficacy are strain- dependent; formulation stability must be controlled	Genome safety, mucosal interaction assays, inflammation markers, GI survival, human outcome validation	1,2,20
<i>Lactobacillus helveticus</i>	Fermented milk, yogurt, cheese, dairy beverages	Bioactive peptides, GABA-related activity, stress-response modulation, possible anxiolytic-like effects through dairy peptide systems	Strong proteolytic activity; useful for peptide-rich fermented dairy	Dairy matrix dependence; peptide profile varies with fermentation and ripening	Peptidomics, GABA quantification, viable count, dairy matrix stability, cortisol/stress and mood endpoints	1,21,76
<i>Bifidobacterium longum</i>	Fermented milk, synbiotic dairy, encapsulated foods, functional powders	SCFA modulation, tryptophan metabolism interaction, immune modulation, gut barrier support, stress and mood-related effects	Strong gut-adapted profile; clinically relevant genus for gut-brain studies	Oxygen sensitivity, poor survival in some food matrices, needs protective technologies	Anaerobic-compatible formulation, viability during storage, microbiome persistence, tryptophan/SCFA profiling, clinical validation	2,20,21
<i>Bifidobacterium breve/B. adolescentis/B. bifidum</i>	Fermented dairy, infant and adult synbiotic foods, encapsulated preparations	Carbohydrate fermentation, acetate production, immune modulation, barrier support, microbiota resilience	Strong relevance for gut ecology and synbiotic design	Sensitive to oxygen, acidity, and processing; matrix protection needed	Strain authentication, oxygen- stability testing, synbiotic compatibility, SCFA profiling, immune and barrier biomarkers	2,10,21
<i>Pediococcus acidilactici/ Pediococcus pentosaceus</i>	Fermented vegetables, cereals, legumes, meat- free fermented foods	Organic acids, antimicrobial peptides, possible GABA production, antioxidant effects	Good acid tolerance; useful in plant fermentations and biopreservation	Some strains may produce undesirable metabolites; safety must be strain-specific	WGS, antibiotic resistance screening, biogenic amine testing, GABA/organic acid profiling, digestion survival	14,21,22
<i>Leuconostoc</i> spp.	Fermented vegetables, kimchi-like products, sourdough, plant fermentations	Organic acids, exopolysaccharides, aroma compounds, possible immune/ barrier effects	Useful for texture, flavor, and EPS formation in fermented foods	Metabolite output is variable; safety and stability need confirmation	EPS characterization, metabolomics, strain safety, microbial stability, matrix-specific performance	13,22,75
<i>Weissella</i> spp.	Fermented vegetables, cereals, sourdough, traditional fermented foods	GABA production in selected strains, EPS production, organic acids, antioxidant potential	Promising for plant-based fermentation and texture improvement	Genus requires caution due to safety variability; not automatically probiotic	Genome-based safety, hemolysis testing, antimicrobial resistance, biogenic amines, GABA confirmation by HPLC/LC-MS	14,22,30
<i>Saccharomyces</i> and kefir- associated yeasts	Kefir, kombucha, sourdough, fermented beverages	B vitamins, volatile compounds, microbial cross-feeding, matrix transformation, possible microbiota modulation	Improve flavor, fermentation complexity, and microbial consortium stability	Ethanol formation, batch variability, uncontrolled fermentation risk	Yeast identification, ethanol monitoring, microbial ecology profiling, metabolomics, safety and sensory validation	13,15,22
Kefir microbial consortia	Milk kefir, water kefir, kefir- like plant beverages	Organic acids, peptides, EPS/kefiran, microbial diversity, GABA-related and immunometabolic metabolites	Natural multispecies system; strong fermentation complexity; potential whole- food intervention	Highly variable composition depending on grain origin and fermentation conditions	Metagenomics, metabolomics, viable count standardization, safety screening, serving-size definition, clinical validation	13,15,22
Sourdough microbial consortia	Wheat, rye, barley, oat, gluten-free cereal fermentations	Organic acids, peptides, phenolic transformation, improved fiber bioaccessibility, post-digestion SCFA potential	Improves flavor, texture, digestibility, and cereal bioactive availability	Baking reduces live cells; psychobiotic effects may depend more on metabolites/ postbiotics than live strains	Peptidomics, phenolic profiling, post-digestion fermentation, SCFA output, distinction between live and postbiotic effects	13,15,22

Table 3 (continued) | Psychobiotic microorganisms, food matrices, metabolites, technological value, safety concerns, and validation requirements

Microbial group/ candidate	Typical food reservoirs or delivery matrices	Main neuroactive/gut-brain-relevant metabolites or functions	Technological advantages	Main safety or formulation concerns	Required validation before psychobiotic claim	References
Vegetable-fermentation LAB consortia	Kimchi, sauerkraut, fermented pickles, traditional vegetable fermentations	Organic acids, GABA in selected systems, phenolic metabolites, EPS, immune and barrier-related metabolites	Acid- and salt-adapted strains; strong plant-food compatibility	Salt level, microbial variability, biogenic amines, batch inconsistency	LAB profiling, salt/pH control, metabolite fingerprints, biogenic amine monitoring, clinical relevance testing	13,22,75
Legume-fermentation LAB	Soy, lentil, chickpea, pea, lupin, faba bean, fermented plant-protein foods	GABA from glutamate-rich matrices, peptides, SCFA precursors, phenolic transformation, reduced antinutritional factors	Strong for vegan psychobiotic products; high protein/fiber/ glutamate potential	Beany flavor, texture instability, antinutritional residues, strain- dependent performance	GABA quantification, peptide profiling, antinutrient reduction, sensory testing, digestion/ bioaccessibility, safety screening	14,15,21
Polyphenol-rich fermentation systems	Kombucha, cocoa, coffee, fruit beverages, tea-based fermentations	Phenolic metabolites, organic acids, antioxidant and anti-inflammatory activity, microbial cross-feeding substrates	Strong metabolite diversity; potential antioxidant and anti- inflammatory relevance	Caffeine, ethanol, acidity, microbial instability, high batch variability	LC-MS/MS phenolic profiling, ethanol/acidity monitoring, microbial safety, antioxidant and anti- inflammatory assays	13,21,22
Postbiotic/paraprobiotic microbial preparations	Heat-treated fermented dairy, pasteurized fermented beverages, inactivated microbial fractions	Cell-wall components, microbial fragments, EPS, peptides, organic acids, immune and barrier modulation	Higher stability than live probiotics; useful for heat- processed foods	Must not be confused with purified metabolites; biological activity must remain reproducible after inactivation	Defined microbial origin, inactivation control, compositional reproducibility, immune/barrier assays, safety evaluation	1,21,22

early-life ecological relevance does not automatically establish adult psychobiotic efficacy. In food systems, bifidobacteria are generally more oxygen-sensitive than many lactic acid bacteria, creating challenges for processing, storage, and delivery in oxygen-exposed matrices. Protective matrices, oxygen-controlled manufacturing, encapsulation, or compatible co-cultures may improve viability, but these technological measures must be evaluated separately from mental-health efficacy². Other microorganisms may contribute to psychobiotic food systems, but they should be approached with greater caution. *Pediococcus*, *Leuconostoc*, *Weissella*, and selected yeasts may produce organic acids, peptides, exopolysaccharides, volatile compounds, or GABA-related metabolites. At present, most should be considered discovery-stage candidates because evidence is commonly limited to metabolite production, in vitro activity, or technological performance. Detection of GABA or another neuroactive compound alone does not validate psychobiotic function.

Non-conventional candidates should undergo genome-based assessment of virulence determinants and transferable antimicrobial resistance, together with evaluation of biogenic-amine formation, undesirable enzymatic activities, product stability, and host-relevant functional effects⁴³. Table 3 consequently highlights a major translational mismatch: lactic acid bacteria generally offer the strongest food-processing compatibility, bifidobacteria require greater formulation protection, and non-conventional taxa have the least developed human evidence. Figure 3 summarizes these differences according to microbial group, food compatibility, proposed function, evidence level, and principal limitation.

Dairy matrices: fermented milk, yogurt, kefir, and cheese

Dairy products are among the most established matrices for probiotic and psychobiotic delivery because milk proteins, lactose, minerals, buffering capacity, and fat-protein structures can protect microbial cells during fermentation, storage, and gastrointestinal transit. Fermented milk and yogurt can support lactic acid bacteria and bifidobacteria while generating peptides, organic acids, and GABA-related compounds through proteolysis and amino acid metabolism. A recent randomized controlled trial evaluated a cultured milk drink containing *Lactobacillus acidophilus* LA-5 and *Lactocaseibacillus paracasei* L. CASEI-01 in individuals with irritable bowel syndrome and subthreshold depression, illustrating the importance of testing the complete fermented product rather than extrapolating efficacy from the strains alone⁴⁴. However, technological protection and metabolite production do not themselves demonstrate psychobiotic efficacy.

Fermented milk and yogurt are comparatively easy to standardize because starter composition, fermentation time, processing conditions, and serving size can be controlled. Kefir is more complex because its bacterial and yeast consortium varies with grain origin, milk type, back-slopping, temperature, and storage. In a randomized crossover trial, traditionally fermented kefir and commercial kefir produced different inflammatory and metabolic responses, supporting the view that microbial composition and manufacturing method materially influence product functionality⁴⁵. Kefir should therefore be described as a matrix with psychobiotic potential unless its microbial composition, metabolite profile, serving dose, and human effects are reproducibly characterized.

Cheese may provide peptides, free amino acids, organic acids, and ripening-derived metabolites. Nevertheless, extended proteolysis and microbial amino acid decarboxylation can favor tyramine, histamine, putrescine, and cadaverine accumulation. A recent analysis of white-brined cheeses detected biogenic amines in multiple commercial samples and identified tyramine as a major concern in some products, confirming the need for strain selection and end-of-shelf-life monitoring⁴⁶. Dairy-product development must therefore balance cell viability and beneficial metabolite production against oxygen sensitivity, biogenic-amine formation, sensory quality, and storage stability.

Cereal, sourdough, and legume-based matrices

Cereal-based matrices provide fermentable carbohydrates, resistant starch, β -glucans, arabinoxylans, phenolic acids, minerals, and proteins

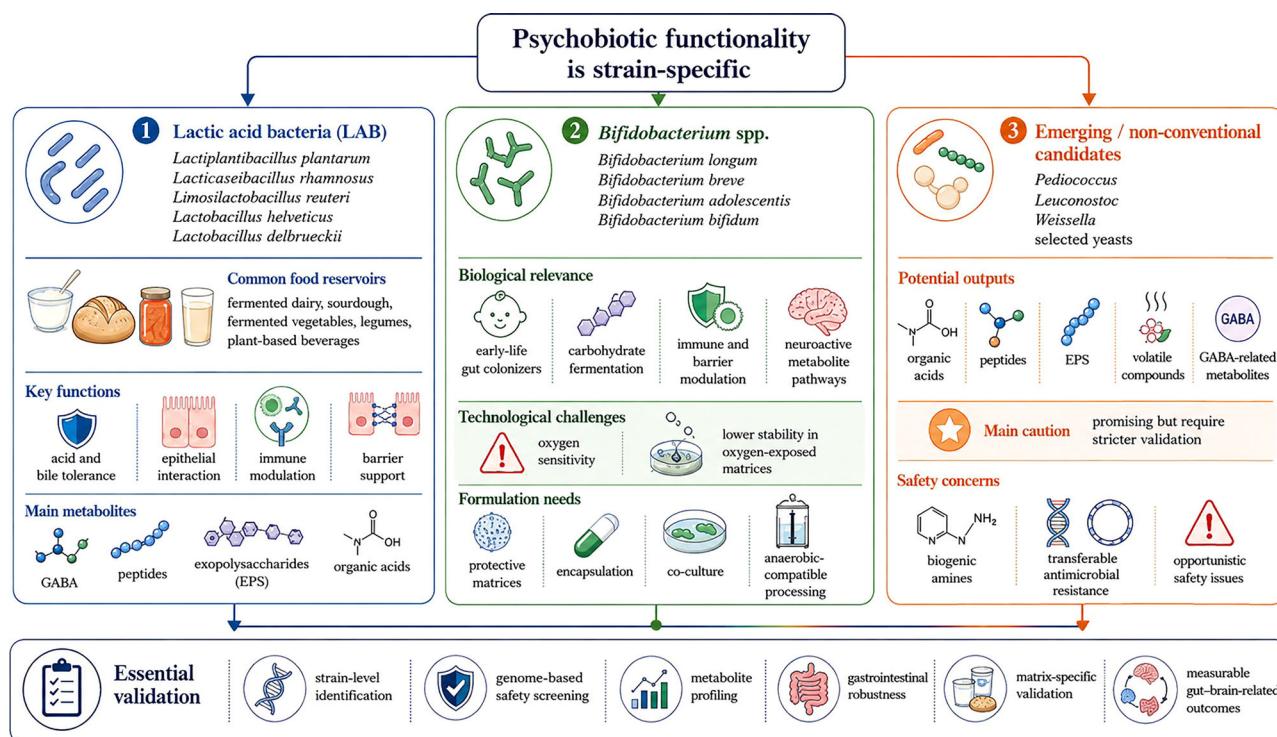


Fig. 3 | Major psychobiotic microbial candidates and essential validation criteria.

that support microbial growth and subsequent colonic fermentation. Sourdough fermentation can modify starch and protein accessibility, release peptides, transform phenolic compounds, reduce some anti-nutritional factors, and generate organic acids. The effects of fermentation must, however, be distinguished from those of subsequent processing. Baking generally prevents the final product from being considered a live-cell delivery system unless post-baking viability is demonstrated. It may also alter the retention and accessibility of GABA, organic acids, peptides, and phenolic metabolites. Xuan et al.⁴⁷ demonstrated that sourdough prepared with the GABA-producing strain *Pediococcus pentosaceus* JC30 yielded bread containing measurable GABA after baking, showing that metabolite retention must be quantified in the consumed product rather than inferred from fermented dough.

Even when viable microorganisms are lost, baked sourdough may retain microbial cell components, organic acids, fermentation-modified substrates, and bioactive metabolites. Such products should be evaluated as fermented matrices or potential postbiotic systems rather than automatically classified as live psychobiotic foods. For each product, viable-cell counts and metabolite concentrations should be measured after baking and throughout storage. Legume-based matrices contain proteins, fibers, resistant starch, glutamate, phenolics, minerals, and antinutritional compounds that can be modified during fermentation. Montagano et al.⁴⁸ showed that GABA formation by *Lactiplantibacillus plantarum* was strongly strain-dependent and that yields measured in laboratory medium were substantially different from those obtained in the actual legume beverage. This directly supports matrix-specific validation⁴⁸.

Legume processing can also affect metabolite formation after ingestion. Purwandari et al.⁴⁹ reported that tempeh fermentation modified protein and starch digestibility and influenced the production of SCFAs, GABA, and tryptophan-related metabolites during subsequent in vitro colonic fermentation. Thus, metabolites contained in the food and those generated by the resident microbiota after ingestion should be clearly distinguished. Legume products may nevertheless present beany flavor, texture instability, variable protein solubility, and strain-dependent fermentation performance. Fermentation can improve the nutritional, microbiological, and sensory characteristics of chickpea-based beverages, but the outcome

depends on the selected starter and formulation conditions⁵⁰. Each final formulation should therefore be assessed for fermentation kinetics, cell retention, metabolite production, sensory quality, digestion stability, and safety.

Fermented vegetables, fruits, and polyphenol-rich matrices

Fermented vegetables such as kimchi, sauerkraut, and fermented pickles may provide acid- and salt-tolerant lactic acid bacteria, organic acids, exopolysaccharides, and transformed plant metabolites. Nevertheless, these products vary considerably in salt content, starter composition, spontaneous microbial succession, serving size, and storage conditions.

In a pilot randomized study, consumption of ~100 g/day of fermented vegetables for six weeks altered selected bacterial taxa but did not significantly change the measured inflammatory markers. This finding indicates that microbiota modification should not automatically be interpreted as an anti-inflammatory or psychobiotic effect⁵¹. Fruit and vegetable fermentations provide sugars, fibers, carotenoids, anthocyanins, flavonoids, and phenolic acids. Microbial activity may transform these compounds and generate GABA, organic acids, and other metabolites. For example, co-culture fermentation with *Levilactobacillus brevis* YSJ3 and *Lactiplantibacillus plantarum* JLSC2-6 increased GABA production and extensively modified the volatile and non-volatile metabolite profiles of cauliflower by-products⁵². However, metabolite enrichment or antioxidant activity alone does not establish a mental-health effect.

Tea, cocoa, coffee, and kombucha should be considered separately from vegetable lactic fermentations because they involve different substrates, microorganisms, and processing conditions. Kombucha additionally involves interactions among yeasts, lactic acid bacteria, and acetic acid bacteria, with variable acid and ethanol formation. Analysis of retail kombucha beverages showed substantial differences in viable microbial populations, with only eight of twelve products containing viable traditional kombucha cultures. These findings demonstrate that label claims or detection of microbial DNA cannot substitute for viable counts and product-level characterization⁵³. Accordingly, polyphenol-rich fermented products require microbial profiling, targeted metabolomics, gastrointestinal bioaccessibility assessment, and batch-to-batch standardization.

Residual sugar, caffeine, ethanol, acidity, and viable-cell stability should also be quantified in the final product.

Production, retention, and delivery of neuroactive metabolites

The biological actions of GABA, SCFAs, and tryptophan metabolites are discussed in Section “microbial metabolites as biochemical mediators”; the present section focuses on their formation, retention, and delivery within food systems. GABA can be produced when selected lactic acid bacteria convert glutamate through the glutamate decarboxylase system. Production depends on strain identity, glutamate availability, pH, temperature, fermentation duration, and matrix composition. Recent food-system studies confirm strong strain and matrix dependence in sourdough bread and legume-based beverages^{47,48}. A GABA-enriched product should report concentration per serving, batch variability, retention during storage and processing, gastrointestinal release, and bioaccessibility. The large difference between GABA yields obtained in laboratory medium and in a real legume beverage further demonstrates that *in vitro* screening cannot replace final-product measurement⁴⁸.

SCFAs are mainly generated after ingestion through colonic fermentation of fibers and resistant carbohydrates. Product-borne SCFAs, SCFA-production potential in a colonic model, fecal concentrations, and systemic exposure represent different measurements and should not be used interchangeably. The tempeh study of Purwandari *et al.*⁴⁹ illustrates how processing can modify subsequent colonic SCFA production without implying that the food itself contains an equivalent SCFA dose. Tryptophan-derived indoles, peptides, exopolysaccharides, organic acids, vitamins, and phenolic metabolites may also be generated or modified during fermentation. Their evaluation should include the final-product concentration, analytical identification confidence, processing and storage stability, exposure per serving, digestive release, and concentrations achieved in intestinal or systemic compartments.

Undesirable metabolites and matrix-dependent safety risks

The same fermentation processes that generate beneficial compounds can produce undesirable metabolites. Biogenic amines may accumulate when amino acid decarboxylase-positive strains grow under favorable conditions. Safety risks differ by matrix. Ripened cheeses are particularly vulnerable to tyramine and histamine accumulation; fermented legumes may retain antinutritional compounds or develop amines; vegetable fermentations require control of salt, hygiene, and microbial succession; and kombucha-like beverages require monitoring of ethanol, acidity, and contamination.

Buňková *et al.*⁴⁶ identified tyramine-producing microorganisms and potentially concerning amine concentrations in commercial white-brined cheeses. In low-salt fermented vegetables, Zhang *et al.*⁵⁴ showed that modified-atmosphere fermentation markedly reduced biogenic-amine formation by suppressing amine-producing microorganisms and associated metabolic genes. Commercial kombucha products also differ in the identity and viability of their microbial populations, reinforcing the need for microbiological testing at the end of shelf life rather than relying only on starter composition⁵³.

Other concerns include transferable antimicrobial-resistance genes, virulence determinants, excessive D-lactate production, toxin formation, and microbial instability. Product assessment should therefore focus on matrix-specific hazards, critical processing controls, serving-size exposure, and end-of-shelf-life safety. Traditional use is informative but insufficient, particularly for children, older adults, pregnant women, immunocompromised individuals, and patients with severe gastrointestinal or metabolic disease.

Integrative design of psychobiotic food systems

Psychobiotic food development should follow a product-level sequence: select a characterized strain or microbial preparation, match it with a compatible substrate, optimize fermentation and processing, quantify retained microorganisms and metabolites, exclude matrix-specific hazards, and validate the final formulation after digestion and storage. Recent

evidence demonstrates why this final-product approach is essential: microbial composition changed the effects of kefir⁴⁵, strain performance differed between culture medium and a legume beverage⁴⁸, and cultured milk effects depended on the complete formulation, population, and clinical context⁴⁴.

Analytical, Technological, and Safety Requirements for Psychobiotic Food Development

Strain authentication and microbial identity control

Psychobiotic functionality must be assigned to an authenticated strain rather than inferred from genus or species identity. Preliminary identification may combine phenotypic characterization, 16S rRNA sequencing, MALDI-TOF MS, and strain-specific PCR. For strains intended for health-oriented applications, whole-genome sequencing (WGS) provides higher-resolution authentication and enables simultaneous screening for antimicrobial-resistance genes, virulence determinants, toxin-associated genes, and amino acid decarboxylases linked to biogenic-amine formation⁵⁵.

WGS can also identify genes associated with GABA synthesis, carbohydrate utilization, stress resistance, adhesion, and exopolysaccharide production. However, genomic potential does not demonstrate functional expression in the food matrix. Candidate functions should therefore be confirmed using phenotype- and product-level assays, including metabolite measurements where relevant. Strain identity, genomic safety, and traceability should be established before technological or clinical evaluation.

Viability, stability, and gastrointestinal survival

For live psychobiotic foods, viability should be assessed during processing, storage, distribution, and gastrointestinal exposure. Counts should be expressed as colony-forming units (CFU) per consumed serving and verified at the end of shelf life rather than only immediately after manufacture. No universal CFU threshold establishes psychobiotic efficacy; the delivered dose should instead match the formulation and dose supported by the relevant efficacy study.

Culture-based enumeration remains essential, but strain-specific molecular methods can complement plate counts in multi-strain or complex fermented products. Conventional qPCR should not be interpreted as a direct viability measure because DNA from non-viable cells may also be detected; viability-PCR approaches such as PMA-qPCR can provide complementary information when appropriately validated⁵⁶. Gastrointestinal robustness should be assessed in the final food rather than in culture medium alone because matrix composition strongly modifies survival. Protective technologies such as encapsulation or protein-polysaccharide carriers are useful only when their benefit persists under realistic storage and digestion conditions⁵⁷.

Targeted metabolite profiling

Targeted analysis should focus on compounds directly linked to the proposed mechanism of the product, including GABA, organic acids, selected SCFAs, tryptophan-kynurenine metabolites, indole derivatives, bioactive peptides, phenolic metabolites, and bile acid derivatives. Quantitative methods should report calibration range, authentic or isotope-labeled standards where appropriate, extraction recovery, matrix effects, precision, limit of detection, limit of quantification, and concentration per gram, milliliter, and consumed serving. This is particularly important because analytical detection alone does not establish physiologically relevant exposure. For GABA-enriched foods, validation should include GABA concentration, precursor availability, batch variability, processing and storage stability, and gastrointestinal bioaccessibility. Detection of glutamate-decarboxylase genes or high GABA production in broth cannot substitute for quantification in the final consumed product. Xuan *et al.*⁴⁷, for example, quantified GABA in the baked sourdough product, illustrating the need to assess metabolite retention after processing.

Untargeted metabolomics, peptidomics, and multi-omics integration

Untargeted LC-MS/MS, UHPLC-QTOF-MS, GC-MS, NMR, peptidomics, lipidomics, and volatile profiling are useful when fermentation generates

complex or incompletely characterized chemical changes. Exploratory metabolomics should include procedural blanks, pooled quality-control samples, randomized analytical sequences, drift and batch correction, multiple-testing control, and transparent metabolite-identification confidence. Candidate biomarkers identified by untargeted analysis should subsequently be confirmed using targeted quantitative methods⁵⁸.

Multi-omics integration can connect microbial composition or function with metabolite production and host response. However, microbiome, metabolome, and clinical datasets should be integrated around prespecified biological hypotheses rather than used to generate large numbers of unstructured associations. Independent validation is particularly important when predictive responder signatures are proposed.

In vitro digestion, colonic fermentation, and bioaccessibility

Psychobiotic foods should be evaluated under simulated gastrointestinal conditions because processing and digestion can alter microbial survival, matrix structure, and metabolite release. The INFOGEST framework provides a standardized approach for oral, gastric, and small-intestinal digestion, while semi-dynamic or dynamic models may be selected when gastric emptying, gradual acidification, or time-dependent digestive events are central to the hypothesis^{59,60}.

Model selection should therefore depend on the biological question rather than on analytical complexity alone. INFOGEST is particularly suitable for standardized and reproducible screening of digestion and bioaccessibility, whereas dynamic systems provide greater physiological resolution. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) reproduces sequential gastrointestinal compartments and is particularly useful for investigating longer-term, region-specific microbial responses and colonic fermentation. The TNO gastrointestinal models (TIM-1 and TIM-2) dynamically simulate gastrointestinal transit, secretion, pH changes, and removal of digested products, with TIM-1 primarily representing the stomach and small intestine and TIM-2 focusing on colonic microbial fermentation. The bionic gastrointestinal reactor (BGR) additionally incorporates gastrointestinal geometry, mixing, and mechanically induced contractions to more closely reproduce physical digestion. However, these dynamic systems are more technically demanding, costly, and less suited to high-throughput screening than standardized static models^{59,60}. SHIME is a multicompartment dynamic system designed for sustained microbiota–substrate interaction, while TIM models incorporate dynamic gastrointestinal conditions and, in TIM-2, colonic fermentation; BGR was developed to reproduce gastrointestinal physical features and mechanical mixing more closely.

Fermentation optimization and process reproducibility

Fermentation temperature, pH, duration, inoculum size, oxygen availability, salt concentration, substrate composition, glutamate availability, and starter interactions can influence microbial growth, metabolite production, sensory quality, and safety. Optimization should therefore use multiple response criteria rather than maximizing cell density or a single metabolite. Conditions that increase GABA or biomass, for example, may simultaneously increase acidity, ethanol, off-flavors, or undesirable metabolites.

Post-fermentation processing must also be incorporated into validation. Heating, baking, pasteurization, drying, freezing, thawing, and oxygen exposure may alter cell viability and the retention or accessibility of GABA, peptides, organic acids, and phenolic metabolites. Microbial counts and key metabolites should therefore be reassessed after processing and at the end of storage⁴⁷. Batch-to-batch reproducibility should include predefined specifications for strain abundance, key metabolites, pH, microbial contaminants, safety markers, and relevant sensory characteristics. Variation among commercial fermented products demonstrates that starter identity alone does not guarantee a reproducible final product⁵³.

Safety assessment and undesirable metabolite control

Safety assessment should combine genomic and phenotypic evidence. Candidate strains should be evaluated for antimicrobial susceptibility,

relevant virulence traits, toxin production, hemolytic activity where appropriate, undesirable metabolic activities, and biogenic-amine-forming capacity. The absence of recognized virulence or resistance genes alone is insufficient to establish safety, particularly for novel microorganisms. Genomic findings should be interpreted together with phenotype, intended dose, food matrix, target population, and history of safe use⁵⁵. Matrix-specific hazards should be assessed in the final product. Histamine, tyramine, putrescine, cadaverine, ethanol, toxins, or relevant microbial contaminants should be quantified when their formation is plausible. Safety testing should include end-of-shelf-life samples because undesirable metabolites may continue to accumulate during storage even when production batches initially meet specifications. Acceptance limits should follow applicable regulatory or toxicological criteria for the specific compound, serving size, matrix, and target population rather than arbitrary universal thresholds.

Quality control and evidence standards for psychobiotic claims

A psychobiotic food claim should be supported by the exact final formulation used in the efficacy study rather than extrapolated from an isolated strain or precursor matrix. Minimum product-level evidence should include authenticated microbial identity or defined postbiotic composition, end-of-shelf-life dose, reproducible chemical profile, matrix-specific safety, and gastrointestinal survival or bioaccessibility appropriate to the proposed mechanism. Human trials should define one primary clinical endpoint, use an a priori power calculation, preregister the protocol, and employ an appropriate placebo or matrix-matched comparator. Randomization, allocation concealment, blinding where feasible, intention-to-treat analysis, adverse-event reporting, and documentation of habitual diet, medications, and recent antibiotic exposure should also be considered in study design and reporting^{27,61}. Mechanistic biomarkers should be selected according to the proposed mode of action rather than requiring every study to measure microbiome composition, SCFAs, GABA, cortisol, cytokines, permeability, and metabolomics simultaneously. For example, a GABA-oriented product may prioritize product GABA exposure and neuroendocrine endpoints, whereas a fiber-rich fermented food may prioritize colonic fermentation, SCFAs, inflammatory markers, and microbiome function.

Accordingly, the available evidence can be interpreted across three progressive levels. A candidate psychobiotic food refers to a product containing an authenticated microorganism or preparation with plausible gut–brain-related functionality. A mechanistically validated psychobiotic food represents a reproducible final product for which exposure and relevant biological effects have been experimentally demonstrated. Finally, a clinically validated psychobiotic food is defined as the same standardized formulation that has demonstrated efficacy in adequately powered human clinical trials. The analytical targets, recommended methods, reporting requirements, decision criteria, and principal limitations for each validation stage are summarized in Table 4.

Evidence from preclinical and human studies

Preclinical evidence: mechanistic plausibility but limited food translation

Preclinical models remain valuable for identifying causal microbiota–gut–brain pathways because they permit controlled manipulation of microbial exposure, diet, stress, and host physiology. Animal studies have linked selected psychobiotic strains to changes in HPA-axis activity, inflammatory signaling, tryptophan metabolism, intestinal barrier integrity, brain-derived neurotrophic factor (BDNF), and behavior. For example, *Bifidobacterium breve* CCFM1025 reduced anxiety- and depression-like behaviors in chronically stressed mice while attenuating corticosterone and inflammatory responses and modifying microbial and SCFA-related pathways⁶².

Such experiments are particularly informative when microbiome, metabolite, endocrine, immune, and neural endpoints are measured together. However, behavioral tests in rodents do not reproduce human psychiatric disorders, and differences in microbiota composition, diet, dose

Table 4 | Analytical, technological, and safety requirements for validating psychobiotic food products

Validation level	Recommended methods/tools	Main outputs	Psychobiotic claims	References
Strain identity and traceability	Phenotyping, 16S rRNA sequencing, MALDI-TOF MS, strain-specific PCR/qPCR, WGS	Accurate strain authentication, taxonomy, strain traceability	Prevents generic claims based only on genus/species name; supports reproducibility	1,2
Genome-based safety screening	WGS, AMR gene screening, virulence gene detection, toxin genes, amino acid decarboxylase genes	Safety qualification, absence of transferable AMR/virulence risk	Essential for food-derived isolates and industrial scale-up	10,22
Viability and shelf-life stability	CFU counts, qPCR, live/dead staining, storage testing, stress challenge assays	Viable count during processing, storage, and consumption	Confirms that live psychobiotic delivery is maintained until intake	10,13
Gastrointestinal survival	Acid/bile tolerance, INFOGEST-like digestion, simulated gastric/intestinal fluids	Survival after gastric and intestinal stress	Determines whether live cells can reach the gut environment	10,59
Targeted neuroactive metabolite profiling	HPLC, LC-MS/MS, GC-MS, enzymatic assays	GABA, SCFAs, organic acids, tryptophan metabolites, biogenic amines	Links food composition to gut–brain-relevant mechanisms	14,21
Untargeted metabolomics and peptidomics	UHPLC-QTOF-MS, LC-MS/MS, GC-MS, NMR, peptidomics, volatile profiling	Metabolite fingerprints, peptide profiles, phenolic derivatives, volatiles	Captures matrix-specific transformations and unknown bioactives	13,22
Colonic fermentation and bioaccessibility	INFOGEST digestion followed by fecal fermentation, SHIME/TIM-type models, SCFA profiling	Post-digestion metabolite release, SCFA production, microbial transformations	Determines intestinal exposure and resident microbiota response	15,59
Fermentation process control	pH, water activity, temperature, inoculum size, oxygen, salt, fermentation time, batch profiling	Controlled and reproducible product manufacturing	Prevents uncontrolled variability in microbes and metabolites	14,22
Undesirable metabolite control	HPLC/LC-MS/MS for histamine, tyramine, putrescine, cadaverine; D-lactate assays	Toxicological safety profile	Prevents harmful fermentation outcomes from being marketed as functional	10,22
Host-response validation	Psychological scales, cortisol, cytokines, gut permeability markers, microbiome, metabolomics	Mechanistic and clinical response evidence	Separates validated psychobiotic foods from generic fermented/probiotic products	2,20
Regulatory and claim support	Label accuracy, serving size, strain declaration, stability data, human evidence	Credible and compliant product claims	Prevents overclaiming and supports consumer transparency	20,22

scaling, metabolism, and exposure duration limit direct translation. Pre-clinical evidence should therefore establish mechanistic plausibility rather than clinical efficacy. For food-oriented psychobiotics, an additional translational gap exists because most animal studies administer isolated strains or suspensions rather than the standardized fermented food ultimately intended for human consumption.

Human evidence in depression and depressive symptoms

Human evidence is most developed for depression, although studies differ substantially in diagnosis, strain, dose, treatment background, and outcome measures. A recent systematic review identified 47 publications from 42 clinical studies across psychiatric disorders, with major depressive disorder (MDD) accounting for 19 studies, substantially more than other diagnoses²⁰. Strain-specific randomized trials illustrate both the potential and the limitations of this evidence. Tian et al.¹⁸ randomized 45 patients with MDD to receive *B. breve* CCFM1025 (10¹⁰ CFU/day) or placebo for four weeks. The intervention improved psychiatric and gastrointestinal symptom scores and was associated with changes in gut microbial tryptophan metabolism, providing one of the clearer examples linking a defined strain, clinical response, microbiome function, and a candidate metabolic mechanism. However, the sample was small and the intervention was a freeze-dried supplement rather than a food matrix.

Food-based evidence remains more limited. Sarkawi et al.⁴⁴ evaluated a cultured milk drink containing *Lactobacillus acidophilus* LA-5 and *Lactocaseibacillus paracasei* L. CASEI-01 in 110 participants with irritable bowel syndrome, including individuals with subthreshold depression. Depression scores decreased from baseline in both probiotic and placebo groups, whereas serum serotonin increased significantly only in the probiotic-treated subthreshold-depression group. These results provide product-level evidence but do not establish a clear antidepressant effect independent of placebo response. Recent meta-analysis supports cautious optimism. Asad et al.⁶³ included 23 randomized trials involving 1401 clinically diagnosed participants and found significant reductions in depressive symptoms with probiotics, whereas evidence for prebiotics was weaker. Nevertheless, substantial heterogeneity in strains, duration, diagnosis, and formulation limits generalization of a pooled “probiotic effect” to individual psychobiotic products. Accordingly, current evidence supports selected probiotics as potentially useful adjunctive interventions, but it does not justify treating psychobiotics as substitutes for established psychological or pharmacological therapies.

Human evidence in anxiety and stress

Evidence for anxiety and stress is promising but more heterogeneous than for depression. Asad et al.⁶³ reported a moderate pooled reduction in anxiety symptoms with probiotic interventions, although formulation, intervention duration, and clinical population contributed substantially to heterogeneity. Whole-diet interventions are particularly relevant to food-based psychobiotics. Berding et al.¹⁷ randomized 45 healthy adults to a four-week diet rich in prebiotic foods and fermented foods or a control diet. Perceived stress decreased by 32% in the psychobiotic-diet group versus 17% in controls, and greater dietary adherence was associated with a stronger stress reduction. However, the between-group difference was not significant and biological stress markers were unchanged.

This trial is important because it extends psychobiotic research beyond capsules, but it also illustrates a central interpretive problem: changes in perceived stress, microbiome stability, and metabolic profiles do not necessarily demonstrate that one specific fermented food or microbial strain caused the psychological response. Thus, future stress trials should distinguish strain-defined probiotic effects, whole-diet effects, and fermented-food effects, while controlling for baseline diet, stress severity, medication, and adherence.

Human evidence in sleep, cognition, and fatigue

Evidence for sleep, cognition, and fatigue is emerging but remains outcome-specific. Moloney et al.⁴¹ reported that *Bifidobacterium longum*

1714 improved sleep quality and sleep duration during examination stress in 20 healthy male students, but did not improve chronic stress, depression, working memory, visual memory, attention, or perception. This finding is important because it demonstrates that benefit in one gut–brain-related domain should not be extrapolated to other psychological or cognitive outcomes.

In a larger randomized trial of 89 adults with impaired sleep, Patterson et al.⁶⁴ found that *B. longum* 1714 accelerated improvement in selected subjective sleep-quality components and daytime functioning, although the primary global PSQI score improved in both groups and objective actigraphy outcomes did not differ significantly. Similarly, Ho et al.³⁹ reported reductions in depressive symptoms, fatigue, awakenings during deep sleep, and selected electrophysiological measures after 30 days of *Lactiplantibacillus plantarum* PS128 in 40 adults with self-reported insomnia. However, the small sample size and pilot design limit generalizability. These studies show that sleep may represent a responsive psychobiotic endpoint, but objective sleep measures and cognition remain less consistently affected than subjective outcomes.

Evidence for fermented foods specifically

Direct evidence for fermented foods as clinically validated psychobiotic interventions remains substantially weaker than evidence for strain-defined probiotic supplements. Fermented foods may simultaneously provide live microorganisms, microbial cell components, organic acids, peptides, phenolic metabolites, vitamins, and fermentable substrates, making attribution of an effect to one component difficult.

Wastyk et al.¹⁶ showed in a 17-week randomized dietary study that a high-fermented-food diet increased gut microbial diversity and reduced several inflammatory markers in healthy adults. However, mental-health outcomes were not the primary focus; the study therefore supports a fermented-food–microbiome–immune relationship rather than direct psychobiotic efficacy. The psychobiotic-diet study of Berding et al.¹⁷ provides more direct stress-related evidence, but fermented foods were consumed together with increased prebiotic and fiber-rich foods, preventing attribution to fermentation alone. Sarkawi et al.⁴⁴ provides a more product-specific example because a defined cultured milk drink was administered, although improvements in depressive scores were not uniquely observed in the probiotic group.

Why clinical results remain inconsistent

Clinical heterogeneity can be reduced to three major sources: intervention heterogeneity, participant heterogeneity, and endpoint heterogeneity. Interventions vary in strain, dose, number of strains, matrix, viability, treatment duration, and concomitant substrates. Participants differ in diagnosis, symptom severity, baseline microbiota, habitual diet, age, sex, medication exposure, and metabolic or gastrointestinal comorbidities. Outcomes range from clinician-rated depression to self-reported stress, sleep questionnaires, cognition, inflammatory biomarkers, and metabolomics.

These sources of variation are reflected in meta-analyses. Asad et al.⁶³ reported substantial heterogeneity despite significant pooled effects for depression and anxiety, whereas Ribera et al.²⁰ showed that evidence remains unevenly distributed across psychiatric diagnoses. Pooling different strains, formulations, populations, and endpoints may therefore generate an average effect that does not accurately predict the response to a particular psychobiotic product. A further limitation is that clinical improvement and biological mechanism are often measured separately. Future trials should therefore prioritize a prespecified clinical endpoint together with a focused biomarker panel capable of testing the proposed mechanism, rather than measuring numerous unrelated biomarkers.

Future evidence standards for psychobiotic food trials

Future research should move from efficacy-only trials toward product-specific causal validation. Priority should be given to standardized food interventions for which the exact strain or microbial preparation, matrix,

serving size, processing conditions, shelf-life composition, and metabolite exposure are known. Clinical trials should use one prespecified primary mental-health endpoint, adequate statistical power, matrix-matched controls, dietary and medication monitoring, and a mechanistically relevant biomarker panel. The next major evidence gap is not whether “probiotics” or “fermented foods” can influence mental-health-related outcomes in general, but which defined strain–matrix combinations work, in which populations, at what dose, through which mechanism, and with what reproducibility. Independent replication of the same final product should therefore be considered a critical step before classification as a clinically validated psychobiotic food. Table 5 should consequently compare actual interventions according to strain/formulation, food matrix, population, sample size, dose, duration, clinical outcome, mechanistic evidence, and major limitation rather than simply listing reported benefits.

Challenges, future perspectives, and conclusion

Main challenges

Despite rapid progress, psychobiotic food research remains constrained by insufficient integration of product characterization, mechanistic evidence, and clinical efficacy. Many interventions are still described broadly as probiotic, fermented, or microbiota-modulating despite incomplete strain-level authentication, product standardization, or mechanistic validation^{1,2,22}. Psychobiotic efficacy should therefore be considered product- and host-dependent rather than an inherent property of a microbial species or fermented food, because strain identity, matrix composition, processing, microbial metabolism, and host characteristics can modify biological responses^{2,13,21}.

A second challenge is the limited standardization of fermented-food interventions. Microbial composition and metabolite profiles may vary with raw materials, starter cultures, spontaneous fermentation, back-slopping, processing, storage, and serving size^{13,22}. Fermented foods may also contain variable combinations of live and non-viable microorganisms, enzymes, organic acids, peptides, phenolic metabolites, vitamins, and fermentable substrates, further complicating exposure assessment^{13,22}. Consequently, apparently similar products may provide substantially different microbial and biochemical exposures, limiting comparison across trials and reproducibility of health effects^{11,22}. The final consumed product, rather than the strain or fermentation process alone, should therefore constitute the unit of validation².

Clinical heterogeneity represents an additional barrier. Evidence is concentrated mainly in depression and related affective outcomes, whereas evidence for other psychiatric, sleep, and cognitive domains remains less mature^{20,63,65}. Trials also differ considerably in strain composition, dose, intervention duration, participant phenotype, medication exposure, habitual diet, and outcome measures^{20,63}. Accordingly, pooled evidence for “probiotics” or “psychobiotics” cannot automatically predict the efficacy of an individual strain, formulation, or food product^{2,63}.

A persistent translational gap also separates food chemistry from clinical interpretation. Fermentation studies frequently identify promising strains or neuroactive metabolites without establishing human mental-health relevance, whereas clinical studies may report psychological outcomes without adequately characterizing microbial exposure, product chemistry, or the biological pathway responsible^{13,21}. For example, whole-diet interventions have demonstrated changes in perceived stress, microbial ecology, or immune markers, but attribution to a specific microorganism or fermented product remains difficult^{16,17}. Current evidence therefore supports selected strain-defined supplements more strongly than clinically validated psychobiotic foods^{20,22}.

Future perspectives

Future psychobiotic development should prioritize product-specific optimization of microbial strain or preparation, food matrix, processing conditions, metabolite exposure, safety profile, and intended clinical target^{2,22}. The most promising formulation will not necessarily be that with the highest viable count or concentration of a single metabolite, but one that provides a

Table 5 | Clinical evidence, endpoints, mechanistic biomarkers, and evidence gaps for psychobiotic interventions

Evidence domain	Current evidence strength	Main reported findings	Recommended clinical endpoints	Mechanistic biomarkers to include	Main limitations	References
Depression/depressive symptoms	Moderate	Most consistent human evidence; probiotics show adjunctive benefit in several analyses	PHQ-9, BDI, HAM-D, MADRS, quality-of-life scales	SCFAs, tryptophan/kyurenine ratio, cytokines, cortisol, microbiome profile	Heterogeneous strains, small trials, limited biomarker integration	20,63,65
Anxiety	Promising but heterogeneous	Probiotics and synbiotics show beneficial signals; prebiotic-only evidence weaker	GAD-7, STAI, HAM-A, anxiety subscales	Cortisol, inflammatory cytokines, SCFAs, GABA-related metabolites, gut permeability	Variable diagnoses, symptom severity, intervention duration, and scales	63,65
Stress/stress resilience	Emerging	Psychobiotic diet and microbiota-targeted approaches suggest potential stress reduction	PSS, perceived stress scales, stress reactivity tests	Cortisol rhythm, SCFAs, microbiome function, metabolomics, cytokines	Few diet-based trials; limited between-group effects in some studies	2,17
Sleep quality	Emerging	Some signals for sleep improvement, often as secondary outcomes	PSQI, ISI, actigraphy, polysomnography where feasible	Cortisol rhythm, inflammatory markers, tryptophan metabolites, microbiome changes	Few dedicated sleep trials; inconsistent measures	20,65
Cognition	Emerging/population-specific	Possible benefits in selected populations, but evidence remains limited	MoCA, memory tests, executive-function batteries, attention tasks	SCFAs, inflammatory markers, tryptophan/kyurenine metabolites, neurotrophic markers	Often secondary endpoint; limited mechanistic confirmation	2,20
Fermented foods specifically	Underdeveloped	Strong mechanistic rationale, but fewer standardized product-specific trials	Mood, stress, sleep, cognition, quality of life	Microbial profile, metabolite fingerprint, SCFAs, GABA, phenolic metabolites, safety markers	Poor batch standardization, weak serving-size control, few direct trials	13,20,22
Mechanistic validation	Weak to moderate	SCFAs, inflammation, tryptophan metabolism, barrier function are plausible mechanisms	Clinical scores plus mechanistic endpoints	Microbiome, metabolomics, cytokines, cortisol, permeability markers, strain persistence	Often missing from clinical trials	2,21
Precision/responder analysis	Early but important	Response likely depends on baseline microbiome, diet, medication, age, sex, and health status	Stratified clinical outcomes, responder/non-responder analysis	Baseline microbiome, diet records, metabolomics, medication profile	Rarely included; limits reproducibility	2,65

reproducible and physiologically relevant exposure associated with measurable biological and clinical responses^{2,21}. Multi-omics approaches may accelerate this transition by connecting microbial identity and function with food-derived metabolites and host responses^{11,21}. Whole-genome sequencing can support strain authentication and functional prediction, whereas metabolomics, peptidomics, and complementary omics approaches can identify matrix-dependent biochemical transformations¹¹. However, these datasets should be used to test prespecified mechanistic hypotheses rather than generate large numbers of exploratory associations. Analytical quality control, independent validation, and replication across batches and populations are essential before molecular signatures can support product claims⁵⁸.

Artificial intelligence (AI) may further accelerate psychobiotic discovery and fermentation design. Machine-learning approaches are increasingly being explored for probiotic-trait prediction, microbial resource screening, metabolic-pathway prediction, starter-culture selection, and optimization of fermentation conditions^{66,67}. AI-supported fermentation systems may also facilitate strain–substrate matching, process monitoring, sensory prediction, and optimization of bioactive-metabolite production^{68,69}. However, their translational value will depend on standardized training datasets, algorithmic interpretability, external validation, and prospective experimental confirmation; computational prediction should guide rather than replace biological and clinical validation^{66,68}. Precision psychobiotics also require moving beyond retrospective responder analyses. Variability in baseline microbiota, habitual diet, medication exposure, age, sex, and host physiology may modify microbiota-targeted responses^{2,70}. Baseline microbial composition may influence substrate utilization, cross-feeding, colonization resistance, and metabolite production, thereby producing heterogeneous responses to the same dietary intervention¹⁶. Medication exposure is another important source of variation because psychotropic and non-psychotropic drugs may alter microbial community structure and confound associations between microbiota composition and psychiatric phenotype⁷¹.

Future precision approaches should therefore evaluate prospectively defined predictors of response rather than relying solely on post hoc responder/non-responder classification^{2,70}. A microbiome or metabolomic biomarker becomes clinically meaningful only when it reproducibly improves intervention selection or outcome prediction in an independent population.

Future trials should consequently prioritize standardized food-based interventions, adequately powered designs, matrix-matched controls, pre-specified primary endpoints, focused mechanistic biomarker panels, dietary and medication monitoring, and independent replication of the same final formulation^{2,27,61}. Head-to-head studies delivering the same strain through different food matrices would be particularly informative for determining whether food structure and processing materially alter gastrointestinal exposure and psychobiotic efficacy^{13,22}.

Conclusion

Psychobiotic foods represent a promising interface between food fermentation, microbial ecology, nutrition, and microbiota–gut–brain research. Current evidence indicates that selected microbiota-targeted interventions may improve depression, anxiety, stress, and sleep-related outcomes, although effects remain highly dependent on the strain, product, population, and measured endpoint. Importantly, the evidence base is currently stronger for defined probiotic strains and supplement-type formulations than for standardized fermented foods. Fermentation, the presence of a probiotic microorganism, or detection of a neuroactive metabolite alone is therefore insufficient to justify a psychobiotic claim.

A practical industry-oriented validation framework should include five sequential requirements: (i) authenticated microbial identity and genomic safety; (ii) standardized final-product composition, processing conditions, serving dose, and shelf-life stability; (iii) reproducible microbial or metabolite exposure after gastrointestinal digestion; (iv) mechanistic evidence linking the product to a defined

microbiota–gut–brain pathway; and (v) clinically meaningful efficacy and safety demonstrated for the same final formulation in adequately powered human trials. Products that satisfy only technological or mechanistic criteria should be considered candidate psychobiotic foods, whereas a clinically validated psychobiotic claim should require reproducible evidence from the standardized consumed product.

Over the next five years, research should address four priority questions: which strain–matrix combinations provide reproducible benefits; what dose and intervention duration are required for clinically meaningful effects; which microbial, metabolic, or host biomarkers reliably predict response; and whether the same standardized product can reproduce efficacy across independent populations and production batches. Head-to-head matrix comparisons, prospective responder-stratification studies, and focused microbiome–metabolome–clinical integration will be particularly important. Finally, psychobiotic foods should currently be positioned as complementary or adjunctive dietary strategies rather than replacements for established psychiatric, psychological, or pharmacological treatments. Their future clinical value will depend on demonstrating added benefit, safety, mechanistic plausibility, and reproducibility alongside standard care. The central translational challenge is no longer simply to show that the microbiota–gut–brain axis can be modulated, but to determine which standardized psychobiotic foods work, for whom, at what dose, through which mechanism, and with what degree of reproducibility.

Data availability

No datasets were generated or analyzed during the current study.

Code availability

No custom code or software was developed or used for the preparation of this review article.

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References

1. Binda, S. et al. Psychobiotics and the microbiota–gut–brain axis: where do we go from here? *Microorganisms* **12**, 634 (2024).
2. Slykerman, R. F. et al. Precision psychobiotics for gut–brain axis health: advancing the discovery pipelines to deliver mechanistic pathways and proven health efficacy. *Microb. Biotechnol.* **18**, e70079 (2025).
3. Hill, C. et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 506–514 (2014).
4. Dinan, T. G., Stanton, C. & Cryan, J. F. Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry* **74**, 720–726 (2013).
5. Gibson, G. R. et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 491–502 (2017).
6. Swanson, K. S. et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 687–701 (2020).
7. Salminen, S. et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 649–667 (2021).
8. Dahmouni, S. et al. Postbiotics and paraprobiotics in food biochemistry mechanisms stability and nutritional applications. *npj Sci. Food* **10**, 242 (2026).
9. Marco, M. L. et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 196–208 (2021).

10. Cichońska, P., Kowalska, E. & Ziarno, M. The survival of psychobiotics in fermented food and the gastrointestinal tract: a review. *Microorganisms* **11**, 996 (2023).
11. Walsh, A. M. et al. Integrated molecular approaches for fermented food microbiome research. *FEMS Microbiol. Rev.* **47**, fuad001 (2023).
12. Bentahar, M. C. et al. Evaluation of probiotic potential and functional properties of *Lactobacillus* strains isolated from Dhan, Traditional Algerian Goat Milk Butter. *Foods* **13**, 3781 (2024).
13. Balasubramanian, R., Schneider, E., Gunnigle, E., Cotter, P. D. & Cryan, J. F. Fermented foods: harnessing their potential to modulate the microbiota-gut-brain axis for mental health. *Neurosci. Biobehav. Rev.* **158**, 105562 (2024).
14. Icer, M. A. et al. Contributions of gamma-aminobutyric acid (GABA) produced by lactic acid bacteria on food quality and human health: current applications and future prospects. *Foods* **13**, 2437 (2024).
15. Schneider, E. et al. Fibre & fermented foods: differential effects on the microbiota-gut-brain axis. *Proc. Nutr. Soc.* **84**, 365–380 (2025).
16. Wastyk, H. C. et al. Gut-microbiota-targeted diets modulate human immune status. *Cell* **184**, 4137–4153 (2021).
17. Berding, K. et al. Feed your microbes to deal with stress: a psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population. *Mol. Psychiatry* **28**, 601–610 (2023).
18. Tian, P. et al. *Bifidobacterium breve* CCFM1025 attenuates major depression disorder via regulating gut microbiome and tryptophan metabolism: a randomized clinical trial. *Brain. Behav. Immun.* **100**, 233–241 (2022).
19. Kelly, J. R. et al. Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain. Behav. Immun.* **61**, 50–59 (2017).
20. Ribera, C. et al. Probiotic, prebiotic, synbiotic and fermented food supplementation in psychiatric disorders: a systematic review of clinical trials. *Neurosci. Biobehav. Rev.* **158**, 105561 (2024).
21. Kyei-Baffour, V. O., Vijaya, A. K., Burokas, A. & Daliri, E. B.-M. Psychobiotics and the gut-brain axis: advances in metabolite quantification and their implications for mental health. *Crit. Rev. Food Sci. Nutr.* **65**, 7085–7104 (2025).
22. Caffrey, E. B. et al. Unpacking food fermentation: clinically relevant tools for fermented food identification and consumption. *Adv. Nutr.* **16**, 100412 (2025).
23. Qadi, W. S. M. et al. Characterization of physicochemical, biological, and chemical changes associated with coconut milk fermentation and correlation revealed by ¹H NMR-based metabolomics. *Foods* **12**, 1971 (2023).
24. Liu, W., Li, H., Liu, L., Ko, K. & Kim, I. Screening of gamma-aminobutyric acid-producing lactic acid bacteria and the characteristic of glutamate decarboxylase from *Levilactobacillus brevis* F109-MD3 isolated from kimchi. *J. Appl. Microbiol.* **132**, 1967–1977 (2022).
25. EFSA. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA J.* **19**, e06506 (2021).
26. Percie du Sert, N. et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **18**, e3000411 (2020).
27. Hopewell, S. et al. CONSORT 2025 statement: updated guideline for reporting randomised trials. *Lancet* **405**, 1633–1640 (2025).
28. Landis, E. A. et al. The diversity and function of sourdough starter microbiomes. *Elife* **10**, e61644 (2021).
29. Liao, T. et al. Nature of back slopping kombucha fermentation process: insights from the microbial succession, metabolites composition changes and their correlations. *Front. Microbiol.* **15**, 1433127 (2024).
30. Devi, P. B. et al. Gamma-aminobutyric acid (GABA) production by potential probiotic strains of indigenous fermented foods origin and RSM based production optimization. *Lwt* **176**, 114511 (2023).
31. Uriho, A. et al. Screening of LAB strains and their co-culture fermentation with *Bacillus subtilis* of Cili fruit substrate: impact on γ -aminobutyric acid enrichment, key enzyme activities, bioactive and functional properties. *Front. Nutr.* **12**, 1622745 (2025).
32. Pawul-Gruba, M., Denis, E., Kiljanek, T. & Osek, J. Prevalence of biogenic amines and their relation to the bacterial content in ripened cheeses on the retail market in Poland. *Foods* **14**, 2478 (2025).
33. Jiang, M. et al. Mechanisms of microbiota-gut-brain axis communication in anxiety disorders. *Front. Neurosci.* **18**, 1501134 (2024).
34. Xu, J. & Lu, Y. The microbiota-gut-brain axis and central nervous system diseases: From mechanisms of pathogenesis to therapeutic strategies. *Front. Microbiol.* **16**, 1583562 (2025).
35. O’Riordan, K. J., Moloney, G. M., Keane, L., Clarke, G. & Cryan, J. F. The gut microbiota-immune-brain axis: Therapeutic implications. *Cell Rep. Med.* **6**, 101982 (2025).
36. Xia, H.-H. & Huang, J.-Z. Tryptophan metabolism at the crossroads of the neuro-immuno-microbial axis: implications for precision medicine in chronic diseases. *Front. Cell. Infect. Microbiol.* **15**, 1707850 (2025).
37. Zhang, J. et al. Molecular mechanisms and therapeutic potential of tryptophan metabolism in gut-brain signaling transduction: a narrative review. *J. Neuroinflamm.* **23**, 131 (2026).
38. Loh, J. S. et al. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct. Target. Ther.* **9**, 37 (2024).
39. Ho, Y.-T., Tsai, Y.-C., Kuo, T. B. J. & Yang, C. C. H. Effects of *Lactobacillus plantarum* PS128 on depressive symptoms and sleep quality in self-reported insomniacs: a randomized, double-blind, placebo-controlled pilot trial. *Nutrients* **13**, 2820 (2021).
40. Lin, S.-K. K., Kuo, P.-H., Hsu, C.-Y., Chiu, Y.-H. & Chen, C.-H. The effects of *Lactobacillus plantarum* PS128 in patients with major depressive disorder: an eight-week double-blind, placebo-controlled study. *Asian J. Psychiatr.* **101**, 104210 (2024).
41. Moloney, G. M. et al. Improvements in sleep indices during exam stress due to consumption of a *Bifidobacterium longum*. *Brain. Behav. Immun-Health* **10**, 100174 (2021).
42. Walden, K. E. et al. A randomized controlled trial to examine the impact of a multi-strain probiotic on self-reported indicators of depression, anxiety, mood, and associated biomarkers. *Front. Nutr.* **10**, 1219313 (2023).
43. Javaid, A. et al. Prevalence and stable acquisition of biogenic amine-synthesizing genes in lactic acid bacteria across diverse niches: implications for food safety and human health. *npj Sci. Food* **9**, 268 (2025).
44. Sarkawi, M., Raja Ali, R. A., Abdul Wahab, N., Abdul Rath, N. D. & Mokhtar, N. M. A randomized, double-blinded, placebo-controlled clinical trial on *Lactobacillus*-containing cultured milk drink as adjuvant therapy for depression in irritable bowel syndrome. *Sci. Rep.* **14**, 9478 (2024).
45. Bourrie, B. C. T. et al. Consumption of kefir made with traditional microorganisms resulted in greater improvements in LDL cholesterol and plasma markers of inflammation in males when compared to a commercial kefir: a randomized pilot study. *Appl. Physiol. Nutr. Metab.* **48**, 668–677 (2023).
46. Buňková, L. et al. Biogenic amines in white brined cheeses. *Foods* **14**, 369 (2025).
47. Xuan, J. et al. Production of γ -aminobutyric acid-enriched sourdough bread using an isolated *Pediococcus pentosaceus* strain JC30. *Heliyon* **10**, (2024).
48. Montagano, F. et al. Enrichment of γ -aminobutyric acid (GABA) in a legume-based beverage through the fermentation by *Lactiplantibacillus plantarum*. *Curr. Res. Food Sci.* **11**, 101176 (2025).
49. Purwandari, F. A., Fogliano, V. & Capuano, E. Tempeh fermentation improves the nutritional and functional characteristics of Jack beans (*Canavalia ensiformis* (L.) DC). *Food Funct* **15**, 3680–3691 (2024).

50. Mefleh, M. et al. Enhancing nutritional and sensory properties of plant-based beverages: A study on chickpea and Kamut® flours fermentation using *Lactococcus lactis*. *Front. Nutr.* **11**, 1269154 (2024).
51. Galena, A. E. et al. The effects of fermented vegetable consumption on the composition of the intestinal microbiota and levels of inflammatory markers in women: a pilot and feasibility study. *PLoS One* **17**, e0275275 (2022).
52. Zhang, J. et al. The impact of *Levilactobacillus brevis* YSJ3 and *Lactiplantibacillus plantarum* JLSC2-6 co-culture on gamma-aminobutyric acid yield, volatile and non-volatile metabolites, antioxidant activity, and bacterial community in fermented cauliflower byproducts. *Food Chem.* **432**, 137169 (2024).
53. O'Sullivan, E. N. & O'Sullivan, D. J. Viability and diversity of the microbial cultures available in retail kombucha beverages in the USA. *Foods* **13**, 1707 (2024).
54. Zhang, C. et al. Reduced formation of biogenic amines in low-salt Zhacai via fermentation under CO₂-modified atmosphere. *Food Res. Int.* **163**, 112256 (2023).
55. Deng, L. et al. Genome sequence and evaluation of safety and probiotic potential of *Lactiplantibacillus plantarum* LPJZ-658. *Microorganisms* **11**, 1620 (2023).
56. Guo, L. et al. Identification and quantification of viable *Lactocaseibacillus rhamnosus* in probiotics using validated PMA-qPCR method. *Front. Microbiol.* **15**, 1341884 (2024).
57. Treven, P., Paveljšek, D., Bogovič Matijašić, B. & Mohar Lorbec, P. The effect of food matrix taken with probiotics on the survival of commercial probiotics in simulation of gastrointestinal digestion. *Foods* **13**, 3135 (2024).
58. Mosley, J. D. et al. Establishing a framework for best practices for quality assurance and quality control in untargeted metabolomics. *Metabolomics* **20**, 20 (2024).
59. Brodkorb, A. et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat. Protoc.* **14**, 991–1014 (2019).
60. Ménard, O. et al. Static in vitro digestion model adapted to the general older adult population: an INFOGEST international consensus. *Food Funct.* **14**, 4569–4582 (2023).
61. Whelan, K. et al. Design and reporting of prebiotic and probiotic clinical trials in the context of diet and the gut microbiome. *Nat. Microbiol.* **9**, 2785–2794 (2024).
62. Tian, P. et al. Towards a psychobiotic therapy for depression: *Bifidobacterium breve* CCFM1025 reverses chronic stress-induced depressive symptoms and gut microbial abnormalities in mice. *Neurobiol. Stress* **12**, 100216 (2020).
63. Asad, A., Kirk, M., Zhu, S., Dong, X. & Gao, M. Effects of prebiotics and probiotics on symptoms of depression and anxiety in clinically diagnosed samples: systematic review and meta-analysis of randomized controlled trials. *Nutr. Rev.* **83**, e1504–e1520 (2025).
64. Patterson, E. et al. *Bifidobacterium longum* 1714 improves sleep quality and aspects of well-being in healthy adults: a randomized, double-blind, placebo-controlled clinical trial. *Sci. Rep.* **14**, 3725 (2024).
65. Lian, J., Yu, W., Feng, G. & Miao, D. Pre-/pro and synbiotics on anxiety and depression symptoms: a GRADE assessed systematic review and meta-analysis of randomized controlled trials. *Ann. Gen. Psychiatry* <https://doi.org/10.1186/s12991-026-00661-6> (2026).
66. Jo, D.-M. et al. Artificial intelligence-driven strategies to enhance the application of lactic acid bacteria as functional probiotics: Health promotion and optimization for industrial applications. *Trends Food Sci. Technol.* **165**, 105309 (2025).
67. Shi, P. et al. AI-driven exploration of microbial resources in fermented foods. *Trends Food Sci. Technol.* **167**, 105450 (2025).
68. Priyadarshini, D., Muthuvel, I., Saraswathy, S., Kavitha, P. S. & Jegadeeswari, V. Precision to plate: AI-driven innovations in fermentation and hyper-personalized diets. *Front. Nutr.* **12**, 1659511 (2025).
69. Yee, C. S. et al. Smart fermentation technologies: microbial process control in traditional fermented foods. *Fermentation* **11**, 323 (2025).
70. Rosas-Sánchez, G. U., Muñoz-Carrillo, J. L. & Soria-Fregozo, C. Transforming mental health: the future of personalized psychobiotics in anxiety and depression therapy. *Front. Neurosci.* **19**, 1711846 (2025).
71. Dilmore, A. H. et al. Medication use is associated with distinct microbial features in anxiety and depression. *Mol. Psychiatry* **30**, 2545–2557 (2025).
72. Zhang, J., Zhu, L., Meng, Q., Wang, Z. & Zhu, H. The efficacy of probiotics, prebiotics, and synbiotics on anxiety, depression, and sleep: a systematic review and meta-analysis of randomized controlled trials. *BMC Psychiatry* **25**, 1199 (2025).
73. Theriraja, T. S. H. et al. Engineering gut–brain therapeutics: advances in psychobiotics and postbiotic technologies. *Food Humanit.* **6**, 101119 (2026).
74. Park, I. & Mannaa, M. Fermented foods as functional systems: microbial communities and metabolites influencing gut health and systemic outcomes. *Foods* **14**, 2292 (2025).
75. Shawky, E., Surendran, S. & El-Khair, R. M. A. Fermented vegetables as a source of psychobiotics: a review of the evidence for mental health benefits. *Probiotics Antimicrob. Proteins* 1–15, <https://doi.org/10.1007/s12602-025-10592-5> (2025).
76. Jena, R. & Choudhury, P. K. Lactic acid bacteria in fermented dairy foods: gamma-aminobutyric acid (GABA) production and its therapeutic implications. *Food Biosci.* **62**, 105276 (2024).

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Competing interests

The authors declare no competing interests.

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