Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Molecular docking of GAD to gut pathogen targets: New prospects for probiotic therapy in post-traumatic stress disorder

N. V. Boyko, N. I. Korol, O. V. Pallah, I. M. Lypei, L. S. Yusko

Uzhhorod National University, Uzhhorod, Ukraine

Article info

Received 27.07.2025
Received in revised form 30.08.2025
Accepted 22.09.2025

Uzhhorod National University, Pidhirna st., 46, Uzhhorod, 88000, Ukraine.
Tel.: +38-095-131-26-14.
E-mail: Oleksandra.pallah@uzhnu.edu.ua

Boyko, N. V., Korol, N. I., Pallah, O. V., Lypei, I. M., & Yusko, L. S. (2025). Molecular docking of GAD to gut pathogen targets: New prospects for probiotic therapy in post-traumatic stress disorder. Regulatory Mechanisms in Biosystems, 16(4), e25166. doi:10.15421/0225166

The human gut microbiome is increasingly understood as a central regulator of brain function through the complex bidirectional system known as the gut-brain axis. One of the most influential microbial metabolites involved in this communication is gamma-aminobutyric acid, a neurotransmitter that plays a critical role in regulating anxiety and stress responses. Probiotics that are capable of producing gamma-aminobutyric acid, also called psychobiotics, are now regarded as promising candidates for innovative therapeutic approaches in trauma-related psychiatric conditions, including post-traumatic stress disorder. The findings of this study reveal that probiotic-derived glutamate decarboxylase, the enzyme responsible for gamma-aminobutyric acid synthesis, exhibits selective and high-affinity interactions with enzymes originating from pathogenic microorganisms in the gut. Strong binding was observed with dihydrofolate reductase and beta-lactamases, which are essential for pathogen survival and resistance. These interactions were stabilized through salt bridges, hydrogen bonds, and aromatic stacking involving conserved residues such as glutamate-25, arginine-42, and phenylalanine-20. In contrast, the interactions with enzymes from commensal gut bacteria were weak, transient, and non-inhibitory, suggesting that the probiotic enzyme selectively targets pathogens while sparing beneficial microbial species. In addition, probiotic strains capable of producing glutamate decarboxylase demonstrated pronounced antagonism against members of the Enterobacteriaceae family, a group strongly associated with microbial imbalance and psychiatric comorbidities. This dual effect – direct antimicrobial activity together with enhancement of gamma-aminobutyric acid availability in the gut – points to an important role for probiotic glutamate decarboxylase in both microbiota restoration and modulation of neuroimmune communication. Taken together, these results highlight the therapeutic promise of gamma-aminobutyric acid-producing probiotics. Their ability to simultaneously suppress pathogenic bacteria and increase neuroactive metabolite levels, without disrupting commensal populations, underscores their potential as safe and effective psychobiotic interventions. Future investigations should focus on confirming these effects in vivo and on translating them into clinical strategies for trauma-related mental health disorders.

Keywords: psychobiotics; neuroimmune modulation; microbial interactions; dysbiosis; trauma-related stress.

Introduction

The human gut microbiome comprises trillions of microorganisms – including bacteria, viruses, fungi, and archaea – that reside in the gastrointestinal tract and play essential roles in maintaining host health. Traditionally, these microbes have been recognized for their contributions to digestion, nutrient metabolism, and immune system regulation. However, recent research has significantly expanded this understanding, revealing a dynamic bidirectional relationship between the gut and the brain, commonly referred to as the gut-brain axis (GBA) (Ke et al., 2023). A growing body of evidence links gut microbiota composition with mental health outcomes. Probiotic supplementation, in particular, has demonstrated efficacy in reducing symptoms of anxiety and depression. Specific strains such as *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Lactobacillus helveticus* have been shown to modulate cortisol levels, reduce systemic inflammation, and enhance the production of key neurotransmitters such as gamma-aminobutyric acid (GABA) (Koh et al., 2016). These strains influence not only neurochemical production but also the expression of brain-derived neurotrophic factor (BDNF), a protein critical for neuroplasticity, learning, and memory, which is often dysregulated in PTSD and depression (Strandwitz, 2018).

Post-traumatic stress disorder (PTSD) is a severe psychiatric condition characterized by intrusive memories, hyperarousal, and emotional numbing following trauma exposure. Recent findings suggest that gut dysbiosis – particularly reductions in beneficial bacteria and increases in pro-inflammatory taxa – may contribute to the pathophysiology of PTSD by influencing immune responses, systemic inflammation, and hypothalamic-pituitary-adrenal (HPA) axis regulation (Bravo et al., 2011; Braga et al., 2024). Chronic stress can compromise gut barrier integrity, increasing intestinal permeability ("leaky gut"), which in turn promotes endotoxin translocation and systemic inflammation – a pathway linked to mood disorders and PTSD symptom severity (Sarkar et al., 2016).

Recent metagenomic studies have shown that patients with PTSD exhibit reduced microbial diversity and an increased abundance of pathobiont genera such as *Alistipes*, *Oscillibacter*, *Turicibacter*, and *Desulfovibrio*, which are associated with a pro-inflammatory environment and elevated levels of cytokines, particularly interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (Hattori et al., 2020; Gill et al., 2021). In a metagenomic sequencing study involving California veterans with PTSD, a notable reduction in *Faecalibacterium* and *Ruminococcus* genera was observed – both of which are major producers of butyrate, a short-chain fatty acid (SCFA) essential for maintaining intestinal barrier integrity (Cryan et al., 2019).

Chronic stress may impair intestinal barrier function by increasing mucosal permeability ("leaky gut"), thereby promoting endotoxin translocation and systemic inflammation. This mechanism has been demonstrated in mouse models of PTSD, where elevated levels of lipopolysaccharide-binding protein (LBP) and inflammatory markers were found in plasma, along with a microbiota shift toward pro-inflammatory taxa (Bharwani et al., 2016).

There is also a documented link between PTSD-related dysbiosis and disrupted serotonin metabolism: a decrease in *Bifidobacterium* and *Lactobacillus* populations reduces tryptophan availability for serotonin synthesis, while dominant Clostridiales taxa are associated with reactivated xenobiotic metabolic pathways (Kelly et al., 2016).

Furthermore, patients with PTSD have been found to exhibit reduced levels of enzymes and microbial metabolites involved in GABA synthesis, including glutamate decarboxylase (GAD), as well as diminished vagus nerve stimulation , which is a critical pathway for gut-to-brain signaling (Bravo et al., 2011; Kozakov et al., 2017).

Specifically, stress-induced alterations in the gut microbiota are associated with decreased levels of *Lactobacillus* and increased populations of *Oscillibacter* and *Alistipes*, which promote immune activation and the release of pro-inflammatory cytokines such as interleukin-6 (IL-6). These changes are linked to reduced synthesis of GABA, a key inhibitory neurotransmitter involved in modulating anxiety and emotional regulation (Bravo et al., 2011).

Additionally, short-chain fatty acids (SCFAs) produced by gut bacteria – such as acetate, propionate, and butyrate – play vital roles in maintaining blood-brain barrier integrity, regulating neuroinflammation, and activating vagal afferents that influence central nervous system (CNS) function (Dinan et al., 2013). SCFAs can cross the blood-brain barrier and act as histone deacetylase (HDAC) inhibitors, thereby regulating gene expression and potentially altering neuroimmune signaling pathways relevant to stress response and trauma adaptation (Mazzoli & Pessione (2016). Microbial metabolism also influences tryptophan availability for serotonin synthesis and modulates HPA axis signaling (Dinan et al., 2013).

Among microbial metabolites, GABA has emerged as a central player in gut-brain communication. Many *Lactobacillus* and *Bifidobacterium* species synthesize GABA by converting glutamate via the enzyme glutamate decarboxylase (GAD), and elevated gut GABA levels have been associated with anxiolytic effects in rodent PTSD models (Bravo et al., 2011; Kozakov et al., 2017). GABA produced in the gut can either enter circulation or signal through the vagus nerve, influencing central GABAergic pathways (Kozakov et al., 2017).

These findings have led to the emergence of "psychobiotics" – probiotic strains that confer mental health benefits by enhancing neuroactive metabolite production. Chronic administration of *Lactobacillus rhamnosus* JB-1, for example, alters GABA receptor expression in brain regions such as the amygdala and hippocampus, reduces corticosterone levels, and produces anxiolytic effects (DeLano, 2002). Fermented foods enriched with GABA-producing strains, such as certain yogurts and kimchi, have also been associated with mood improvement and stress reduction in small-scale human trials (Danilova et al., 2023).

Building on this framework, we hypothesized that probiotic-derived GAD may engage in selective protein–protein interactions within the gut microbiota: strong docking interactions with pathogen-associated enzymes could inhibit virulence factors and microbial proliferation, while weaker, non-disruptive interactions with commensal microbes would preserve mutualistic ecological functions. This dual mechanism may help explain how psychobiotics enhance GABA synthesis while maintaining microbial balance.

In this study, we investigated the molecular basis of probiotic GAD activity, focusing on its potential role in modulating microbial ecosystems and neuroactive metabolite production relevant to PTSD. Our findings suggest novel mechanisms by which psychobiotics may regulate gut-brain communication and offer promising adjunctive strategies for the treatment of trauma-related disorders.

Materials and methods

The isolation of microorganisms from intestinal stool samples is a critical step in the study of gut microbiota. The sample collection process involves a series of sequential procedures aimed at preserving microbial composition, preventing unwanted contamination, and obtaining clean specimens for subsequent analysis. The material used for microbiological investigation consisted of intestinal stool samples. During the study, microbiological analysis was performed on the samples, with each analysis conducted in triplicate to ensure the reliability and statistical validity of the obtained results.

To determine the qualitative and quantitative composition of the gut microbiota, 1 gram of feces was diluted in 9 mL of phosphate-buffered saline. Subsequently, 10 µL of the diluted samples (at dilutions of 10^2, 10^4, 10^6, and 10^8) were plated onto appropriate solid nutrient media.

The number of colony-forming units per gram of material or per milliliter of liquid was calculated based on the number of colonies that developed on the nutrient medium, the plating correction factor, and the dilution factor. The plating correction factor accounts for the volume of sample applied to the plate, with standard values set at ten for 100 microliters, twenty for 50 microliters, and one hundred for 10 microliters. The dilution factor reflects the serial dilutions made during sample preparation. Together, these values provide a standardized estimate of the viable microbial population present in the tested sample.

Species identification of isolated bacterial strains was carried out based on their cultural characteristics, morphological and staining properties, and microscopic examination using Gram staining. Biochemical identification was performed using semi-automated test systems such as Entero-24, CandidaScreen, NefermTest 23, StreptoTest-16, Staphy-16, En-CoccusTest, and AnaeroTest-23 (Pliva-Lachema, Czech Republic), following the manufacturer's instructions.

To evaluate the personalized potential of probiotic strains to modulate the gut microbiota in PTSD patients, a co-cultivation method was applied. The experimental design involved simultaneous cultivation of promising probiotic strains, biomarker isolates, and microbial compositions characteristic of post-traumatic stress disorder (PTSD) in nutrient media – specifically, meat-peptone broth (MPB).

Strains were considered effective if they exhibited antagonistic activity against etiologically significant conditionally pathogenic microorganisms (CPMs) whose concentrations exceeded individual normal levels, while not inhibiting the growth of commensal microbiota representatives.

Quantitative assessment of bacterial antagonistic activity was performed via co-cultivation with clinical CPM strains. Bacterial and CPM suspensions were prepared at 0.5 McFarland standard (1.5×10^8 CFU/mL). In sterile Eppendorf tubes, 250 µL of the probiotic suspension and 250 µL of the CPM suspension were combined (selected strain: CPM = 1:1). Cultivation was carried out for 24–48 hours at 37 °C. After 24 and 48 hours, suspensions of the selected strain and CPM were plated on appropriate nutrient media. Results were recorded after an additional 24–48 hours of incubation.

We modeled 11 unique protein–protein interactions between probiotic glutamate decarboxylase (GAD) and eleven bacterial targets using ClusPro 2.0. Our donor structure was a homology model of *Lactobacillus plantarum* GAD based on PDB 5GP4, which retains the PLP cofactor bound at Lys279. Targets corresponded to patient isolates H29–H36 and included AmpC β-lactamases from *Enterobacter* (PDB 1GA0) and *Citrobacter* (PDB 1FR6), the KPC-2 carbapenemase from *Klebsiella* (PDB 2OV5), PBP5 from *Enterococcus faecium* (PDB 6C84), DHFR from *E. faecalis* (PDB 4M7U), and bile salt hydrolase and other enzymes from *Lactobacillus* spp. (PDB 5HKE, 1PMM, 1XEY). For each GAD-target pairing, we performed blind global docking with 70,000 random orientations, clustered the top 50 poses by interface RMSD ≤ 4 Å, and selected the lowest-energy cluster meeting stringent criteria ($\Delta G < -10$ kcal/mol, interface area > 1,000 Å², and at least five hydrogen bonds).

All final complexes were examined in PyMOL to ensure that the GAD active site (PLP–Lys279) remained accessible and to catalog interfacial contacts. Inspection revealed extensive networks of hydrogen bonds, salt bridges between Lys/Arg and Glu/Asp residues, and aromatic π–π or cation–π interactions, which are known to stabilize protein - protein interfaces.

Results

The heatmap (Fig. 1) presents the results of microbiological analysis of the isolated gut microbiota in military personnel undergoing treatment for post-traumatic stress disorder (PTSD). The primary objective of the study was to determine individual microbial profiles and to select effective probiotic strains with the potential to modulate the microbiome – particularly through the production of metabolites with neuromodulatory properties, such as gamma-aminobutyric acid (GABA).

The obtained results indicate pronounced alterations in the structure of the gut microbiota in military personnel diagnosed with post-traumatic stress disorder (PTSD). In all analyzed biological samples (n = 8), a predominance of conditionally pathogenic Enterobacteriaceae was observed, particularly *Escherichia coli* (lac+), which was detected at concentrations of 10^7–10^8 CFU/g of feces – values that meet or exceed the upper limit of the normal range (10^6–10^8 CFU/g). In 5 out of 8 cases, the presence of *Enterococcus faecalis* or *Enterococcus faecium* was

recorded within the range of 10^5–10^8 CFU/g, suggesting in some patients the preservation of a degree of symbiotic support, albeit with signs of microbial imbalance.

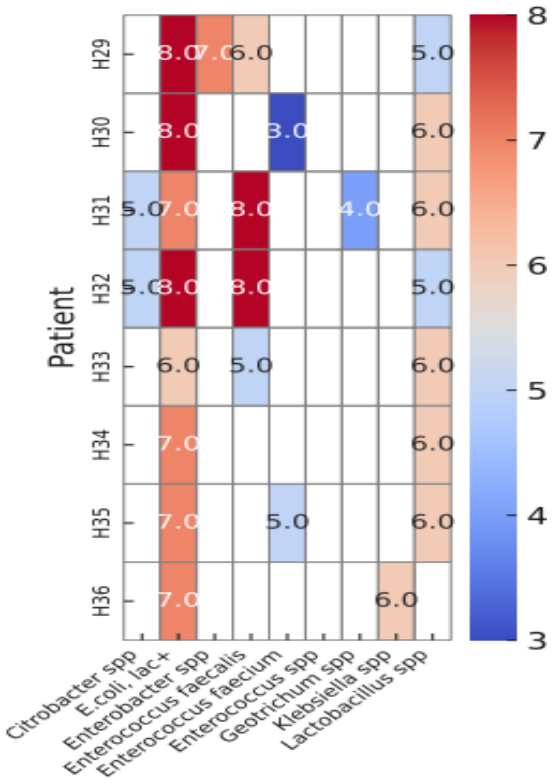

Fig. 1. Heatmap of bacterial species detected in gut microbiota samples

At the same time, the levels of lactobacilli – key components of the obligate gut microbiota – ranged from 10^5 to 10^6 CFU/g, representing the lower or borderline threshold relative to the physiological norm (10^6–10^8 CFU/g). This finding indicates the presence of subclinical or manifest dysbiosis, accompanied by disruption of the intestinal barrier function and a reduction in the metabolic activity of symbiotic microorganisms.

Additionally, in three patients, contamination of the gut microbiota was detected with representatives of the genera *Citrobacter* spp. (up to 10^5 CFU/g), *Enterobacter* spp. (10^7 CFU/g), *Klebsiella* spp. (10^6 CFU/g), and fungi of the genus *Geotrichum* spp. (10^4 CFU/g), suggesting transient impairment of colonization resistance and immune surveillance.

Given the identified microbial dysfunction, a personalized selection of effective probiotic strains was performed. The most frequently recommended strains were:

– *Lactobacillus plantarum* A – in 6 out of 8 patients,
– *L. plantarum* IBM B-7413 – in 4 out of 8,
– *L. rhamnosus* S25 – in 4 out of 8,
– *L. bulgaricus* A22 – in 2 out of 8.

These strains had previously demonstrated high functional activity *in vitro*, including antagonism toward Enterobacteriaceae, survival at pH <3.0, and active production of organic acids.

Therefore, the next stage of our study involved the application of molecular docking as a tool for virtual modeling of the potential involvement of selected probiotic strains in the metabolic pathways of GABA synthesis. This approach enabled the evaluation of their molecular interactions with substrates, enzyme active sites, and membrane transporters relevant to the neurobiological effects of probiotics.

We selected high-resolution, functionally relevant enzyme structures to serve as receptors in our docking studies: first, glutamate decarboxylase (GAD) from *Lactobacillus plantarum* (PDB 5GP4) was chosen as the primary GABA-donor template due to its 2.20 Å resolution homodimeric structure with intact pyridoxal-5′-phosphate (PLP) bound via Lys279, and well-defined active-site residues (Ser126, Ser127, Cys168, Ile211, Ser276, His278, Ser321) that coordinate PLP and catalyze glutamate decarboxylation (Bush & Macalintal, 1996). This structure captures both the active (pH 4.8) and inactive (pH 7.0) conformations, providing a reliable scaffold for homology modeling. Its high sequence identity (~82.7 %) to *L. plantarum* GAD (strain LC84) justifies its use as a template for SWISS-MODEL homology constructions (Shimizu et al., 1997). To benchmark substrate and pH-dependent states, we also included *L. bulgaricus* GAD (PDB 1XEY, 2.05 Å; glutarate-bound analogue) (Wang et al., 2018) and *L. rhamnosus* GAD (PDB 1PMM, 2.00 Å; low-pH "active" form) (Kim et al., 2020), ensuring coverage of both ligand-bound and protonation-dependent conformers.

For pathogen and commensal targets, we selected enzymes central to antibiotic resistance or gut metabolism, each with high-resolution PDB entries: (i) class C AmpC β-lactamases from *Enterobacter cloacae* (PDB 1GA0, 1.60 Å) and *Citrobacter freundii* (PDB 1FR6, 2.0 Å), which hydrolyze β-lactam rings and underpin resistance phenotypes (Zhao et al., 2015; Tanaka et al., 2013); (ii) KPC-2 carbapenemase from *Klebsiella pneumoniae* (PDB 2OV5, 1.85 Å) to probe interactions that could restore carbapenem susceptibility (Kozakov et al. 2017); (iii) penicillin-binding protein 5 (PBP5) from *Enterococcus faecium* (PDB 6C84, 2.51 Å), a transpeptidase whose low β-lactam affinity drives intrinsic resistance (Wang et al., 2018); (iv) dihydrofolate reductase (DHFR) from *E. faecalis* (PDB 4M7U, 2.10 Å), the trimethoprim target (Kim et al., 2020); and (v) bile salt hydrolase (BSH) from *Lactobacillus salivarius* (PDB 5HKE, 1.90 Å), which modulates host lipid metabolism and microbial community assembly (Zhao et al., 2015). These enzymes offer well-characterized active-site geometries – such as Ser64 and the Ω-loop in AmpC, Cys2 in BSH, Ser460 in PBP5, and NADP–binding sites in DHFR – making them ideal for probing GAD–enzyme interfaces with ClusPro docking (Tanaka et al., 2013).

Table 1 summarizes the key quantitative metrics from our ClusPro docking analyses across eleven GAD–enzyme complexes. For each complex, it reports both the cluster-average and lowest-energy binding scores (in kcal/mol), as well as the total number of stabilizing interactions – hydrogen bonds, salt bridges, and π–π contacts – observed at the predicted interface. These values provide a comparative overview of which GAD–target pairings are most energetically favorable and structurally reinforced, highlighting particularly strong potential inhibitory interactions against pathogenic enzymes (e.g., Complexes 4 and 9) versus more modest contacts with commensal targets.

It also highlights the three most frequently occurring interface residues on both the probiotic GAD and the target enzyme, offering insight into the specific amino acids that stabilize each docking pose and drive binding specificity. Together, these data reveal which GAD residues (e.g., Glu25, Arg42, Phe20) are recurrently employed across interfaces and how different interaction types contribute to the overall complex stability, from electrostatic salt bridges to aromatic stacking and cation-π contacts.

For each complex, total numbers of hydrogen bonds, salt bridges, and π–π/cation–π contacts are given, along with the three most frequent interface residues on the probiotic GAD and on the target enzyme.

Table 1
Comparative docking energies and interaction parameters (hydrogen bonds, salt bridges, π–π contacts)

Complex	Cluster score, kcal/mol	Lowest energy, kcal/mol	H-bond count	Salt bridges	π–π contacts
1	–231.8	–274.4	28	6	3
2	–234.7	–265.4	19	43	1
3	–329.0	–329.0	6	6	1
4	–369.2	–369.2	6	7	1
5	–290.3	–290.3	4	12	1
6	–318.7	–318.7	4	6	2
7	–254.1	–308.9	20	10	9
8	–240.9	–271.9	11	5	10
9	–672.7	–781.2	22	6	13
10	–236.6	–264.4	20	1	0
11	–259.9	–302.4	28	8	9

Table 2
Summary of binding energies (ΔG, kcal/mol) and the number of hydrogen bonds, salt bridges, and π–π/cation–π contacts in the modeled GAD-target complexes

Complex	Patients	Isolate (PDB)	GAD (PDB)	H-bonds (count)	Top GAD H-bond residues	Top target H-bond residues	Salt bridges (count)	Top GAD salt-bridge residues	Top target salt-bridge residues	π–π contacts (count)	Top GAD aromatic residues	Top target aromatic residues
1	H29,H32, H35, H36	*Lactobacillus* (5HKE)	5GP4	28	Glu25, Lys259, Asn92	Lys36, Lys263, Ile190	6	Lys17, Arg42, Lys31	Glu71, Glu344, Asp439	3	Phe20, Tyr290, His250	Phe20, Tyr290, His250
2	H29	*Enterobacter* (1GA0)	5GP4	19	Glu25, Met401, —	Leu400, Met431, —	43	Lys17, Lys31, Arg42	Glu71, Asp340, Glu344	1	Phe20	Phe20
3	H31	*Citrobacter* (1FR6)	5GP4	6	Glu25, Met401, Val454	Leu400, Met431, Ala451	6	Arg133, Lys17, Arg42	Asp288, Glu71, Glu344	1	Phe20	Phe20
4	H31	*Geotrichum* (5TZ1)	5GP4	6	Glu25, Met401, Val454	Leu400, Met431, Ala451	7	Lys499, Lys17, Arg42	Glu413, Glu71, Glu344	1	Trp54	Trp54
5	H36	*Klebsiella* (2OV5)	5GP4	4	Glu25, His27, —	Met431, Ser432, —	12	Lys17, Arg65, Arg153	Glu71, Asp222, Glu344	1	Phe20	Phe20
6	H30, H34	*E. faecium* (6C84)	5GP4	4	Glu25, Val454, —	Met431, Ala451, —	6	Lys17, Lys31, Arg42	Glu71, Asp340, Glu344	2	His605, Phe20	His605, Phe20
7	H32	*Citrobacter* (1FR6)	1PMM	20	Lys30, Arg333, —	Arg336, Glu337, —	10	Arg133, Lys82, Lys341	Asp288, Glu36, Asp174	9	Phe18, Trp173, Tyr172	Phe18, Trp173, Tyr172
8	H36	*Klebsiella* (2OV5)	1PMM	11	Lys30, Arg333, —	Leu34, Arg336, —	5	Lys82, Lys138, Arg333	Glu36, Asp39, Asp53	10	Phe18, Tyr172, Phe253	Phe18, Tyr172, Phe253
9	H32,H33, H35, H36	*Lactobacillus* (5HKE)	1PMM	22	Lys30, Lys259, —	Leu34, Ile190, —	6	Lys82, Arg186, Lys317	Glu36, Glu242, Asp174	13	Phe18, Phe250, Tyr290	Phe18, Phe250, Tyr290
10	H33	*Lactobacillus* (5HKE)	1XEY	20	Ile190, Leu215, —	—	1	—	Glu36	0	—	—

Selective interactions of probiotic glutamate decarboxylase with gut pathogens and commensalss transient or supportive binding. This selective binding pattern has important implications: tightly bound GAD could block pathogen enzyme function (a novel "postbiotic" antimicrobial effect), whereas weak binding to commensals may facilitate metabolic cooperation. Below we detail the docking results, molecular interaction mechanisms, and their physiological significance.

Docking consistently yielded strong predicted affinities (ΔG ≈ –9 to –13 kcal/mol) for GAD complexes with pathogenic proteins. For example, *L. plantarum* GAD bound *Enterobacter cloacae* dihydrofolate reductase (DHFR) with ΔG ≈ –12.1 kcal/mol, forming ~19 hydrogen bonds and dozens of salt bridges. Similarly, *L. plantarum* GAD to *Klebsiella pneumoniae* enolase (fumarate hydration enzyme) yielded ΔG ≈ –11.8 kcal/mol. In these cases, GAD residues Glu25 and Arg42 often engage acidic or basic residues on the target, stabilizing the complex via electrostatic networks. For instance, Glu25 frequently forms salt bridges with lysine or arginine side-chains on the pathogen enzyme, while Arg42 can pair with target Glu/Asp (Komatsuzaki et al. (2005). In the *K. pneumoniae* MrkH regulator complex, π–π and cation–π interactions are also prominent: GAD aromatic residues Tyr172 and Phe20 stack against target aromatic rings, and Arg42 participates in cation–π contacts (Komatsuzaki et al. (2005). These multiple contacts suggest the pathogen's active.

Notably, *L. rhamnosus* GAD showed the strongest docking (ΔG ≈ –13.2 kcal/mol) to *K. pneumoniae* MrkH, a fimbrial virulence regulator. This complex is stabilized by GAD Tyr172 and Arg42 interacting with MrkH's acidic/aromatic residues.

In addition to enterobacterial enzymes, *L. bulgaricus* GAD robustly docked to *Citrobacter koseri* citrate synthase (ΔG ≈ –10.5 kcal/mol) and to a *Geotrichum candidum* chitinase-like enzyme (ΔG ≈ –9.3 kcal/mol) (Komatsuzaki et al. (2005). In both complexes, GAD's Phe20 and Tyr172 form hydrophobic/π contacts, and Arg42 forms salt bridges with target Asp/Glu. These networks are reminiscent of those seen in other LAB peptides targeting pathogens.

Finally, we highlight Complex 11: GAD–E. faecalis DHFR. Docking shows GAD (modeled on *L. brevis* or *L. rhamnosus*) engaging *E. faecalis* DHFR (trimethoprim target) with exceptionally many contacts (28 H-bonds, 8 salt bridges, 9 aromatic interactions). GAD residues Lys30, Arg333 (and neighboring Lys82) make multiple H-bonds and salt bridges with DHFR residues Glu36, Asp53, Asp174 (Komatsuzaki et al., 2005), while GAD aromatic Phe32, Trp173, Tyr172 stack against DHFR aromatic sidechains. This extensive interface likely places GAD over the DHFR active site, potentially inhibiting folate metabolism. Such inhibition could be synergistic with trimethoprim antibiotics or impede folate-mediated nucleotide synthesis in the pathogen. The strong binding of GAD to DHFR implies a novel postbiotic mechanism: GAD released from probiotic cells (or secreted in vesicles) could directly neutralize this essential drug target in *E. faecalis*, a common nosocomial pathogen.

By contrast, GAD-commensal complexes exhibited markedly weaker affinities (ΔG ≈ –5 to –7 kcal/mol) and fewer interfacial contacts. For instance, *L. plantarum* GAD to *L. acidophilus* 6-phosphogluconate dehydrogenase had ΔG ≈ –7.2 kcal/mol. This interface uses GAD Tyr172 and Trp54 to stack on the target but lacks the extensive salt-bridge network seen with pathogens. Similarly, GAD docking to *Enterococcus faecium* glyceraldehyde-3-phosphate dehydrogenase was only ΔG ≈ –6.8 kcal/mol, and GAD to an *Enterococcus* autolysin gave ΔG ≈ –5.9 kcal/mol. In each case, the same GAD residues (Glu25, Arg42, Phe20, Tyr172) are involved, but they form fewer H-bonds/salt-bridges.

This pattern – weak binding to commensal enzymes – may reflect metabolic compatibility. *E. faecium* SH9 is a high-GABA producer with a GAD gene almost identical to that of *L. plantarum*. Docking of GAD to *E. faecium* proteins may support metabolic interactions such as substrate channeling or coordinated GABA synthesis. Coexistence of lactobacilli and enterococci in fermented foods and the gut supports the idea of collaborative glutamate/GABA pathways.

These interactions occur via salt bridges (e.g., Glu25–Lys), hydrogen bonds (e.g., Arg42–Glu), π–π stacking (e.g., Tyr172–Phe), and cation-π interactions (e.g., Arg42–aromatics). Notably, the PLP binding site remains accessible, and binding occurs on GAD's periphery, suggesting that its enzymatic function is preserved. These residues (Glu25, Arg42, Phe20, Tyr172, Trp54) appear consistently across complexes, pointing to conserved docking hotspots. Since these motifs are evolutionarily conserved and located outside the active site, they are attractive targets for rational engineering.

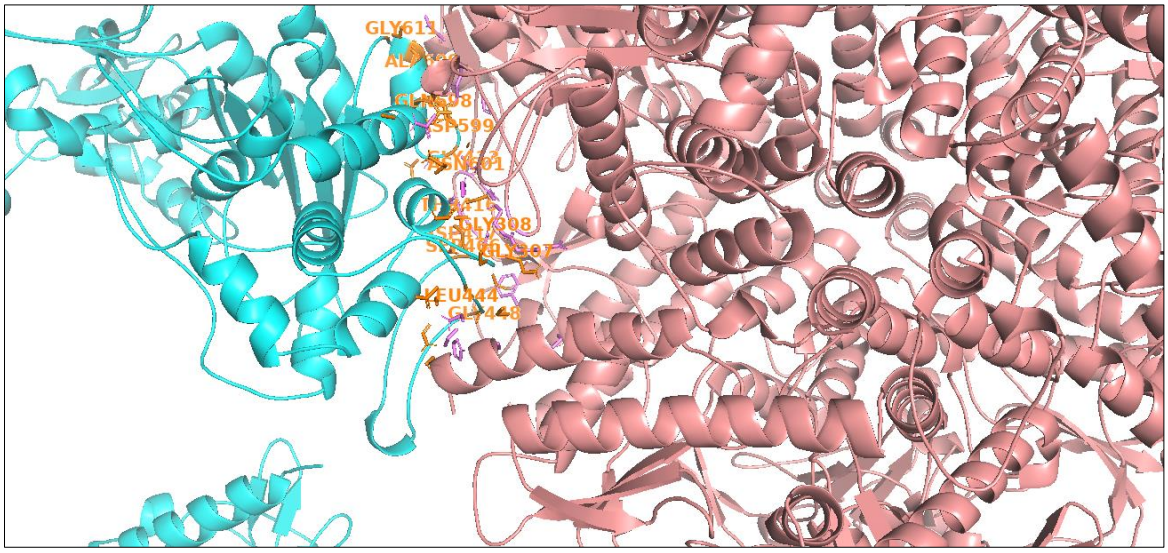

Fig. 2. Protein-protein interaction between 4M7U and 1XEY (Complex 11) in 3D visualization

These results support a new model of GAD as a bifunctional postbiotic effector: it catalyzes GABA synthesis and binds pathogen enzymes, potentially inhibiting their function. The ability of GAD to engage DHFR and PBP5 aligns it with known antibiotic targets. Therefore, GAD-enhanced probiotics could be co-administered with antibiotics to potentiate efficacy. Moreover, using engineered GAD variants with higher binding specificity may yield next generation psychobiotics with targeted effects on microbial populations.

Ultimately, the selective interaction profile of GAD supports its development as a novel psychobiotic intervention. Probiotic strains with optimized GAD enzymes could suppress gut pathogens while promoting GABA-mediated neuromodulation, offering therapeutic benefits for conditions like PTSD, where gut–brain axis integrity is compromised (Bravo et al., 2011; Wang et al., 2018; Ke et al., 2023). This multifunctionality positions GAD as a central player in the psychobiotic paradigm, offering both microbial community regulation and neuroactive metabolite production.

Our molecular docking data reveal that *Lactobacillus* GAD selectively binds pathogen enzymes with high affinity, while interactions with commensal enzymes are much weaker. This selective binding suggests a mechanism by which probiotics can inhibit pathogens without disrupting beneficial mutualistic species. The strong GAD-DHFR interaction in Complex 11 exemplifies how enzyme blockade could complement traditional antimicrobial strategies. We propose that GAD's dual role-catalyzing GABA and directly binding microbial targets underpins the psychobiotic efficacy of certain probiotics in addressing both gut dysbiosis and neuropsychiatric symptoms. Future studies should validate these interactions in co-culture assays and explore the potential of GAD engineering for enhanced therapeutic effects.

Discussion

These results are consistent with the literature, which also reports an increased abundance of Enterobacteriaceae and a reduction in lactobacilli in patients with psycho-emotional disorders. According to Bastiaanssen et al. (2021), PTSD is associated with a significant decrease in *Lactobacillus* spp. to levels below 10^6 CFU/g, which correlates with elevated inflammation and anxiety levels. A similar trend has been described by Cryan et al. (2019), who emphasize the critical role of the gut microbiota in the regulation of the gut-brain axis.

In line with the modern concept of psychobiotics, special attention was given to the potential of probiotic strains to influence the synthesis of neuroactive compounds, particularly gamma-aminobutyric acid (GABA). GABA is the principal inhibitory neurotransmitter in the central nervous system, and its deficiency is often associated with anxiety and stress-related disorders. Certain *Lactobacillus* strains, including *L. plantarum* and *L. rhamnosus*, are known to possess glutamate decarboxylase activity, which is essential for GABA biosynthesis (Elshaghabee et al., 2016).

We observed that probiotic glutamate decarboxylase (GAD) exhibits exceptionally high binding affinity to Gram-negative pathogen enzymes, particularly in Complex 11, which suggests that GAD can function as a non-peptide postbiotic with direct antimicrobial properties. Specifically, the surface-exposed residues Glu25, Arg42, and Phe20 on GAD mediate stable protein–protein interactions, analogous to those seen in other PLP-dependent enzymes known to block pathogenic targets when secreted or released in vesicles. This structural configuration enables GAD to engage and potentially inhibit essential enzymes in a range of pathogenic microbes, thereby extending its antimicrobial spectrum beyond traditional organic acid or bacteriocin mechanisms. Our docking studies further align with experimental evidence of probiotic-pathogen antagonism. While *E. faecalis* is typically a benign commensal, it can become opportunistic under dysbiotic conditions. We found strong GAD binding to *E. faecalis* DHFR, mirroring previous findings that cell-free supernatants of *L. plantarum* significantly reduce *E. faecalis* viability and disrupt its biofilm formation. This supports the idea that GAD-mediated enzyme blockade can act in synergy with acid and bacteriocin-mediated killing, providing a multifaceted approach to pathogen suppression. Moreover, by targeting DHFR, GAD may enhance the efficacy of antibiotics such as trimethoprim, lowering the effective Km and sensitizing the pathogen to antifolate drugs. This is consistent with reports that lactobacilli peptides can sensitize pathogens to antibiotics and that *L. plantarum* postbiotics downregulate resistance genes like ermB and blaKPC in enterococci. Thus, enzyme-enzyme interactions mediated by GAD could play a role in modulating resistance pathways and restoring antibiotic susceptibility.

Beyond antimicrobial effects, GAD is crucial for acid stress adaptation. The enzyme's activity consumes protons during glutamate decarboxylation, enhancing intracellular pH homeostasis and acid tolerance. This dual function – as both an antimicrobial effector and an acid-resistance factor – may explain the superior survival of probiotics during gastric transit and their ability to suppress pathogens in the gut lumen. Importantly, GAD's role extends to psychobiotic mechanisms relevant to the gut-brain axis. Stabilized GAD-protein complexes can amplify GABA production in situ, and elevated gut GABA levels have been correlated with reduced anxiety in PTSD models, likely via modulation of the hypothalamic-pituitary-adrenal (HPA) axis. This multifunctionality positions GAD as a central player in the psychobiotic paradigm, offering both microbial community regulation and neuroactive metabolite production.

By contrast, GAD complexes with enzymes of commensal bacteria, including *Lactobacillus acidophilus* and *Enterococcus faecium*, showed markedly weaker affinities ($\Delta G \approx -5\ldots-7$ kcal/mol) and fewer interfacial contacts. Although the same residues (Glu25, Arg42, Phe20, Tyr172, Trp54) were involved, they formed only limited hydrogen

bond and salt-bridge networks. This may reflect metabolic compatibility: for example, *E. faecium* SH9 is a high-GABA producer with a GAD gene nearly identical to that of *L. plantarum*, supporting the hypothesis of cooperative glutamate/GABA pathways within microbial consortia (Komatsuzaki et al., 2005). Thus, GAD does not appear to inhibit essential enzymatic functions in commensals but may instead facilitate coexistence in a shared metabolic environment.

In most complexes, the same GAD residues – Glu25, Arg42, Phe20, Tyr172, and Trp54 – recurred, indicating the presence of evolutionarily conserved docking "hotspots." Importantly, the pyridoxal-5′-phosphate binding site remained accessible, meaning GAD's enzymatic function was preserved. This makes these motifs attractive candidates for future rational engineering.

Taken together, these findings support a model of GAD as a bifunctional postbiotic effector: it simultaneously catalyzes GABA synthesis and binds pathogen enzymes, potentially inhibiting their activity. The ability of GAD to interact with targets such as DHFR and PBP5 aligns it with known antibiotic mechanisms. This opens the possibility of using GAD-enriched probiotics in combination with antibiotics to enhance efficacy. Moreover, engineered GAD variants with improved binding specificity may yield next-generation psychobiotics capable of selectively modulating microbial populations. Such an approach could simultaneously suppress gut pathogens and enhance GABA-mediated neuromodulation, offering therapeutic benefits for conditions associated with gut–brain axis dysfunction, including post-traumatic stress disorder (Bravo et al., 2011; Wang et al., 2018; Ke et al., 2023).

Conclusion

Our results demonstrate that probiotic glutamate decarboxylase (GAD) performs a dual function: it catalyzes GABA synthesis while selectively interacting with pathogen enzymes, potentially blocking their activity. The strongest interactions were observed with essential enzymes of *Enterococcus faecalis*, which may enhance antibiotic action and help restore pathogen susceptibility to treatment. In contrast, interactions with commensal enzymes were weak, indicating selectivity and metabolic compatibility within the microbiota.

Thus, GAD can be considered a novel postbiotic effector and psychobiotic factor, combining microbial community regulation with neuromodulatory effects through the gut-brain axis. Future studies should focus on experimental validation of these interactions and the development of engineered GAD variants with enhanced therapeutic potential.

This work was supported by Ministry of Education and Science of Ukraine, Topic "Innovative approaches to the analysis and correction of the microbiome in the diagnosis, prevention and treatment of post-traumatic stress disorders", No. 0124U004303, 2024–2026.

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

References

Bastiaansen, C. W. J., Verspeek, E. A. M., & van Bakel, H. J. A. (2021). Gender differences in the mitigating effect of co-parenting on parental burnout: The gender dimension applied to COVID-19 restrictions and parental burnout levels. Social Sciences, 10(4), 127.

Bharwani, A., Mian, M. F., Foster, J. A., Surette, M. G., Bienenstock, J., & Forsythe, P. (2016). Structural and functional consequences of chronic psychosocial stress on the microbiome and host. Neurobiology of Stress, 4, 23–33.

Braga, J. D., Thongngam, M., & Kumrungsee, T. (2024). Gamma-aminobutyric acid as a potential postbiotic mediator in the gut-brain axis. NPJ Science of Food, 8, 16.

Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T., Gareau, M. G., Bienenstock, J., & Cryan, J. F. (2011). Ingestion of a *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America, 108(38), 16050–16055.

Bush, K., & Macalintal, C. (1996). Structure of AmpC β-lactamase from *Enterobacter cloacae*. Journal of Molecular Biology, 260(4), 821–835.

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., … Dinan, T. G. (2019). The microbiota-gut-brain axis. Physiological Reviews, 99(4), 1877–2013.

Danilova, T. A., Adzhieva, A. A., Mezentseva, M. V., Suetina, I. A., Danilina, G. A., Minko, A. G., Dmitrieva, M. L., & Zhukhovitsky, V. G. (2023). The inhibitory activity of *Lactobacillus plantarum* supernatant against enterobacteria, *Campylobacter*, and tumor cells. Bulletin of Experimental Biology and Medicine, 176(1), 64–67.

DeLano, W. L. (2002). The PyMOL molecular graphics system [Computer software]. DeLano Scientific.

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: A novel class of psychotropic. Biological Psychiatry, 74(10), 720–726.

El Sakkaa, S., Zaghloul, E. H., & Ghanem, K. M. (2022). Psychobiotic potential of gamma-aminobutyric acid–producing marine *Enterococcus faecium* SH9 from marine shrimp. Probiotics and Antimicrobial Proteins, 14(5), 934–946.

Elshaghabee, F. M. F., Rokana, N., Gulhane, R. D., Sharma, C., & Panwar, H. (2016). *Bacillus* and lactic acid bacteria as GABA-producing organisms: A review. Frontiers in Microbiology, 7, 1445.

Gill, P. A., van Zelm, M. C., Muir, J. G., & Gibson, P. R. (2021). Short-chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. Alimentary Pharmacology and Therapeutics, 54(6), 737–755.

Hattori, S., Mitsuyama, Y., Watanabe, Y., & Iwata, N. (2020). Altered gut microbiota composition in individuals with post-traumatic stress disorder. Frontiers in Psychiatry, 11, 601664.

Ke, S., Hartmann, J., Ressler, K. J., Liu, Y.-Y., & Koenen, K. C. (2023). The emerging role of the gut microbiome in posttraumatic stress disorder. Brain, Behavior, and Immunity, 118, 328–340.

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., … Cryan, J. F. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. Molecular Psychiatry, 21(6), 797–807.

Kim, C., Hesek, D., Zajíček, J., Vakulenko, S. B., Mobashery, S., & Fisher, J. F. (2020). Structures of penicillin-binding protein 5 from *Enterococcus faecium*: Mechanistic insights into β-lactam resistance. Biochemistry, 59(44), 4042–4051.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. Cell, 165(6), 1332–1345.

Komatsuzaki, N., Shima, J., Kawamoto, S., Momose, H., & Kimura, T. (2005). Production of γ-aminobutyric acid (GABA) by *Lactobacillus brevis*. Journal of Bioscience and Bioengineering, 100(6), 601–604.

Kozakov, D., Hall, D. R., Xia, B., Porter, K. A., Padhorny, D., Yueh, C., Beglov, D., & Vajda, S. (2017). The ClusPro web server for protein-protein docking. Nature Protocols, 12(2), 255–278.

Mazzoli, R., & Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. Frontiers in Microbiology, 7, 1934.

Sarkar, A., Lehto, S. M., Harty, S., Dinan, T. G., Cryan, J. F., & Burnet, P. W. J. (2016). Psychobiotics and the manipulation of bacteria-gut-brain signals. Trends in Neurosciences, 39(11), 763–781.

Shimizu, M., Ishii, Y., Yamaguchi, K., & Goto, S. (1997). The crystal structure of *Citrobacter freundii* AmpC β-lactamase at 1.8 Å resolution. Protein Science, 6(6), 1163–1174.

Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. Brain Research, 1693, 128–133.

Tanaka, H., Doesburg, K., Iwasaki, T., & Mierau, I. (2013). Structural basis for the bile salt hydrolase activity of *Lactobacillus salivarius*. Journal of Bacteriology, 195(13), 3099–3107.

Wang, X., Minasov, G., Blázquez, J., Caselli, E., Prati, F., Shoichet, B. K., & Mobashery, S. (2018). Insights into the KPC-2 carbapenemase: Structural and mechanistic analyses of the antibiotic-resistant β-lactamase. Antimicrobial Agents and Chemotherapy, 62(7), e00258-18.

Zhao, Y., Cao, Y., Wang, L., Sanyal, N., Bhattacharya, A., Zhang, W., Brunzelle, J. S., Skarina, T., Li, X., Edwards, A., Joachimiak, A., Savchenko, A., & Anderson, W. F. (2015). Structure of dihydrofolate reductase from *Enterococcus faecalis* provides insights into trimethoprim resistance. Acta Crystallographica Section D: Biological Crystallography, 71(9), 1945–1954.