RESEARCH PAPER

Exploring Enterococcus inesii as a Psychobiotic Candidate for Next-Generation Mental Health Therapies

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Received: 13th Dec, 2025; Revised: 8th Feb 2026; Accepted: 10th Feb, 2026; Available Online: 30th March, 2026

ABSTRACT
Depression is a neuropsychiatric disorder that results from a multitude of biological and environmental determinants. In spite of the renewed interest in the gut-brain axis in modulating mental health, the specific microbial targets that have the capacity to modulate the synthesis of neurotransmitters remains limited. In particular, the majority of the research in psychobiotics has focused on the traditional probiotic genera *Lactobacillus* and *Bifidobacterium,* while the therapeutic potential of other microbial groups remains unexplored. In this regard, the present study employed a novel integration of machine learning-based analytics of available depression-related datasets from Kaggle and experimental approaches in modulating the microbiome.
Data-driven analysis has identified chronic stress, unhealthy dietary patterns, obesity, inflammation, and social isolation as key predictors of depression. All these factors have been found to have a strong association with gut microbiota dysbiosis and alterations in the gut-brain axis, which can interfere with neurotransmitter homeostasis. Experimental findings have demonstrated that phytobiotics and essential oil compounds can selectively enhance the growth of poultry gut bacteria that are responsible for the production of neurotransmitter compounds. There has been a notable connection between the newly identified bacterium, *Enterococcus inesii*, and the production of neuroactive compounds.
Through the bridging of human depression risk analytics with experimental approaches of microbiota enrichment, this study has provided new insights into the complex relationship between the human microbiome and neurotransmitters, and has identified *Enterococcus inesii* as a potential candidate for the next generation of psychobiotics, which can be used as a novel approach to therapy for mental health disorders.
Keywords: *Psychobiotics; Gut microbiota; Enterococcus inesii; Neurotransmitters; Depression; Glutamate decarboxylase (GAD).*
How to cite this article: Venkatesan R, Navaraj MM, David S, Soundararajan S, Navaraj SB and Ranjan J, Exploring Enterococcus inesii as a Psychobiotic Candidate for Next-Generation Mental Health Therapies. Int J Drug Deliv Technol. 2026;16(3): 8-17. DOI: 10.25258/ijddt.16.3.2
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Major depressive disorder represents one of the most leading causes of global disability, depending on a complex interplay of genetic, environmental, metabolic, and psychosocial factors. Large-scale clinical and population datasets, enabled by machine learning, curated in public repositories and Kaggle platforms, have allowed for the identification of key predisposing variables, including chronic stress, sleep disturbances, poor dietary habits, obesity, systemic inflammation, and social isolation, all factors significantly increasing the risk and severity of depressive symptoms[2]. Indeed, recent evidence suggests that many of these risk factors converge on alterations in the gut microbiota, implicating the microbiome–gut–brain axis as central to mood regulation. Via multiple mechanisms, the gut microbiota modulates immune responses, produces SCFAs, regulates the HPA axis, and synthesizes neuroactive compounds, such as serotonin, dopamine, and GABA[13].

Certain bacterial species like Lactobacillus have been found to stimulate the secretion of GABA, thereby impacting emotional characteristics. Therefore, in light of dysbiosis resulting from stress, diet, and inflammation, it can possibly affect neurotransmitter homeostasis and thus the pathophysiology of depression. In view of this, phytobiotics like flavonoids, polyphenolic compounds,

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and essential oils have been identified as being beneficial in their ability to influence gut microbiota composition in a favorable way[23]. In poultry models, phytobiotic supplements have been established as being capable of increasing beneficial gut microbiota composition and lowering inflammatory levels, thereby creating a model for analyzing gut microbiota modulation in the central nervous system.

Data-informed identification of depression risk factors and experimental studies on phytobiotic-enriched neurotransmitter-producing bacteria offer promising approaches to establishing novel translational strategies to effectively cope with depression by leveraging the gut microbiome. Data science studies on large-scale clinical and behavior-related datasets have repeatedly identified risk factors like chronic stress, sleep problems, poor dieting habits, metabolic problems, and inflammation as highly correlated with depression-like disorders[24]. These depression risk factors correlate directly with gut microbiome dysbiosis and alterations in gut-brain axis transmissions, which play crucial roles in the production and regulation of neurotransmitters. Phytobiotic-producing gut bacteria or microbes like *Lactobacillus* and *Bifidobacterium* are capable of synthesizing neurotransmitters like GABA and serotonin to regulate moods[16].

Phytobiotics are bioactive compounds derived from plants that selectively enrich such beneficial microbial populations and enhance short-chain fatty acid production, thus modulating both inflammatory and neurochemical pathway[23]. By linking predictive analytics of depression with mechanistic studies of phytobiotic-driven microbial neurotransmitter production, the present integrative approach connects computational epidemiology with experimental microbiology and provides a mechanistically sound route toward microbiota-targeted interventions in depressive disorders[13].

Further, to delve into the epigenetics of these neurological disorders, it has also been investigated how the glutamate decarboxylase (Gad-b1) gene expresses GABA and dopamine. One of the numerous types of glutamic acid decarboxylase, recognized as a significant autoantigen in insulin-dependent diabetes. The enzyme involved is responsible for catalyzing the conversion of a substance called L-glutamic acid to gamma-aminobutyric acid. GAD1 expression is less frequent in SZ patients, according to numerous studies. The ability of the gut isolate to produce neurotransmitters is confirmed by the gene's existence in the probiotic *Enterococcus inesii.*

METHODOLOGY

The resources that were used in this investigation were from both the laboratory and Hi Media.

Datasets from Kaggle

The data used in the study has been taken from Kaggle datasets on depression factors, Global Trends in Mental Health Disorder, From Schizophrenia to Depression, Amit Last Updated: 3 years ago (Version 2).The datasets were analyzed as predisposing factors for depression for early detection.

Ethics approval for the study

Animal studies were undertaken with an aim to enrich the animal's gut with phytobiotics to enrich gut bacteria whose metabolites may serve as neurotransmitters to reduce and treat depression. The study was conducted according to the approved procedures after obtaining approval from the Institutional Animal Ethics Committee (IAEC) at Bharathidasan University, Tiruchirappalli, Tamil Nadu, India, for its planning and execution. All procedures adhered to applicable rules and regulations. The registration number for the study was 418/GO/Re/S/01/CPCSEA, dt.. 24.07.2018; (BDU/IAEC/P06/2021).

Experimental design for birds

Forty 1-day-old healthy male broiler chicks, each weighing about 50 g, were purchased from the Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. The chicks were fed with a phytobiotic supplement of *Moringa oleifera* leaf powder (MOLP) at a rate of 10 g/kg, along with a starter meal. From day 15 to 42, the starter meal was replaced with a grower-finisher meal. On the 42nd day, one healthy chicken was chosen for euthanasia. The chicken was euthanized by bloodletting just outside the neck after aseptic abdominal incisions. The weights were measured after rapid cecum excision, and cecal digesta samples were taken to the lab for further analysis. These samples were immediately frozen and stored in liquid nitrogen (−80◦C) [21] .

Sample Collection

The probiotic bacterium was isolated and identified by sacrificing the phytobiotically pretreated chicken gut and caecum samples were collected in sterile screw capped tubes and ground using a mortar and pestle in an aseptic setting [21].

Identification of Isolates

The crushed cecum samples were serially diluted and inoculated into MRS broth containing amino acid precursors and incubated in the anaerobic compartment at the ambient temperature. The grown culture was streaked into MRS agar plates and further incubated anaerobically for 1-2 days. The isolates obtained were screened for probiotic properties and the selected strain was subject to Gram staining and Biochemical tests were confirmed by 16S rRNA sequencing and a phylogenetic tree was constructed.

Production and Extraction of Dopamine and GABA from isolated Probiotic bacteria

The broth was cultured at 37°C for a week and the necessary amino acid precursors were added. Before centrifugation, the bacterial cells in the broth culture were subjected to cell lysis by ultrasonication for about 20 min with glass beads and then were subject to centrifugation for about 25 min at 8000 rpm. The supernatant was collected with saline water. The presence of extracted

compounds such as Dopamine and GABA (Gamma Amino Butyric Acid) was then subjected to preliminary tests like Thin Layer Chromatography, following the standard protocol[3].

Identification and Analysis of Dopamine and GABA by Thin-Layer Chromatography

Since Dopamine, Serotonin and GABA are all biogenic amines, visualizing agents were applied for their visualization and identification after the TLC fractionation. Various solvent systems were used for the visualization of the compound of interest. Isolated dopamine and GABA from the probiotic bacterium *spp.* were spotted on the silica-coated TLC plate[4] . To analyse Dopamine, Bromophenol blue was prepared in 5% NaOH and the chromatograms developed with the mobile phase, Glacial acetic acid: Butanol: Water (1:4:1v/v) were sprayed with the same. The visualization spots were observed when the silica plate was wet with the reagent[5]. To analyse GABA, the chromatograms developed with the mobile phase Phenol: Chloroform: Isoamyl Alcohol (25:24:1v/v) were kept in the dark for 6-8 hr and then sprayed with 0.1% of Ninhydrin in Butanol before use. Colour development was allowed for 24 hr. in a dark room, and the effect was observed[6,8].

Spectrophotometric methods of analysis of Dopamine and GABA

Analysis of Dopamine

The standard protocol was followed for spectrophotometric analysis[9].

*Standard Dopamine Solution***:** The Dopamine standard stock solution (1mg/ml) was prepared from 10mg/ml dopamine stock solution by dissolving 1ml dopamine solution in 10 ml of saline. The stock solution was used to prepare the different working concentrations in the range from 0.1 to 1 mg/ml in the test tubes with saline. To the working solutions, 0.5 ml of 0.03M ferric chloride followed by 0.5 ml of 0.0081M Potassium hexacyanoferrate were added to obtain a Prussian blue coloured complex. Absorbance was measured at 710nm against the reagent blank. Calibration graphs were constructed by plotting absorbance against the final concentration of dopamine[9] .

Analysis of Gamma Amino Butyric Acid

Glutamate decarboxylase assay:

The cells were centrifuged and washed with 0.5 ml of saline solution and re-suspended in 0.5ml of GAD reagent solution containing 0.1g of L-Glutamic acid, 0.03ml of Triton X-100, 9 g of NaCl solution and 0.005g of bromocresol green in 100ml of double-distilled water adjusted to pH 4. The development of green color and blue color shows the GAD activity. Absorbance was measured at 570 nm against the reagent blank. Calibration graphs were constructed by plotting absorbance against the final concentration of GABA[4] .

Expression of the GAD B gene

To confirm the production of GABA by the probiotic bacteria isolated from gut, the presence of GAD B gene was screened.

Extraction of total RNA

Total RNA isolation was performed using TRIZOL, as per manufacturer's instructions (Qiagen, USA) for all the groups. Briefly, 1 OD culture of the sample was washed with Q-grade water by centrifugation, and 700 µl of TRIZOL was added to the pellet to facilitate cell lysis. The lysate was collected into 1.5 ml tubes and vigorously pipetted, following which 200 µl of chloroform was added and mixed vigorously for 5 min at room temperature. The aqueous layer was separated by centrifugation at 12000 rpm for 25 min at 4ºC organic and collected into a fresh 1.5 ml tube. RNA was precipitated by adding 700 µl of isopropanol. Precipitated RNA was pelleted by centrifugation at 12000 rpm for 20 min at 4ºC

DNase treatment

The possible DNA contamination in the preparation was removed by the DNase treatment. The reaction volume was set to 20 µl containing 1U of DNase (Thermo Scientific, USA). It was incubated at 37°C for 30-45 min, then heat-inactivated at 85 °C for 3 min stored till further processing.

cDNA synthesis

Approximately 1.5 µgm of total RNA was taken and converted to cDNA using a High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific, USA). The synthesis was carried out at 25°C for 10 min, followed by 37°C for 120 min. Denaturation of cDNA & RNA hybrid, along with inactivation of reverse transcriptase, was carried out at 85°C for 2 min. The prepared cDNA was used as a template for gene expression.

Semi-Quantitative PCR

For the current study, all quantitative gene expression analyses were carried out by semi-quantitative PCR on ABI Step One Plus machine (Thermo Scientific, USA) with the primer sequences given in **Table 1**. Amplicons of each PCR sample were resolved in 2% agarose gel with ethidium bromide for the confirmation of amplification.

Table 1: Primer sequence used in the study.

Gene	Forward sequence	Reverse sequence
gadB1	AACCCTGACACAGCCGAGAC	TCGTTTCAAGTGAACCAAGCA
16s rRNA	AGAGTTTGATCMTGGCTCAG	TACGGYTACCTTGTTACGACTT

Construction of protein-protein interaction networks (PPINs)

The PPINs were constructed using String v12.0 to identify a functional overview of key genes involved in glutamate–GABA metabolism, synthesis, and neurotransmission

regulation. The network was predicted using the high confidence score of 0.7 (70% of similarity). The final network was validated using CytoScape v3.8.2 and network properties were predicted using the Network Analyzer module. The genes corresponding to biological processes and metabolic pathways were predicted based on gene ontology enrichments.

RESULTS

Analysis of Predisposing factors for depression through datasets

Depression is a complex and multifaceted disorder, and its development is influenced by a combination of genetic, environmental, and psychological factors. This study examines several key predisposing factors that are frequently associated with the onset and severity of depression, including eating disorders, anxiety, substance use (drugs and alcohol), and comorbid psychiatric conditions like schizophrenia and bipolar disorder[11] .

Eating Disorders: Eating disorders, such as anorexia nervosa, bulimia nervosa, and binge-eating disorder, have long been linked with depression. The relationship between the two is bidirectional; individuals suffering from eating disorders often experience depressive symptoms due to negative body image, low self-esteem, and societal pressures. Conversely, depression may exacerbate unhealthy eating patterns, leading to disordered eating behaviours. The neurobiological changes associated with eating disorders, including altered levels of neurotransmitters such as serotonin, could contribute to the development of depressive symptoms. This visualization explores the relationship between eating disorders and depression (Fig. 1a). It indicates that individuals suffering from eating disorders, such as anorexia or bulimia, often experience higher rates of depression, suggesting a strong psychological link.

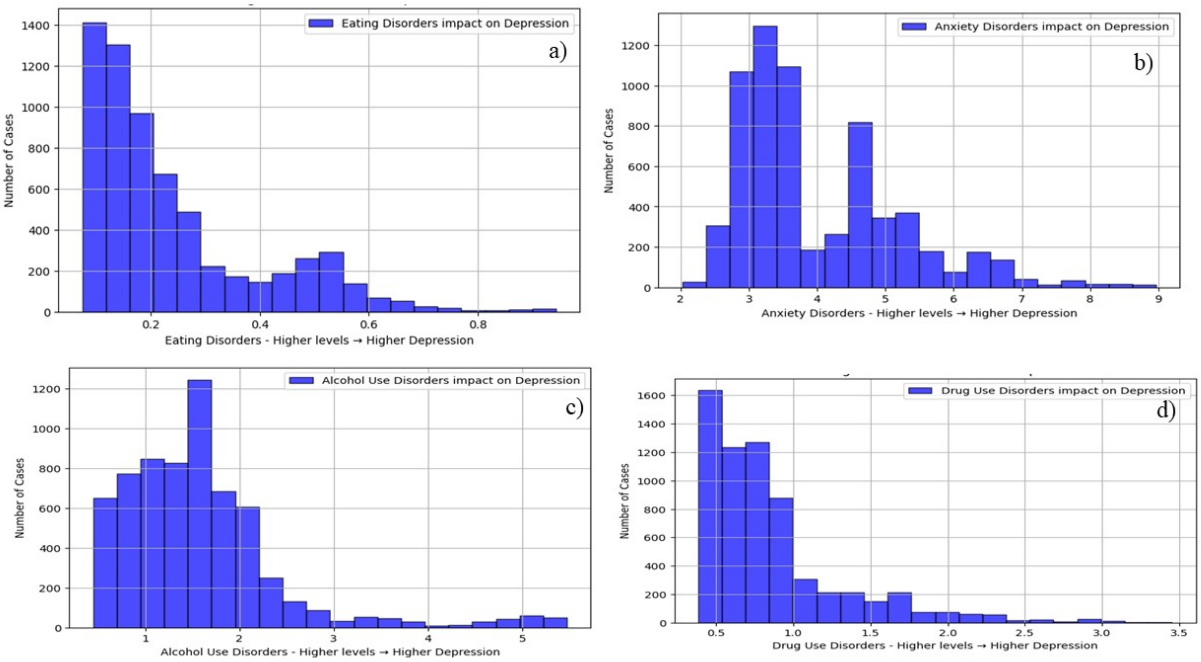

Figure 1 Histogram representation of the influence of eating (a), anxiety (b), alcohol (c), and drug use (c) disorders on depression levels. **Note:** The x-axis in each plot represents the **severity or level of a specific disorder**, and the y-axis represents the **number of cases** or individuals.

Anxiety: Anxiety and depression often co-occur, with anxiety disorders acting as a significant risk factor for depression. The chronic stress and heightened physiological arousal in individuals with anxiety disorders can lead to dysregulation of neurotransmitters such as serotonin, dopamine, and GABA, all of which play crucial roles in mood regulation. Anxiety disorders may also trigger maladaptive coping mechanisms, including substance abuse or avoidance behaviours, which further increase the risk of developing depression. Fig. 1b illustrates the correlation between anxiety and depression, showing how individuals experiencing anxiety disorders are also prone to depressive symptoms. The trend highlights the overlap in symptoms and the prevalence of comorbid conditions.

Substance Use (Drugs and Alcohol): Drug and alcohol use are both common co-occurring factors in depression, often used as coping mechanisms to alleviate emotional distress. However, substance abuse can lead to neurochemical imbalances, such as dopamine dysregulation, which exacerbates depressive symptoms. The chronic use of alcohol and drugs may also interfere with the production of neurotransmitters, further impairing mood regulation and cognitive function. Additionally, withdrawal from substances can induce depressive

episodes, creating a cyclical relationship between substance use and depression.

Fig. 1c represents the connection between alcohol use disorder and depression, showing how excessive alcohol consumption can contribute to depressive symptoms or vice versa. The data highlights the bidirectional influence between these two conditions. This chart depicts the impact of substance abuse on depression (Fig. 1d). It showcases how drug dependency can lead to or worsen depressive episodes, emphasizing the importance of addressing substance abuse in mental health treatment.

The graph presents the prevalence of different mental health disorders, highlighting the factors associated with depression, including schizophrenia, bipolar disorder, eating disorders, anxiety disorders, drug use disorders, depression and alcohol use disorders, and how they coexist with other disorders. The overlapping histogram compares the frequency distribution of five disorders—Depression, Eating, Anxiety, Alcohol Use, and Drug Use Disorders—based on population percentages. Eating (orange) and Drug Use (purple) disorders are highly concentrated at low percentages (0–1%), indicating low prevalence but high frequency in those ranges. Alcohol Use Disorders (red) show a moderate spread, peaking around 1.5%, while anxiety disorders (green) display the broadest distribution (2–6%), suggesting higher prevalence. Depression (blue) peaks between 3–4%, overlapping notably with anxiety, hinting at comorbidity. Overall, the plot underscores the widespread impact of anxiety and depression compared to the narrower yet significant presence of eating and drug use disorders (Fig. 2a).

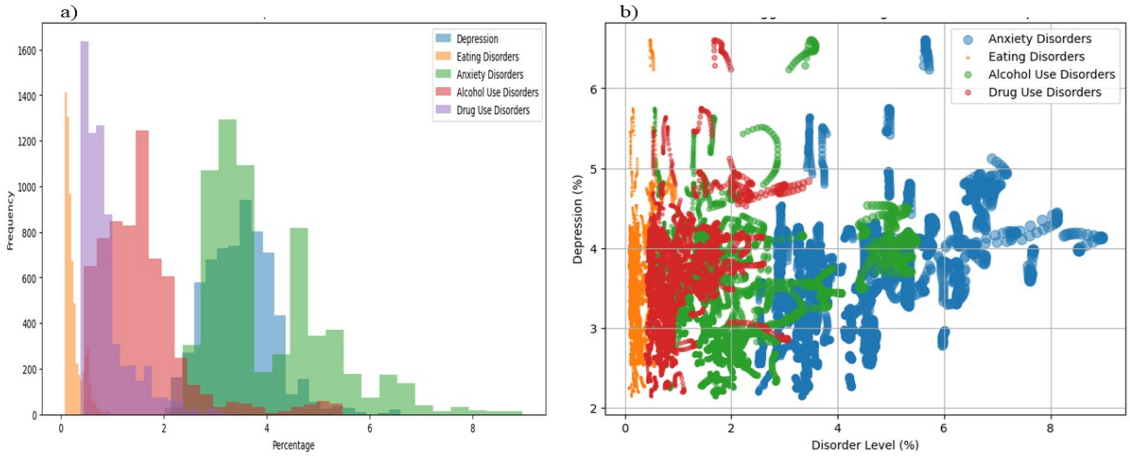

Figure 2 Distribution (a) and correlation (b) of psychological and behavioral disorders in the population.

The scatter plot (Fig. 2b) illustrates the relationship between various mental health and substance use disorders—Anxiety (blue), Eating (orange), Alcohol Use (green), and Drug Use Disorders (red)—and their impact on depression levels (%). Anxiety disorders show a broad and consistent positive correlation with depression, increasing steadily as disorder levels rise. Alcohol use disorders also correlate with depression, though more variably. In contrast, eating and drug use disorders, despite occurring at lower levels (0–2%), are still linked with high depression percentages, indicating a significant impact even at low prevalence. The plot highlights the comorbid nature of these disorders and emphasizes the importance of integrated mental health care. The size of each bubble represents the magnitude of the disorder's effect, making it easy to compare.

Overall, the interaction between these predisposing factors contributes to a complex and dynamic process that may increase an individual's vulnerability to depression. Understanding these factors is crucial for developing effective prevention and intervention strategies, especially in populations at high risk. Further research into the neurobiological mechanisms, particularly in the context of the gut-brain axis and probiotic interventions, may provide novel therapeutic approaches to managing depression and improving mental health outcomes for individuals suffering from these co-occurring conditions.

The predisposing factors of depression could be addressed through neurotransmitter producing probiotic enriched supplements.

Isolation and characterization of probiotic bacterial strains in the sample

The phytobiotic-treated chicken gut and caecum samples, aseptically collected, were ground using a mortar and pestle. To isolate probiotic bacteria, the sample was subjected to serial dilution followed by plating on MRS agar media and the plates were then incubated at 37ºC for 48 hours. After 48 hours of incubation, isolated colonies were obtained. The isolated bacterial strain was morphologically characterized and identified by DNA isolation, 16S rRNA sequencing and construction of a phylogenetic tree. 16S rRNA sequencing revealed the bacterial isolate to be *Enterococcus inesii* strain JasBHCBT001 and was submitted to GenBank and has acquired the accession number OQ536300. The phylogenetic tree illustrates the evolutionary relationship of *Enterococcus inesii* strain JasBHCBT001* with various closely and distantly related bacterial strains based on 16S rRNA gene sequences. The strain JasBHCBT001* clusters

tightly with *Enterococcus inesii* strain GAL7, supported by a 100% bootstrap value, indicating a high level of sequence similarity and confirming its taxonomic placement within the *E. inesii* species.

Thin Layer Chromatographic Analysis of Dopamine and GABA

In the analysis of the neurotransmitters by *Enterococcus sp*. using TLC, spots were visualized when respective reagents were used. The presence of Dopamine and GABA was confirmed by the presence of colored spots on the silica plate with the visualizing agents. Rf values were calculated and matched with the standard, confirming the presence of the neurotransmitters.

UV Spectrophotometric Analysis of Dopamine and GABA

The quantification of the neurotransmitters was done through spectrophotometric analysis following their identification and confirmation. Using the absorbance values obtained through the spectrophotometry and a standard calibration curve was obtained (**Table 2 & 3**).

Table 2 Comparative Standard Curve Data of GABA and Dopamine Based on Concentration vs. Absorbance.

GABA Concentration µg/mL	GABA Absorbance	Dopamine Absorbance
20	0.050	0.075
40	0.100	0.125
60	0.160	0.175
80	0.230	0.220
100	0.350	0.260
120	0.440	0.290
140	0.540	0.340
160	0.611	0.385
180	0.730	0.401
200	0.810	0.413

Table 3 Quantitative analysis of GABA & Dopamine.

S. No.	Sample	OD value @ 630nm	Concentration (µg/ml)
1	**Sample-Dopamine**	**0.4**	**180**
2	**Sample- GABA**	**0.6**	**160**

DNA amplicon has shown a single band with size of 1400 bp and the sample cDNA based amplicon shows similar mild band at 1400 bp which was as expected, supportive to the literature (Fig 3). Hence we can conclude that the gene has been expressed. However gene sequencing and dose dependent studies have to be conducted to validate the result.

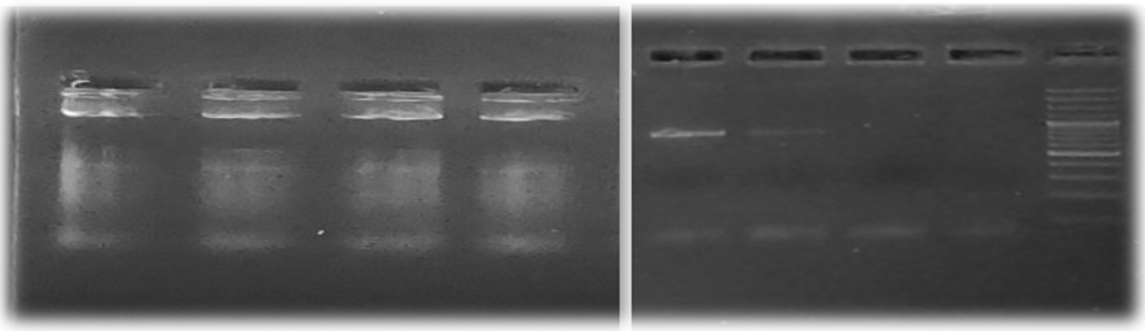

Figure 3 Screening of GAD B

(Gene RNA Quality checking AGE & gadB1 gene PCR product.) **Note:** Lane 1: DNA amplicon; Lane 2: cDNA amplicon; Lane 3, 4: Negative Control Lane 5: 100 bp ladder.

Analysis of PPINs

The PPINs consists of 11 genes, functionally associated with genes involved in glutamate–GABA metabolic and neurotransmission pathways, as depicted in Fig 4a. The network has 11 nodes and 37 edges, with an average local clustering coefficient of 0.861, indicating a high degree of functional connectivity with each other's (Fig 4b), and the p-value (1.61×10^{-9}) confirms that strong biological interrelationships among these genes. The functional description of each gene involved in the network is described in Table 1. The gene ontology enrichment revealed that the genes — GAD1, GAD2, SLC32A1, ABAT, PVALB, MECP2, GLUD1, GLUD2, GLUL, GLS, and GLS2 were predominantly involved in neurotransmitter biosynthesis, synaptic signaling, glutamate and GABA metabolism, and neuronal communication pathways, highlighting their coordinated role in maintaining excitatory–inhibitory balance and

regulating neurochemical signaling within the central nervous system, (Fig 4c & d). Thus, the PPINs provided a systems-level understanding of how key genes involved in glutamate–GABA metabolism and neurotransmission are functionally interconnected with each other.

DISCUSSION

The current study combines the identification of the data-informed markers of depression-predisposing factors and the experimental verification of the probiotic strains of neurotransmitter producers enriched using a phytobiotic supplemented protocol, thereby providing a rationale for the development of a platform for the gut–brain axis. The gut and blood-brain barrier may be crossed by several chemical by-products of the microbiota, including neuroactive metabolites and their related inflammatory mediators, allowing transit through the Brain-Gut-Microbiome system[14].

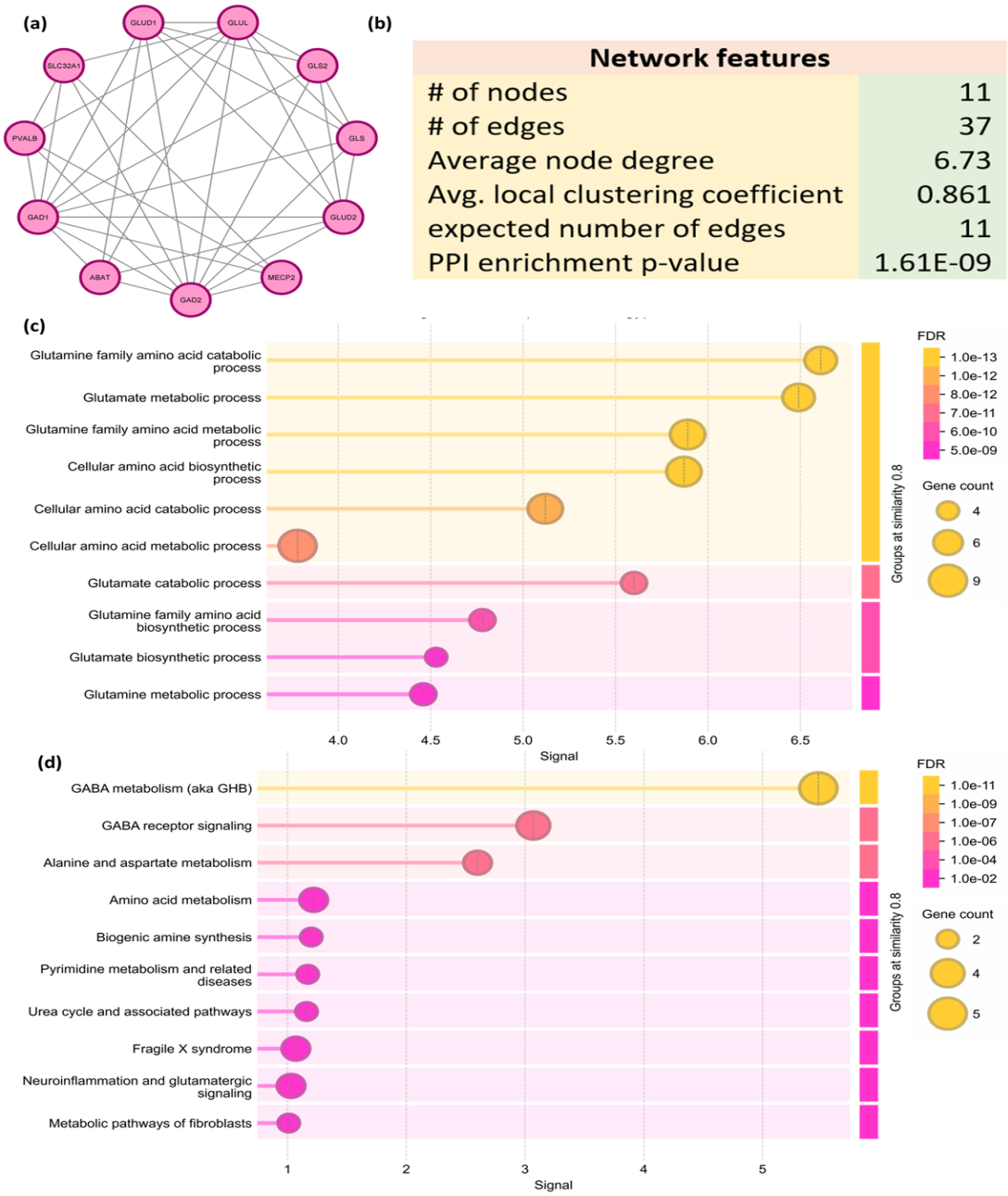

Figure 4 Constructed protein-protein interaction networks (a) of genes associated with GABAergic neurotransmission, (b) predicted network features, biological process based on gene ontology enrichments, and Wiki Pathway (d).

Growing data suggest that the pathogenesis of prevalent neurological disorders such as autism, depression, Alzheimer's, and Parkinson's diseases is significantly influenced by microbial dysbiosis (Mitre) Given the impact of the gut microbiota on brain connections, particularly in terms of enhancing mental health, it would seem that a healthy diet rich in probiotics might preserve the gut-brain connection[19].

Analysis of the dataset exhibited significant correlations between depression and comorbid conditions such as anxiety disorders, eating disorders, alcohol use disorder, and drug use disorder. These correlations are consistent with the epidemiology literature indicating high comorbidity of depression/anxiety and substance use disorders[24]. Among these conditions, anxiety disorders were found to exhibit the highest positive correlation with depression, providing partial verification of the concept of shared neurobiological aspects of these disorders [12].

The pattern of comorbidity between the illnesses suggests that there is a neurochemical overlap between them. Stress and anxiety are known to affect the serotonin and GABA neurochemistry by activating the hypothalamic-pituitary-adrenal axis for a long period. Substance use disorders affect the dopamine reward system, leading to mood swings and depression[15]. In addition, the role of eating disorders in altered serotonin functioning and disrupted microbial composition was identified, which further solidified the role of metabolic pathways in depression[22]. All of these findings converge to point towards neurochemistry imbalance, i.e., serotonin, dopamine, and γ-aminobutyric acid (GABA), as a key bridging link between all the predisposing factors[10].

The experimental results obtained in the present study shed light on the mechanistic role of the microbiota in the control of these neurotransmitters. Phytobiotic supplementation stimulated the levels of probiotic bacteria in the chicken gut, resulting in the isolation and molecular characterization of the novel probiotic bacteria **Enterococcus inesii** JasBHCBT001. Functional biochemical assays revealed the bacteria's capacity for the synthesis of the neurotransmitters, dopamine and GABA, which play a significant role in the control of mood states. Spectrophotometric measurements revealed significant levels of the synthesis of both neurotransmitters, i.e., dopamine (180 microgram per mL) and GABA (160 microgram per mL). The amplification of the gadB1 gene encoding glutamate decarboxylase further confirmed the genetic basis of GABA synthesis[20]. GABA is vitally important as an inhibitor of anxiety and stress; conversely, dopamine is tremendously important as an element of reward that is commonly impaired in depression, as are motivation and behavioral responses[7]. Such results are consistent with the emerging pattern of evidence that the microbiota in the gut may produce neuroactive substances that affect the central nervous system through the microbiome-gut-brain axis. Indigenous microbiota in the gut has been demonstrated to influence the biosynthesis of serotonin in the host, with therapeutic possibility[25].

Phytobiotics such as plant-derived polyphenols and flavonoids have been demonstrated to positively influence the microbial population and gut barrier function as well as the immune system. Thus, phytobiotic-induced enrichment of neurotransmitter-producing probiotic strains could be a promising adjunct strategy to counteract neurochemical imbalances associated with depression and its comorbidities [18].

Nevertheless, some limitations need to be considered. Dataset analysis establishes correlation but does not confirm causality. Moreover, in this paper, experimental validation was performed in a poultry model, which, although highly advantageous for the purpose of controlled microbiota studies, would require translational validation in mammalian systems and in human clinical trials. Further studies using metagenomic sequencing, targeted metabolomics, and dose-dependent phytobiotic interventions will be necessary to enhance mechanistic insights.

CONCLUSION

In summary, this investigation connects computational epidemiology with experimental microbiology by linking machine learning-identified depression risk factors to functional microbial neurotransmitter production. The current findings indicate that *Enterococcus inseii* exhibits potential as a psychobiotic, meaning it could serve as a supportive therapeutic agent alongside conventional medications for mental health disorders such as depression and anxiety. Its ability to produce neuroactive compounds, such as gamma-aminobutyric acid (GABA), suggests a possible mechanism through which it may exert positive effects on the gut-brain axis, influencing mood and emotional regulation. This integrative framework furthers the current knowledge of microbiome-based neurochemical modulation and encourages the possible development of neurotransmitter-producing probiotic-enriched supplements as adjuvant therapeutic interventions in the management of depression. Ultimately, these studies will contribute to the development of targeted psychobiotic therapies that can complement existing pharmacological approaches in managing mental health conditions.

Declaration of Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Funding

This research received no external funding. The authors acknowledge the DST FIST and management support of Bishop Heber College , Vivekanandha College of Arts and Sciences for Women and support from Karunya Institute of Technology

Ethics Approval and Consent to Participate

The study was conducted according to the approved procedures after obtaining approval from the Institutional Animal Ethics Committee (IAEC) at Bharathidasan University, Tiruchirappalli, Tamil Nadu, India, for its planning and execution. All procedures adhered to applicable rules and regulations. The registration number for the study was 418/GO/Re/S/01/CPCSEA, dt.. 24.07.2018; (BDU/IAEC/P06/2021).

Author's Contributions

The authors confirm their contribution to the paper: Study conception and design: **RV, JR**; Data collection: **MMN,**

SD; Methodology: **SS, SBN**; Result analysis and interpretation: **MMN, SS** Drafting and reviewing the manuscript: **RV, JR.** All authors reviewed the results and approved the final version of the manuscript.

AVAILABILITY OF DATA AND MATERIALS
Not applicable

Declarations
Authors declare that all works are original and this manuscript has not been published in any other journal

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