Frontiers in Bioscience Elite

Front. Biosci. (Elite Ed) **2026**; 18(2): 44158
https://doi.org/10.31083/FBE44158

Original Research

Synergistic Enhancement of Fecal GABA Content by *Bifidobacterium adolescentis* 4-2 and Mannooligosaccharides in a Human Intestinal Flora Model

Hend Altaib[1,*], Tohru Suzuki[1,‡], Takumi Morioka[2], Mayuko Abe[2], Yassien Badr[3,4]

[1]The United Graduate School of Agricultural Science, Gifu University, 501-1193 Gifu, Japan
[2]Graduate School of Natural Science and Technology, Gifu University, 501-1193 Gifu, Japan
[3]Department of Infectious Diseases and Epidemics, Faculty of Veterinary Medicine, Damanhour University, 22511 El-Beheira, Egypt
[4]Faculty of Applied Biological Sciences, Gifu University, 501-1193 Gifu, Japan
*Correspondence: hend_altaib@yahoo.com (Hend Altaib)
‡Deceased.

Academic Editor: Baohong Zhang
Submitted: 23 June 2025 Revised: 16 October 2025 Accepted: 6 November 2025 Published: 5 June 2026

Abstract

Background: Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system and exerts multiple health-promoting effects, including antidepressant, hypotensive, immunomodulatory, and antidiabetic actions. Previous studies have reported a positive correlation between the abundance of *Bifidobacterium* species in the gut and fecal GABA levels. However, the direct contribution of specific GABA-producing *Bifidobacterium* strains and the associated interaction with prebiotics remain insufficiently characterized. **Methods**: This study aimed to elucidate the direct effects of a GABA-producing strain, *Bifidobacterium adolescentis* 4-2, in combination with the prebiotic mannooligosaccharides (MOS), on fecal GABA concentrations using the Kobe University Human Intestinal Microbiota Model (KUHIMM), an *in vitro* simulation of the human colonic microbiota. GABA levels were quantified via high-performance liquid chromatography, and changes in microbial composition following oligosaccharide supplementation were assessed by next-generation sequencing of 16S rRNA genes. The presence and activity of the β-mannosidase producing genes, essential for MOS degradation and present in the *B. adolescentis* 4-2 genome, were evaluated using enzymatic assays. **Results**: *B. adolescentis* 4-2 significantly increased fecal GABA levels within the KUHIMM. MOS supplementation elevated GABA levels, reduced fecal culture pH, and increased the relative abundance of Actinobacteria, particularly the *Bifidobacterium* species. Notably, the combined application of MOS and *B. adolescentis* 4-2 produced a synergistic increase in GABA production compared with the individual application of each component. β-mannosidase activity assays confirmed the effective utilization of MOS by *B. adolescentis* 4-2, supporting its role in enhancing GABA biosynthesis. **Conclusion**: These findings demonstrate a synergistic interaction between MOS and *B. adolescentis* 4-2 in promoting microbial GABA production in the KUHIMM *in vitro*. This synbiotic combination shows promise for modulating gut-derived GABA levels *in vitro* and warrants further investigation in animal models and human clinical studies.

Keywords: *Bifidobacterium adolescentis*; mannooligosaccharides; GABA; synergistic interactions; prebiotic; probiotic; gut microbiota

1. Introduction

Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the central nervous system and plays a pivotal role in maintaining neurological homeostasis. Beyond its function in the brain, GABA exhibits a wide range of health-promoting effects, including antidepressant, hypotensive, immunomodulatory, and antidiabetic activities [1]. Emerging evidence suggests that the gut microbiota plays a significant role in GABA biosynthesis, with specific bacterial taxa demonstrating the ability to produce this bioactive compound [2,3]. Among these, *Bifidobacterium* has been identified as a key genus involved in intestinal GABA production.

Bifidobacteria are beneficial symbionts that predominantly colonize the gastrointestinal tract of mammals, including humans. However, their relative abundance declines with age, often accompanied by an increase in genera such as *Escherichia coli* and *Clostridium* [4]. This age-related shift in microbiota composition can adversely affect host health by altering the gut metabolite landscape [5,6]. These findings underscore the significance of *Bifidobacterium* supplementation in maintaining microbial balance and promoting host health and well-being. In addition to their gastrointestinal benefits, *Bifidobacteria* exert systemic physiological and psychological effects [7]. One of their notable metabolites is GABA, categorized as a “postbiotic”, a non-viable bacterial product or metabolic byproduct that confers health benefits to the host [8,9]. Microbially derived GABA may influence the host via systemic circulation or through gut-brain neural pathways [10].

Prebiotics, particularly oligosaccharides, are known to enhance the growth and metabolic activity of beneficial gut microbes such as *Bifidobacterium* [11]. Certain strains possess the enzymatic machinery necessary to me-

tabolize oligosaccharides, improving their colonization potential and functional outputs [12,13]. As indigestible dietary components, prebiotics are fermented by the gut microbiota into short-chain fatty acids and other metabolites with systemic effects [14]. Acting as selective substrates, prebiotics modulate the composition and function of the gut microbiota [11–13,15]. For instance, seaweed-derived polysaccharides can enrich *Bacteroides* species [16], while galactooligosaccharides were reported to increase particular *Bifidobacterium* species [17]. These microbial shifts contribute to broader changes in the gut metabolome, including altered production of postbiotics [18]. Given its diverse physiological functions, GABA is considered one of the most valuable postbiotics [19].

Our previous work demonstrated a positive correlation between *Bifidobacterium* abundance and fecal GABA levels in the human gut [20]. Building on these findings, the present study aimed to investigate the direct impact of a GABA-producing *Bifidobacterium* strain, in combination with oligosaccharide-based prebiotics, on fecal GABA production using an *in vitro* fecal fermentation model. Experiments were conducted using the Kobe University Human Intestinal Microbiota Model (KUHIMM), a standardized *in vitro* colonic fermentation system designed to simulate the human gut microbiota under controlled and reproducible conditions [21]. KUHIMM enables longitudinal assessments of microbiota dynamics and metabolite production in response to experimental interventions.

2. Materials and Methods

2.1 Bacterial Strains and Culture Conditions

Several *Bifidobacterium adolescentis* strains were isolated from the gastrointestinal tracts (GIT) of healthy individuals residing in Japan. These isolates are part of the microbial strain collection maintained at the Laboratory of Genome Microbiology, Gifu University, Japan. Additional commercial strains were obtained from the Japan Collection of Microorganisms (JCM).

All strains were cultivated in de Man, Rogosa, and Sharpe (MRS) broth (BD, Cat# MD21152, USA) at 37 °C under anaerobic conditions using a BUG Box anaerobic chamber (Ruskinn Technology Ltd., Bridgend, Wales, UK) and a gas mixture composed of 80% N_2, 10% CO_2, and 10% H_2. To support GABA production, 1% (v/v) monosodium glutamate (MSG; Sigma-Aldrich, Cat# G1626, St. Louis, MO, USA) was added to the MRS broth. For standard cultivation, 30–50 µL of frozen stock (stored at –80 °C) was inoculated into 12 mL of MRS broth and incubated at 37 °C for 24 hours. The culture was subsequently sub-cultured in fresh MRS broth containing 1% MSG and incubated for an additional 48 hours. Cells were then harvested by centrifugation at 6000 ×g for 10 minutes, and the supernatant was collected for high-performance liquid chromatography (HPLC) analysis.

2.2 Fecal Samples Collection and Handling

Fecal samples were obtained from healthy adult volunteers with no history of systemic or psychiatric disorders and no antibiotic usage for at least three months prior to sample collection. Donors (n = 8) were healthy adults aged 21–36 years, including both males and females, with no known dietary restrictions. All reported following a mixed diet typical of the local population. Samples were collected using BD BBL CultureSwab Plus (BD Co., New Jersey, US, Cat# 212550), immediately stored at 4 °C, and processed within 12 hours of collection. Written informed consent was obtained from all participants. The study protocol was approved by the institutional ethics review board of Gifu University (Certificate No. 2019-283).

2.3 Kobe University Human Intestinal Microbiota Model (KUHIMM)

Batch fermentation was conducted using a pH-controlled, multi-channel fermentation system (Kobe University Human Intestinal Microbiota Model, KUHIMM; Kobe, Hyogo, Japan) [22]. The simulator consisted of six parallel and independent vessels. The working volume per vessel was 100 mL of Gifu Anaerobic Medium (GAM broth; Nissui Pharmaceutical Co., Ltd., Code: 05422, Tokyo, Japan) with an initial pH adjusted to 6.5 using manual acid/base titration. KUHIMM is equipped with a pH sensor that continuously monitors and records pH values throughout the entire cultivation period. No fixed pH set point was applied, as the system was designed to record the natural pH changes resulting from microbial fermentation. Anaerobic conditions were maintained by continuous flushing with an N_2/CO_2 (80:20) gas mixture at a rate of 10 mL min^{-1} through a 0.2 µm polytetrafluoroethylene membrane (Pall Corporation, Port Washington, NY, USA) for 30 minutes at 37 °C prior to fermentation. The pH was continuously monitored for each vessel, and continuous stirring was maintained at 300 rpm to ensure homogeneous microbial distribution.

For inoculation, fecal samples were suspended in 2 mL of physiological saline and introduced through the side port of each vessel. Based on the experimental design, three additives were prepared: mannooligosaccharides (MOS; van wankum ingredients BV, Maarssen, Netherlands; powder form), fructooligosaccharides (FOS; SigmaAldrich, Cat. F8052, St. Louis, MO, USA) and dextrin (Dex; Shandong Bailong Chuangyuan BioTech Co., Ltd., Dezhou, Shandong, China), each dissolved in 10 mL of sterilized water at a final concentration of 0.5%. These additives were then added to the test vessels. Additionally, sterile water was added to the control vessels. Fully grown *B. adolescentis* 4-2 was added to designated vessels at 0.1% (v/v) of the total volume.

2.4 HPLC Analysis of GABA

Cell-free supernatants were obtained by membrane filtration (0.45 µm) from both bacterial and fecal cultures. GABA concentrations were quantified using HPLC (1100 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a fluorescence detector (excitation at 350 nm and emission at 450 nm) and a COSMOSIL packed column (5C18-MS-II, 3.0 mm ID × 150 mm; Nacalai Tesque, Kyoto, Japan, cat#34245-31). Prior to analysis, each sample was derivatized with O-phthalaldehyde (OPA; Sigma-Aldrich, St. Louis, MO, USA, cat#P0657) reagent [20,23]. The mobile phase consisted of A ($CH_3CN/CH_3OH/H_2O$, 45/40/15, v/v/v) and B (20 mM KH_2PO_4, pH 6.9, H_3PO_4). Compounds were eluted using the following gradient program: 0–9 min, 100% B; 9–12 min, 89% B; and 12–21 min, 78% B. The column temperature was maintained at 35 °C with a flow rate of 0.7 mL/min. GABA concentrations in unknown samples were calculated by comparing peak area and retention time to those of standard curves prepared using known concentrations.

2.5 DNA Extraction and Bioinformatics Analysis

DNA extraction and next-generation sequencing (NGS) were performed as previously described [20]. Raw NGS data were analyzed using QIIME 2 (version 2020.2; QIIME 2 Development Team) [24]. Additionally, bar plots displaying microbial composition were generated at both the phylum and species levels based on identified operational taxonomic units (OTUs).

2.6 β-mannosidase Activity Assay

Apart from the KUHIMM culture, liquid MRS culture (1 mL) was disrupted by sonication. Sonication was performed using a Fisherbrand™ Sonicator with probe (Fisher Scientific, Waltham, MA, USA) for cell disruption prior to enzyme assay. The disrupted cells were then centrifuged at 13,000 rpm for 15 min at 4 °C, and the supernatant (cell-free extract) was used for the β-mannosidase assay. For the assay, the reaction mixture consisted of 0.4 M sodium acetate buffer (pH 6.0, containing 4 mM $CaCl_2$; Sigma-Aldrich, Cat# C4901, St. Louis, MO, USA), 10 mM p-nitrophenyl-β-D-mannopyranoside (PNPβM; Sigma-Aldrich, Cat# N2127, St. Louis, MO, USA) and distilled water in a 3:1:6 (v/v/v) ratio. A total of 280 µL of this mixture was combined with 46.7 µL of the cell-free extract and incubated at 37 °C for 1 hour. The reaction was terminated by adding 490 µL of 0.2 M Na_2CO_3 (Sigma-Aldrich, Cat# S7899, St. Louis, MO, USA). A 200 µL aliquot of the mixture was then used for spectrophotometric analysis at 450 nm.

Enzymatic activity was quantified based on the concentration of p-nitrophenol released, using a standard curve prepared from known concentrations of p-nitrophenol (Sigma-Aldrich, Cat# N7660, St. Louis, MO, USA), under identical assay conditions.

2.7 Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 9; GraphPad Software, San Diego, CA, USA). Data were presented as mean ± standard deviation (SD) unless otherwise specified. Comparisons between two groups were assessed using unpaired two-tailed Student's *t*-tests. For multiple group comparisons, one-way analysis of variance (ANOVA) was followed by Tukey's post-hoc test to identify significant differences among groups. Moreover, a *p*-value of less than 0.05 was considered statistically significant. All experiments were conducted with biological replicates obtained from five to ten individual volunteers, as specified. Additionally, microbial community analysis was performed using QIIME2 (QIIME 2 Development Team, Flagstaff, AZ, USA).

3. Results

3.1 Targeted Modulation of GABA and Glutamate via Glutamate Conversion by B. adolescentis 4-2 in Human Fecal Microbiota Cultures

To identify strains with strong GABA-producing capabilities, a collection of *Bifidobacterium* isolates, comprising both fecal-derived and commercially available strains (Table 1), was screened. Consistent with previous findings [25], GABA production was found to be markedly strain-specific rather than species-dependent. Among the *B. adolescentis* isolates, strain 4-2 demonstrated the highest GABA yield, achieving 1.4 ± 0.15 g/L.

To further assess its functional potential, *B. adolescentis* 4-2 was introduced into the KUHIMM, an *in vitro* system inoculated with human fecal microbiota from donors exhibiting low baseline GABA levels. Monosodium glutamate (MSG) was provided as the precursor substrate. As depicted in Fig. 1A,B, the presence of *B. adolescentis* 4-2 resulted in a significant increase in fecal GABA concentration compared with both the control and MSG-only conditions. Concurrently, Fig. 1B illustrates a marked reduction in glutamate levels in the *B. adolescentis* 4-2 + MSG group, indicating efficient microbial conversion of glutamate to GABA. These results underscore the strain's capacity to modulate key gut neurotransmitter levels by elevating GABA while reducing glutamate, thereby supporting its application in microbiome-based interventions aimed at the gut-brain axis.

3.2 Prebiotic Oligosaccharides Enhance GABA Production and Reshape the Microbiota in KUHIMM

To further investigate the effect of dietary components on microbial activity, prebiotics including MOS, DEX, and FOS were introduced. Supplementation with these oligosaccharides resulted in a significant increase in GABA levels across all treatment groups compared with the control (untreated) group. Among the evaluated prebiotics, MOS and FOS produced the highest GABA concentrations, with statistically significant differences ($p < 0.01$ to $p < 0.001$)

Table 1. GABA production by various *Bifidobacterium* strains used in the current study, including human fecal isolates and commercial strains.

Bacterial strain	GABA (g/L) ± SD	Source	Origin
B. dentium JCM 1195	2.00 ± 0.05	JCM company	Human
B. adolescentis 4-2*	1.40 ± 0.15	Kobe, Japan	Adult human
*B. adolescentis**	0.60 ± 0.03	Kobe, Japan	Adult human
B. adolescentis 4-16*	0.30 ± 0.12	Kobe, Japan	Adult human
B. adolescentis 3-117*	0.20 ± 0.02	Kobe, Japan	Adult human
B. adolescentis JCM 7042	0.10 ± 0.01	JCM company	Human
B. adolescentis 12-111*	0.10 ± 0.05	Kobe, Japan	Adult human
B. ruminantium JCM 8222	0.18 ± 0.09	JCM company	Animal
*B. catenulatum**	0.20 ± 0.01	Kobe, Japan	Human
B. adolescentis JCM 1275	ND	Kobe, Japan	Human
B. animalis subsp. animalis JCM 1190	0.00 ± 0.00	JCM company	Animal
B. animalis subsp. lactis JCM 10602	0.00 ± 0.00	JCM company	Animal
B. indicum JCM 1302	0.00 ± 0.00	JCM company	Human
B. longum 105A	0.00 ± 0.00	JCM company	Human
B. longum subsp. infantis JCM 1222	0.00 ± 0.00	JCM company	Human
B. breve JCM 1192	0.00 ± 0.00	JCM company	Human
B. minimum JCM 5821	0.00 ± 0.00	JCM company	Human

Note: Strains marked with an asterisk (*) are fecal isolates, ND refers to non-detected.

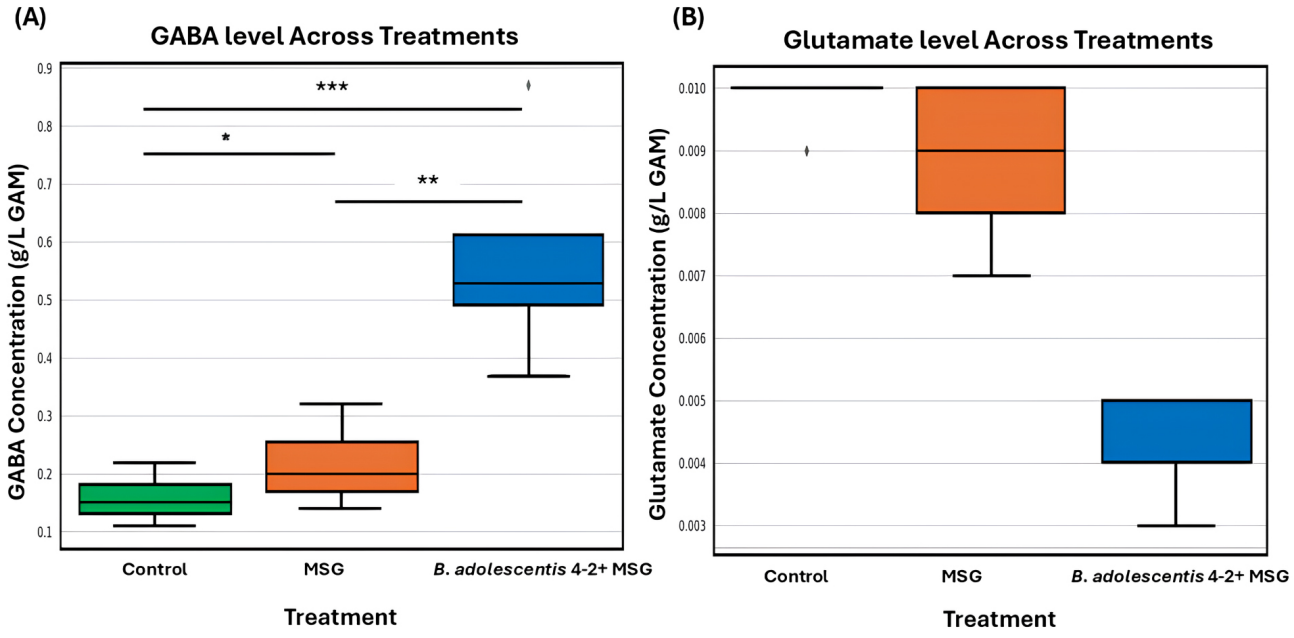

Fig. 1. Gamma-aminobutyric acid (GABA) and glutamate concentrations across different fecal culture conditions. GABA (A) and glutamate (B) concentrations were measured in fecal culture samples obtained from five individual volunteers under three conditions: Control (green), MSG supplementation (orange), and *Bifidobacterium adolescentis* 4-2 with MSG (blue). Boxplots depict the data distribution across volunteers, where the horizontal line represents the median, the box represents the interquartile range (IQR), and whiskers extend to 1.5 × IQR. Diamonds (♦) represent individual statistical outliers falling outside this range. Co-culturing with *B. adolescentis* 4-2 and MSG resulted in a pronounced increase in GABA levels and a reduction in glutamate levels, indicating microbial conversion during fecal fermentation. Statistical significance was determined using one-way analysis of variance followed by Tukey's multiple comparison test, with comparisons indicated above the bars (* = $p < 0.05$; ** = $p < 0.01$; and *** = $p < 0.001$). GABA, Gamma-aminobutyric acid; MSG, monosodium glutamate; ANOVA, one-way analysis of variance.

observed particularly between the control and MOS/FOS groups (Fig. 2A). In contrast, glutamate concentrations remained relatively stable across all treatments (Fig. 2B), suggesting that the increase in GABA was not merely attributed

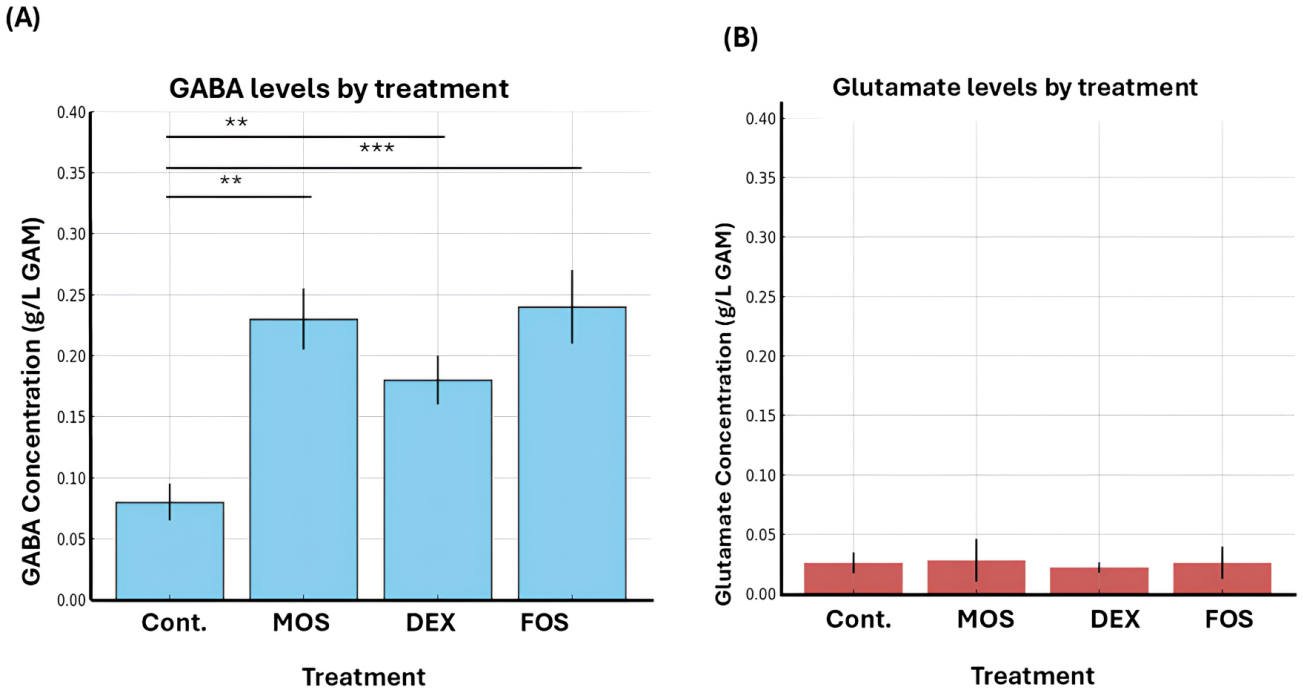

Fig. 2. Effect of different treatments on gamma amino butyric acid (GABA) and glutamate concentrations in the gut microbiota culture. Bar graphs show the mean concentrations (±SD) of GABA (A) and glutamate (B) in response to four treatments: Control (Cont.), mannan oligosaccharides (MOS), dextrin (DEX), and fructo-oligosaccharides (FOS). GABA concentrations were significantly higher in the MOS and FOS groups compared with the control. Although MOS and FOS showed higher average GABA levels than DEX, the differences were not statistically significant ($p > 0.05$). Glutamate levels remained relatively low and stable across treatments. Data are based on pooled measurements from five biological replicates per group. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test, with comparisons indicated above the bars (** = $p < 0.01$; *** = $p < 0.001$). Error bars represent standard deviations.

to elevated substrate availability but rather to enhanced microbial metabolic modulation.

During the 24-hour fermentation, pH values decreased more markedly and remained lower in cultures treated with oligosaccharides, particularly MOS and FOS, compared with the control, which showed only a slight drop followed by partial recovery (Fig. 3A). This sustained acidification indicates enhanced microbial fermentation activity.

Microbiota analysis indicated an enrichment of Actinobacteria across all treatment groups, with the greatest increase observed under MOS and FOS conditions (Fig. 3B). Within this phylum, *Bifidobacterium* species, particularly *B. adolescentis*, became more predominant, especially in donor samples 1 and 2 (Fig. 3C). This taxonomic shift aligns with previous reports demonstrating *B. adolescentis*'s preference for oligosaccharides and its ability to synthesize GABA [25,26].

3.3 B. adolescentis 4-2 Efficiently Assimilates MOS

Mannan hydrolysis requires multiple synergistic glycoside hydrolases. Genomic analysis revealed that *B. adolescentis* 4-2 possesses two key genes involved in this process: *manB*, encoding a β-mannosidase precursor, and *manA*, encoding an exo-acting β-mannosidase (Fig. 4A). The β-mannosidase activity of *B. adolescentis* 4-2 was evaluated under various carbon sources. MOS supplementation significantly increased enzymatic activity compared with glucose, indicating that MOS serves as a preferred substrate (Fig. 4B). These results confirm the strain's capacity to assimilate MOS efficiently.

3.4 Synergistic Enhancement of GABA Production by B. adolescentis 4-2 and Mannan-Oligosaccharides in a Gut Microbiota Model

To assess the potential symbiotic effect between *B. adolescentis* 4-2 and oligosaccharides, a gut microbiota model was employed using fecal samples characterized by low baseline GABA levels. Four experimental setups were tested: control (no additives), 0.5% MOS alone, *B. adolescentis* 4-2 alone, and a combination of 0.5% MOS with *B. adolescentis* 4-2. GABA concentrations increased progressively across the groups in the following order: MOS, *B. adolescentis* 4-2, and MOS + *B. adolescentis* 4-2, while glutamate levels were similar to those in the control group (Fig. 5A). The combined treatment produced the highest GABA yield, demonstrating a synergistic interaction between the probiotic strain and MOS under gut-simulating

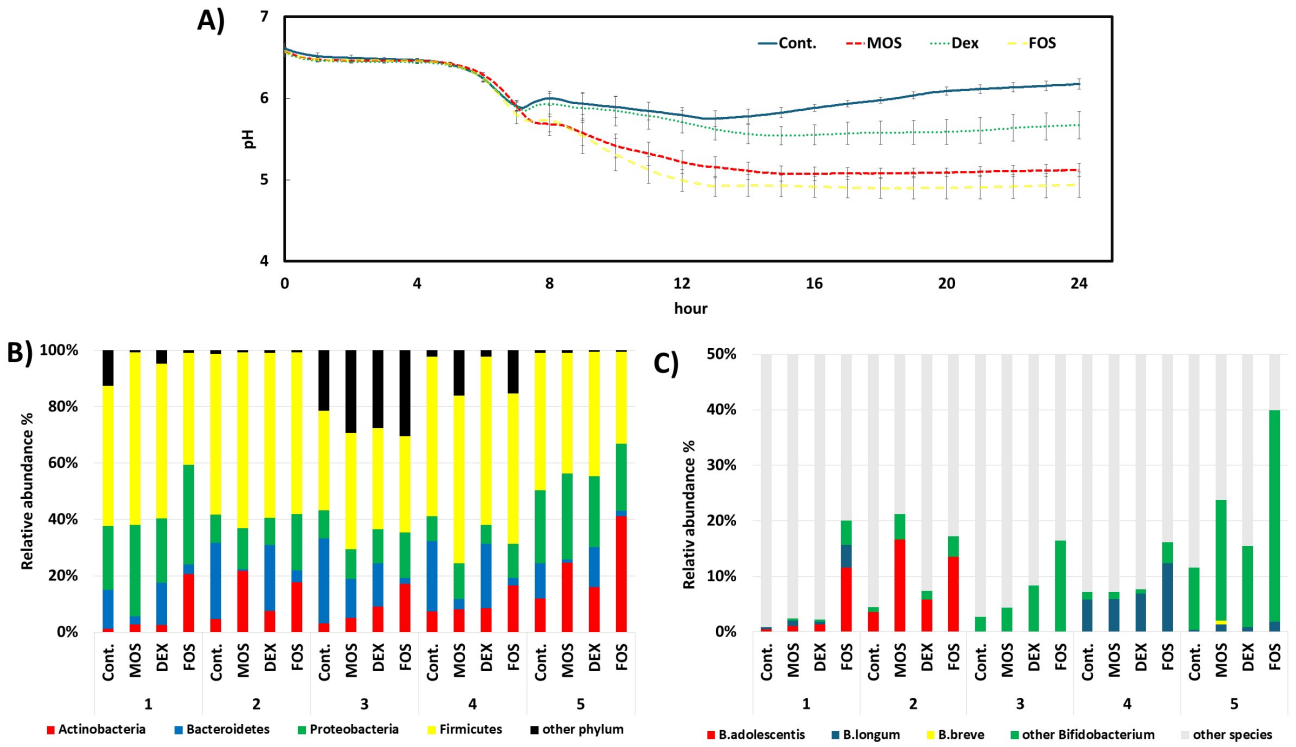

Fig. 3. Impact of different treatments on gut fermentation and microbial composition. (A) pH dynamics over 24 hours of fermentation under different treatments: Control (Cont.), mannooligosaccharides (MOS), dextrin (DEX), and fructooligosaccharides (FOS). A rapid decline in pH occurred in all groups within the first 8 hours, followed by partial recovery in the control and DEX groups, whereas the pH remained low in the MOS and FOS treatments, indicating enhanced fermentation activity. (B) Phylum-level relative abundance of gut microbiota across five biological replicates per treatment. *Firmicutes* and *Proteobacteria* were dominant across most treatments. In most donners, MOS and FOS treatments exhibited increased proportions of *Actinobacteria* and *Proteobacteria* compared to the control. (C) Relative abundance of *Bifidobacterium* species. *B. adolescentis* and other *Bifidobacterium* species were particularly enriched in MOS and FOS treatments, with noticeable inter-individual variation. *B. longum* and *B. breve* were detected in lower but variable proportions.

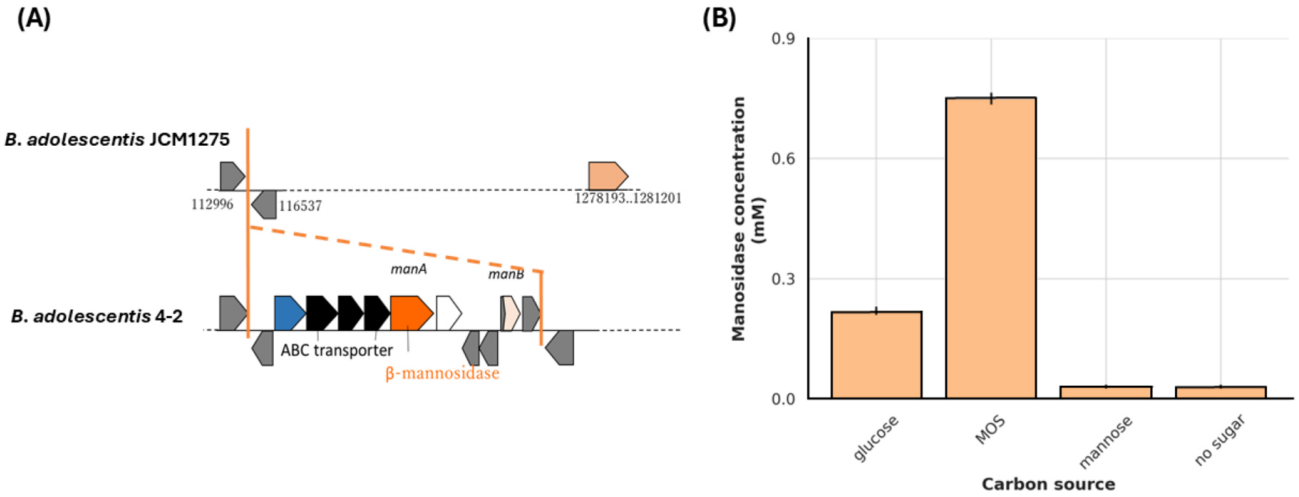

Fig. 4. β**-mannosidase induction by different carbon sources and proposed manno-oligosaccharide (MOS) utilization genes.** (A) Schematic representation of the proposed MOS utilization genes. (B) β-mannosidase activity in response to different carbon sources. Cultures were grown using glucose, MOS, mannose, or no sugar as the sole carbon source. The β-mannosidase concentration (mM) was quantified and plotted. Bars represent the mean $\pm$ standard deviation. MOS induced the highest β-mannosidase production, whereas mannose and no sugar conditions resulted in minimal enzyme activity, indicating that MOS specifically triggers β-mannosidase induction.

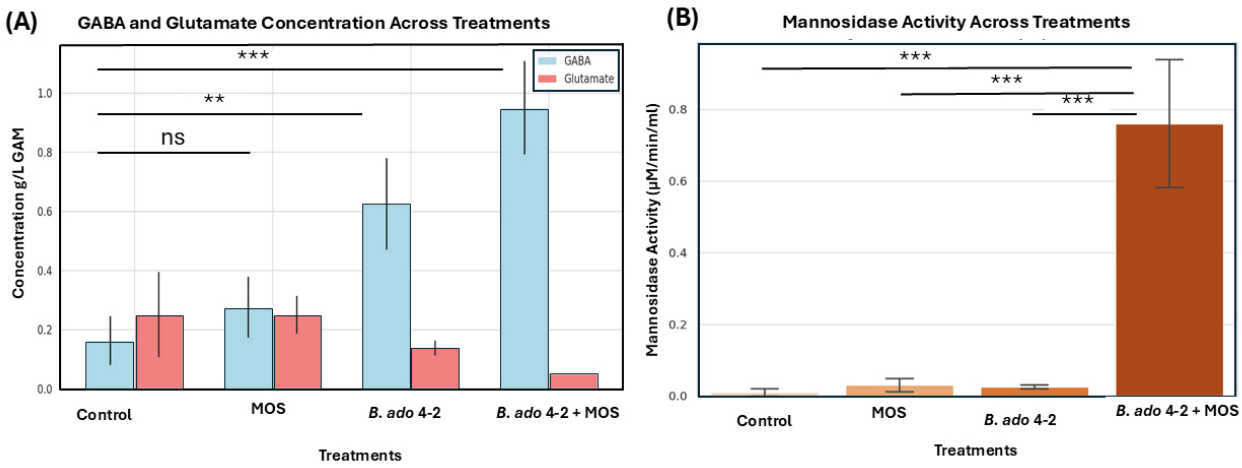

Fig. 5. Effects of treatments on gamma amino butyric acid (GABA) and glutamate concentrations and mannosidase activity. (A) Bar graph displaying the concentrations of GABA (blue bars) and glutamate (red bars) across four treatment groups: Control, MOS (mannan oligosaccharides), *B. adolescentis* 4-2, and *B. adolescentis* 4-2 + MOS. GABA concentrations increased significantly in both the *B. adolescentis* 4-2 and *B. adolescentis* 4-2 + MOS treatments, accompanied by corresponding reductions in glutamate levels. (B) Bar graph showing mannosidase activity (measured in µM/min/mL) in the same groups. The highest enzymatic activity was recorded in the *B. adolescentis* 4-2 + MOS group, indicating a synergistic effect. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test, with comparisons noted above the bars (ns = not significant; ** = $p < 0.01$; and *** = $p < 0.001$). Error bars represent standard deviations.

conditions. To confirm this symbiosis, β-mannosidase activity was analyzed across all treatment groups. The highest activity was detected in cultures treated with both *B. adolescentis* 4-2 and MOS (Fig. 5B), confirming the active utilization of MOS by the strain and reinforcing its role in enhancing GABA biosynthesis through a prebiotic-probiotic synergy. This interaction supports the potential development of symbiotic formulations designed to modulate GABA levels within the gut environment.

4. Discussion

This study elucidates the synergistic interaction between the GABA-producing strain *B. adolescentis* 4-2 and the prebiotic MOS in enhancing GABA production within an *in vitro* model of the colonic microbiota. Our findings align with and extend previous reports that identified *Bifidobacterium* species as key contributors to gut-derived GABA [2,3,27]. In particular, the strain specificity of GABA synthesis observed in our screening is consistent with earlier studies demonstrating heterogeneity in GABA production among strains of *B. adolescentis* and *B. breve* [27–29]. *B. adolescentis* strain 4-2 not only exhibited superior GABA biosynthesis in monoculture but also retained its functional capacity within the complex fecal microbial community simulated by KUHIMM. Although *B. dentium* JCM 1195 showed higher GABA production in monoculture, *B. adolescentis* 4-2 was prioritized for synbiotic development because it was isolated from healthy adults, performed stably in the KUHIMM, and possesses β-mannosidase genes enabling MOS utilization. These properties support its physiological relevance and safety as a probiotic candidate, whereas *B. dentium* is less dominant in healthy microbiota and can behave opportunistically [30,31].

Certain gut microbes, such as *Bacteroides* species, possess GABA transaminase activity, which can degrade GABA and thereby influence net concentrations observed in fecal cultures [32]. The introduction of MSG as a precursor facilitated robust microbial conversion of GABA. Corroborating studies have shown that exogenous glutamate enhances GABA production in various lactobacilli and Bifidobacteria [33]. Notably, the concurrent depletion of glutamate and the rise in GABA levels in the *B. adolescentis* 4-2 treatment group highlight the enzymatic activity of glutamate decarboxylase (GAD), a key enzyme responsible for this metabolic conversion, which has been previously characterized in other probiotic strains [34].

Beyond monoculture effects, our study demonstrated that MOS supplementation significantly boosts fecal GABA levels and supports the proliferation of Actinobacteria, particularly *Bifidobacterium* spp., which aligns with prior research showing selective bifidogenic effects of MOS and similar oligosaccharides [12,15]. Notably, MOS-driven acidification of the culture medium is indicative of active microbial fermentation, a hallmark of oligosaccharide metabolism [13,14]. The enrichment of *B. adolescentis* in MOS-treated cultures, especially in low-GABA fecal donors, highlights the prebiotic potential of MOS to

selectively enhance strains capable of psychobiotic function. While these findings emphasize the selective bifidogenic effects of MOS, it is important to note that other gut commensals may utilize MOS. In particular, members of the genera *Bacteroides* and *Firmicutes* harbor diverse glycoside hydrolases and transport systems that enable the degradation and assimilation of mannan-derived oligosaccharides [22]. Such taxa often act as primary degraders within the gut ecosystem, breaking down complex polysaccharides into smaller metabolites that can be cross-fed to other microorganisms [16]. Consequently, competitive utilization of MOS by these microbes could reduce the relative availability of the substrate for Bifidobacteria, potentially influencing the magnitude of GABA production [22,35]. On the other hand, cross-feeding interactions may generate complementary metabolic outputs, such as short-chain fatty acids, which could synergize with GABA to modulate host physiology [15,36]. Previous reports have demonstrated that the balance between competition and cooperation for prebiotic substrates strongly shapes microbial community structure and functional outcomes both *in vitro* and *in vivo* [15,16,35,37].

The synergistic increase in GABA production observed with the combined administration of *B. adolescentis* 4-2 and MOS marks a pivotal contribution of this study. While earlier works have proposed the benefit of combining prebiotics with probiotics to create synbiotics [38], our study is among the first to mechanistically link this synergy to enhanced GABA biosynthesis in a fecal microbiota model. This finding is further supported by the elevated β-mannosidase activity under co-treatment conditions, suggesting efficient MOS degradation and utilization by strain 4-2. The genomic presence of *manA* and *manB*, which encode key enzymes for β-mannan degradation, supports previous bioinformatics analyses identifying glycoside hydrolase genes in Bifidobacteria adapted for oligosaccharide metabolism [35,39]. Our results extend the findings of Jadhav *et al*. [36], who demonstrated that diet-driven modulation of gut microbiota can influence neurotransmitter pools. Similarly, Strandwitz *et al*. [32,40] reported that microbial production of GABA and other neuroactive metabolites can directly modulate host neurophysiology via the gut-brain axis. Our study provides experimental evidence that prebiotic-probiotic combinations can be optimized to enhance microbial GABA production, thereby highlighting their potential as psychobiotics for both animal and human trials. It is important to note that donor-specific microbiota composition may contribute to variability in GABA production and microbial shifts. Age, sex, and diet are known determinants of baseline microbiota, and such factors may influence responsiveness to MOS and probiotic supplementation.

Importantly, the application of the KUHIMM allowed for controlled evaluation of microbial and metabolic shifts over time. This model mimics key aspects of the human colon environment, providing a reproducible platform to test gut-derived bioactivity and overcoming limitations associated with *in vivo* variability [21,22]. While KUHIMM provides a reproducible platform to assess microbial and metabolic responses, it does not capture host-microbe interactions, absorption, signaling, or immune responses, which should be investigated in subsequent animal and human intervention studies.

5. Conclusion

In conclusion, the synergistic increase in GABA production observed with *B. adolescentis* 4-2 and MOS illustrates the potential of targeted synbiotic strategies to modulate gut neurochemistry under *in vitro* conditions. These findings demonstrate the potential of MOS and *B. adolescentis* 4-2 to synergistically enhance GABA production in the KUHIMM. Further studies in animal and human systems are required to establish whether these effects translate to clinical outcomes.

Availability of Data and Materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

HA and TS designed the research study. TM, YB and HA performed the research. YB, HA, and MA conducted experiments and analyzed the data. HA wrote the first draft of the manuscript. All authors contributed to editorial revisions of the manuscript. All authors read and approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. The late Professor TS contributed to the conception and execution of this study. His scientific insight and dedication were instrumental, and this work is respectfully dedicated to his memory.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. The protocol was approved by the institutional ethics review board of Gifu University (Certificate No. 2019-283). Written informed consent was obtained from all participants.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used AI assistance to check spelling and grammar. After that, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

[1] Rashmi D, Zanan R, John S, Khandagale K, Nadaf A. Chapter 13 - γ-Aminobutyric Acid (GABA): Biosynthesis, Role, Commercial Production, and Applications. Studies in Natural Products Chemistry. 2018; 57: 413–452. https://doi.org/10.1016/B978-0-444-64057-4.00013-2.

[2] Cui Y, Miao K, Niyaphorn S, Qu X. Production of Gamma-Aminobutyric Acid from Lactic Acid Bacteria: A Systematic Review. International Journal of Molecular Sciences. 2020; 21: 995. https://doi.org/10.3390/ijms21030995.

[3] Dhakal R, Bajpai VK, Baek KH. Production of gaba (γ - Aminobutyric acid) by microorganisms: a review. Brazilian Journal of Microbiology. 2012; 43: 1230–1241. https://doi.org/10.1590/S1517-83822012000400001.

[4] Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, *et al*. Gut microbiome and aging: Physiological and mechanistic insights. Nutrition and Healthy Aging. 2018; 4: 267–285. https://doi.org/10.3233/NHA-170030.

[5] Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. PeerJ. 2019; 7: e7502. https://doi.org/10.7717/peerj.7502.

[6] Turroni F, van Sinderen D, Ventura M. Bifidobacteria: insights into the biology of a key microbial group of early life gut microbiota. Microbiome Research Reports. 2021; 1: 2. https://doi.org/10.20517/mrr.2021.02.

[7] Altaib H, Badr Y, Suzuki T. Bifidobacteria and Psychobiotic Therapy: Current Evidence and Future Prospects. Reviews in Agricultural Science. 2021; 9: 74–91. https://doi.org/10.7831/ras.9.0_74.

[8] Savignac HM, Kiely B, Dinan TG, Cryan JF. Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. Neurogastroenterology and Motility. 2014; 26: 1615–1627. https://doi.org/10.1111/nmo.12427.

[9] Braga JD, Thongngam M, Kumrungsee T. Gamma-aminobutyric acid as a potential postbiotic mediator in the gut-brain axis. NPJ Science of Food. 2024; 8: 16. https://doi.org/10.1038/s41538-024-00253-2.

[10] Mazzoli R, Pessione E. The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. Frontiers in Microbiology. 2016; 7: 1934. https://doi.org/10.3389/fmicb.2016.01934.

[11] Wang H, Huang X, Tan H, Chen X, Chen C, Nie S. Interaction between dietary fiber and bifidobacteria in promoting intestinal health. Food Chemistry. 2022; 393: 133407. https://doi.org/10.1016/j.foodchem.2022.133407.

[12] Monteagudo-Mera A, Arthur JC, Jobin C, Keku T, Bruno-Barcena JM, Azcarate-Peril MA. High purity galacto-oligosaccharides enhance specific Bifidobacterium species and their metabolic activity in the mouse gut microbiome. Beneficial Microbes. 2016; 7: 247–264. https://doi.org/10.3920/BM2015.0114.

[13] Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. The American Journal of Clinical Nutrition. 1999; 69: 1052S–1057S. https://doi.org/10.1093/ajcn/69.5.1052s.

[14] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition. 1995; 125: 1401–1412. https://doi.org/10.1093/jn/125.6.1401.

[15] Bedu-Ferrari C, Biscarrat P, Langella P, Cherbuy C. Prebiotics and the Human Gut Microbiota: From Breakdown Mechanisms to the Impact on Metabolic Health. Nutrients. 2022; 14: 2096. https://doi.org/10.3390/nu14102096.

[16] Pudlo NA, Pereira GV, Parnami J, Cid M, Markert S, Tingley JP, *et al*. Diverse events have transferred genes for edible seaweed digestion from marine to human gut bacteria. Cell Host & Microbe. 2022; 30: 314–328.e11. https://doi.org/10.1016/j.chom.2022.02.001.

[17] Kosuwon P, Lao-Araya M, Uthaisangsook S, Lay C, Bindels J, Knol J, *et al*. A synbiotic mixture of scGOS/lcFOS and Bifidobacterium breve M-16V increases faecal Bifidobacterium in healthy young children. Beneficial Microbes. 2018; 9: 541–552. https://doi.org/10.3920/BM2017.0110.

[18] Cheng W, Lu J, Li B, Lin W, Zhang Z, Wei X, *et al*. Effect of Functional Oligosaccharides and Ordinary Dietary Fiber on Intestinal Microbiota Diversity. Frontiers in Microbiology. 2017; 8: 1750. https://doi.org/10.3389/fmicb.2017.01750.

[19] Ngo DH, Vo TS. An Updated Review on Pharmaceutical Properties of Gamma-Aminobutyric Acid. Molecules. 2019; 24: 2678. https://doi.org/10.3390/molecules24152678.

[20] Altaib H, Nakamura K, Abe M, Badr Y, Yanase E, Nomura I, *et al*. Differences in the Concentration of the Fecal Neurotransmitters GABA and Glutamate Are Associated with Microbial Composition among Healthy Human Subjects. Microorganisms. 2021; 9: 378. https://doi.org/10.3390/microorganisms9020378.

[21] Hoshi N, Inoue J, Sasaki D, Sasaki K. The Kobe University Human Intestinal Microbiota Model for gut intervention studies. Applied Microbiology and Biotechnology. 2021; 105: 2625–2632. https://doi.org/10.1007/s00253-021-11217-x.

[22] Oba S, Sunagawa T, Tanihiro R, Awashima K, Sugiyama H, Odani T, *et al*. Prebiotic effects of yeast mannan, which selectively promotes Bacteroides thetaiotaomicron and Bacteroides ovatus in a human colonic microbiota model. Scientific Reports. 2020; 10: 17351. https://doi.org/10.1038/s41598-020-74379-0.

[23] Lindroth P, Mopper K. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. Analytical Chemistry. 1979; 51: 1667–1674.

[24] Estaki M, Jiang L, Bokulich NA, McDonald D, González A, Kosciolek T, *et al*. QIIME 2 Enables Comprehensive End-to-End Analysis of Diverse Microbiome Data and Comparative Studies with Publicly Available Data. Current Protocols in Bioinformatics. 2020; 70: e100. https://doi.org/10.1002/cpbi.100.

[25] Duranti S, Ruiz L, Lugli GA, Tames H, Milani C, Mancabelli L, *et al*. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. Scientific Reports. 2020; 10: 14112. https://doi.org/10.1038/s41598-020-70986-z.

[26] Ruiz E, Gullón B, Moura P, Carvalheiro F, Eibes G, Cara C, *et al*. Bifidobacterial growth stimulation by oligosaccharides generated from olive tree pruning biomass. Carbohydrate Polymers. 2017; 169: 149–156. https://doi.org/10.1016/j.carbpol.2017.04.014.

[27] Altaib H, El-Nouby MAM, Badr Y. An Overview of GABA Production by Microorganisms. Microbial Nutraceuticals: Products and Processes. Microbial Nutraceuticals: Products and Processes (pp. 365–398). Wiley Book: Hoboken, New Jersey, USA. 2025.

[28] Leser T, Baker A. Bifidobacterium adolescentis - a beneficial microbe. Beneficial Microbes. 2023; 14: 525–551. https://doi.org/10.1163/18762891-20230030.

[29] Li J, Li Y, Zhao J, Li L, Wang Y, Chen F, *et al*. Effects of Bifidobacterium breve 207-1 on regulating lifestyle behaviors

and mental wellness in healthy adults based on the microbiome-gut-brain axis: a randomized, double-blind, placebo-controlled trial. European Journal of Nutrition. 2024; 63: 2567–2585. https://doi.org/10.1007/s00394-024-03447-2.

[30] Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, *et al*. Bacteria of dental caries in primary and permanent teeth in children and young adults. Journal of Clinical Microbiology. 2008; 46: 1407–1417. https://doi.org/10.1128/JCM.01410-07.

[31] Hidalgo-Cantabrana C, Delgado S, Ruiz L, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and Their Health-Promoting Effects. Microbiology Spectrum. 2017; 5: 10.1128/microbiolspec.bad-0010-2016. https://doi.org/10.1128/microbiolspec.BAD-0010-2016.

[32] Strandwitz P, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, *et al*. GABA-modulating bacteria of the human gut microbiota. Nature Microbiology. 2019; 4: 396–403. https://doi.org/10.1038/s41564-018-0307-3.

[33] Yunes RA, Poluektova EU, Dyachkova MS, Klimina KM, Kovtun AS, Averina OV, *et al*. GABA production and structure of gadB/gadC genes in Lactobacillus and Bifidobacterium strains from human microbiota. Anaerobe. 2016; 42: 197–204. https://doi.org/10.1016/j.anaerobe.2016.10.011.

[34] Yogeswara IBA, Maneerat S, Haltrich D. Glutamate Decarboxylase from Lactic Acid Bacteria-A Key Enzyme in GABA Synthesis. Microorganisms. 2020; 8: 1923. https://doi.org/10.3390/microorganisms8121923.

[35] Saito Y, Shigehisa A, Watanabe Y, Tsukuda N, Moriyama-Ohara K, Hara T, *et al*. Multiple Transporters and Glycoside Hydrolases Are Involved in Arabinoxylan-Derived Oligosaccharide Utilization in Bifidobacterium pseudocatenulatum. Applied and Environmental Microbiology. 2020; 86: e01782-20. https://doi.org/10.1128/AEM.01782-20.

[36] Jadhav A, Bajaj A, Xiao Y, Markandey M, Ahuja V, Kashyap PC. Role of Diet-Microbiome Interaction in Gastrointestinal Disorders and Strategies to Modulate Them with Microbiome-Targeted Therapies. Annual Review of Nutrition. 2023; 43: 355–383. https://doi.org/10.1146/annurev-nutr-061121-094908.

[37] Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 2012; 3: 289–306. https://doi.org/10.4161/gmic.19897.

[38] Bisht D, Pal D, Shrestha R. Introduction to Probiotics, Prebiotics, and Synbiotics: A Holistic Approach. Probiotics (pp. 1–28). CRC Press: Boca Raton, Florida, USA. 2024.

[39] Wei X, Yu L, Zhang C, Ni Y, Zhang H, Zhai Q, *et al*. Genetic-Phenotype Analysis of *Bifidobacterium bifidum* and Its Glycoside Hydrolase Gene Distribution at Different Age Groups. Foods. 2023; 12: 922. https://doi.org/10.3390/foods12050922.

[40] Strandwitz P. Neurotransmitter modulation by the gut microbiota. Brain Research. 2018; 1693: 128–133. https://doi.org/10.1016/j.brainres.2018.03.015.