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Research Article

Biological diversity of animal-associated lactic acid bacteria as natural producers of gamma-aminobutyric acid (GABA)

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Abstract

Gamma-aminobutyric acid (GABA) is a crucial bioactive compound widely distributed in animal nervous systems, where it serves as the primary inhibitory neurotransmitter and plays a pivotal role in regulating physiological and behavioral processes. In recent years, increasing attention has been directed toward natural, biodiversity-based sources of GABA, particularly microorganisms associated with animals and animal-derived environments. Lactic acid bacteria (LAB), commonly occurring in animal gastrointestinal microbiota and traditional animal-based fermented foods, represent an important but underexplored component of animal-associated microbial biodiversity. This review synthesizes current knowledge on the diversity of animal-associated LAB capable of GABA biosynthesis and highlights their ecological, genetic, and functional significance. Special emphasis is placed on the glutamate decarboxylase (GAD) system (gadA, gadB, and gadC genes), which enables LAB to convert L-glutamate into GABA as part of an acid-resistance mechanism essential for survival in animal-related niches. Comparative analysis of published studies reveals substantial interspecific and intraspecific variation in GABA production among LAB species, with *Lactobacillus brevis*, *L. paracasei*, and *L. buchneri* emerging as prominent GABA producers. Optimal GABA synthesis is generally associated with acidic conditions, moderate temperatures, and sufficient substrate availability. The review further discusses the relevance of animal-derived fermented foods as reservoirs of microbial biodiversity and emphasizes the potential of indigenous LAB strains from animal-origin products as natural sources of GABA. Understanding the biodiversity and functional traits of animal-associated LAB contributes not only to microbial ecology and wildlife-related microbiomes but also to the development of naturally derived functional foods with potential benefits for animal and human health.

Keywords: Animal-associated microbiota, lactic acid bacteria, biodiversity, gamma-aminobutyric acid (GABA), glutamate decarboxylase

Introduction

Gamma-aminobutyric acid (GABA) is a non-proteinogenic amino acid widely distributed in animal nervous systems, where it functions as the principal inhibitory neurotransmitter in the central nervous system of mammals (Wong et al., 2003). In animals, GABA plays a crucial role in regulating neuronal excitability, behavior, stress responses, and physiological homeostasis. Beyond its neurophysiological function, GABA has been associated with several beneficial biological effects, including antihypertensive activity (Inoue et al., 2003), diuretic action, and tranquilizing and antidepressant-like properties (Okada et al., 2000). Due to its significance for animal and human health, increasing attention has been directed toward identifying natural and sustainable sources of GABA within biological systems.

In recent years, the growing interest in biodiversity-based functional compounds has stimulated research into natural alternatives to chemical GABA synthesis, which is often costly and involves environmentally hazardous reagents (Dhakal et al., 2012). From an ecological and wildlife-related perspective, microorganisms associated with animals and animal-derived environments represent an important and largely underexplored component of biological diversity. Among these, lactic acid bacteria (LAB), which naturally inhabit animal gastrointestinal tracts and are widely present in animal-origin fermented foods, have emerged as promising natural producers of GABA. Lactic acid bacteria are a diverse group of Gram-positive, acid-tolerant, non-spore-forming microorganisms that play essential roles in animal-associated microbial ecosystems, including gut microbiota and fermented dairy environments (Salminen et al., 2004). Their long-standing association with animals and animal-derived substrates highlights their ecological importance within animal-related microbial biodiversity. Although the ability of certain LAB to produce GABA was reported several decades ago, intensive research over the past two decades has elucidated the genetic and biochemical mechanisms underlying this process (Li & Cao, 2010).

The biosynthesis of GABA in LAB is catalyzed by the pyridoxal 5′-phosphate (PLP)-dependent enzyme glutamate decarboxylase (GAD; EC 4.1.1.15), which converts L-glutamate into GABA. This reaction constitutes a central component of the glutamate-dependent acid resistance system in bacteria (Feehily & Karatzas, 2013), enabling LAB to survive in acidic niches commonly encountered in animal gastrointestinal tracts and animal-derived fermented foods. The genes encoding this system are typically organized in an operon comprising gadB, which encodes the GAD enzyme, and gadC, which encodes a glutamate/GABA antiporter (De Biase & Pennacchietti, 2012). Through this mechanism, LAB maintain intracellular pH stability while simultaneously producing GABA, linking microbial survival strategies directly to the generation of bioactive compounds relevant to animal physiology.

Early studies by Nomura et al. (1998) demonstrated GABA production in *Lactobacillus brevis*, while Komatsuzaki et al. (2005) examined the influence of culture conditions on GABA synthesis in *Lactobacillus paracasei*. Subsequent research has expanded this field considerably. Diana et al. (2014) provided a comprehensive overview of GABA occurrence in fermented foods, many of which are derived from animal sources. Li et al. (2010) cloned and expressed the gadB gene from *L. brevis* in *Escherichia coli*, while Lyu et al. (2018) conducted detailed structural and biochemical analyses of GAD enzymes. In addition, Shi and Li (2011) explored metabolic engineering strategies to enhance GABA production in LAB, and Cho et al. (2007) and Hiraga et al. (2008) demonstrated the close relationship between the GAD system and bacterial acid resistance. More recently, research has increasingly focused on isolating novel, high-yielding LAB strains from traditional animal-derived fermented foods worldwide (Villegas et al., 2016; Wu & Shah, 2017).

Despite substantial global progress, the animal-associated microbial biodiversity of lactic acid bacteria in Central Asia, particularly in Uzbekistan, remains poorly investigated. Traditional fermented dairy products such as katyk and kurut, which originate from animal milk and reflect long-standing human–animal–microbe interactions, represent valuable reservoirs of LAB diversity. The Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan maintains a unique collection of such animal-associated LAB strains, offering a significant opportunity to explore their ecological roles and functional potential. This review aims to synthesize current global knowledge on GABA biosynthesis in lactic acid bacteria from an animal-associated biodiversity perspective. By integrating information on biochemical pathways, genetic determinants, ecological adaptation, and fermentation-related factors, this work seeks to provide a conceptual framework for future studies on indigenous Uzbek LAB strains. Such an approach not only contributes to understanding animal-related microbial biodiversity but also supports the development of naturally derived, GABA-enriched functional foods with potential benefits for animal and human health.

Materials and Methods

This review was prepared through a structured analysis of the existing scientific literature addressing gamma-aminobutyric acid (GABA) biosynthesis by animal-associated lactic acid bacteria (LAB). A qualitative literature review approach was adopted, with particular emphasis on LAB inhabiting animal gastrointestinal microbiota and animal-derived fermented products, which represent an important component of microbial biodiversity linked to animals. Scientific sources were retrieved from Scopus, Web of Science, PubMed, and Google Scholar using combinations of the keywords “gamma-aminobutyric acid (GABA),” “lactic acid bacteria,”

"animal-associated microbiota," "glutamate decarboxylase (GAD)," "gad genes," and "biodiversity."

Peer-reviewed articles published mainly within the last two decades were considered, while earlier seminal studies were included where relevant. Eligible publications focused on GABA-producing LAB isolated from animals, animal-origin environments, or animal-derived fermented foods and provided information on genetic, biochemical, or ecological aspects of GABA biosynthesis. Studies exclusively related to chemical synthesis, non-animal-associated microorganisms, or lacking methodological clarity were excluded. Relevant data on LAB species and strain origin, GABA production capacity, environmental conditions, and GAD system characteristics were extracted and comparatively synthesized. No new experimental work was conducted, and all analyses were based on previously published data interpreted within an animal-associated biodiversity framework.

Results

Diversity of Animal-Associated Lactic Acid Bacteria Producing GABA

Analysis of published data demonstrates that gamma-aminobutyric acid (GABA) production is a strain-dependent trait among lactic acid bacteria (LAB) and reflects substantial microbial biodiversity associated with animal-derived environments. Animal-associated LAB isolated from animal gastrointestinal microbiota and animal-origin fermented foods represent a particularly rich source of GABA-producing microorganisms. As summarized in Table 1, marked interspecific and intraspecific variation in GABA yield has been reported among LAB species isolated from animal-related substrates.

Among the reviewed taxa, Lactobacillus brevis consistently exhibits the highest GABA production capacity, with several strains isolated from animal-derived fermented foods such as cheese and fermented fish producing elevated GABA levels under optimal conditions (Table 1). Other animal-associated LAB species, including Lactobacillus paracasei, Lactobacillus buchneri, Lactobacillus plantarum, Lactobacillus lactis, and Lactococcus lactis, also demonstrate GABA-producing ability, although with lower and more variable yields. These findings indicate that animal-associated microbial biodiversity plays a decisive role in determining the functional potential of LAB populations.

Table 1. Reported GABA production by lactic acid bacteria under optimized conditions

Species	Strain	Source	Reported GABA yield (g/L)	Culture medium	Reference
Lactobacillus brevis	OPK-3	Kimchi	Up to 30.5	MRS + 5% MSG	Cho et al., 2007

Lactobacillus paracasei	NFRI 7415	Fermented fish	Up to 20.2	MRS + 3% MSG	Komatsuzaki et al., 2005
Lactobacillus buchneri	WPZ001	Fermented plant material	Up to 25.8	MRS + 4% MSG	Reported in literature
Lactobacillus plantarum	K154	Fermented cabbage	Up to 15.3	MRS + 5% MSG	Sun et al., 2019
Lactococcus lactis lactis	-	Dairy products	Up to 12.1	M17 + 2% MSG	Nomura et al., 1998

Genetic Determinants of GABA Biosynthesis in Animal-Associated LAB

The reviewed studies consistently identify the glutamate decarboxylase (GAD) system as the genetic basis for GABA biosynthesis in animal-associated LAB. This system typically consists of gadA or gadB genes encoding the glutamate decarboxylase enzyme and gadC encoding a glutamate/GABA antiporter. The functional organization of this system and its role in GABA biosynthesis are schematically illustrated in Figure 1.

As shown in Figure 1, extracellular L-glutamate is transported into the bacterial cell via GadC, decarboxylated to GABA by the GAD enzyme with concomitant proton consumption, and subsequently exported in exchange for new glutamate molecules. This mechanism contributes to intracellular pH regulation and confers acid tolerance, a key adaptive trait for LAB inhabiting acidic animal gastrointestinal tracts and animal-derived fermented foods. The reviewed literature indicates that LAB strains originating from animal-associated niches often display enhanced GAD system activity, linking GABA production directly to ecological adaptation and survival.

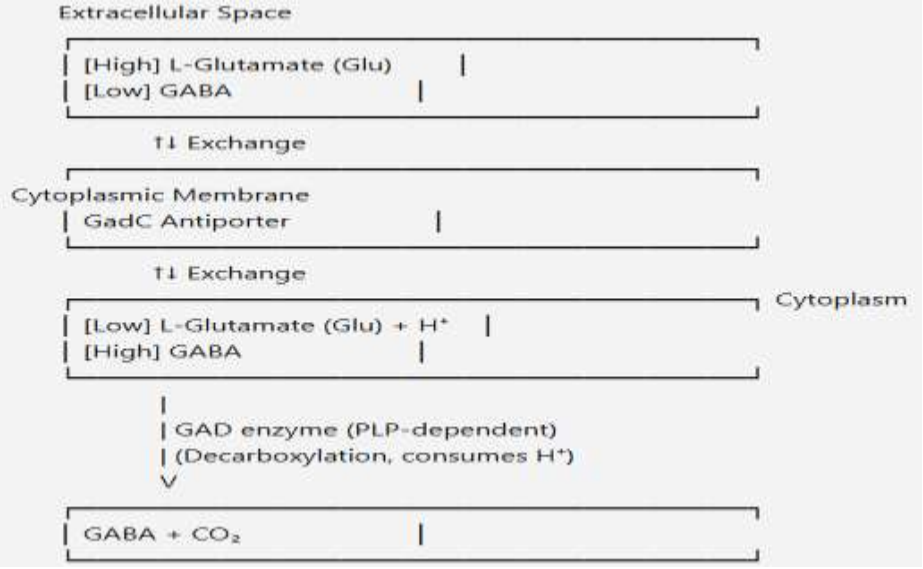

Figure 1. Schematic Representation of the GAD System in Lactic Acid Bacteria.

Environmental and Fermentation Parameters Affecting GABA Production

Synthesis of the reviewed studies reveals that GABA production by animal-associated LAB is strongly influenced by environmental and fermentation-related factors. Optimal GABA yields are consistently reported under acidic conditions, typically within a pH range of 4.5–5.5, which coincides with the induction of gad gene expression. The influence of key parameters on GABA synthesis is summarized in Table 2**.** According to Table 2, mesophilic temperatures between 30 and 37 °C favor both LAB growth and GAD enzymatic activity, while fermentation time plays a critical role in determining final GABA accumulation. In many animal-derived fermentation systems, GABA production continues into the stationary phase, reflecting the stress-responsive nature of the GAD system. Substrate availability, particularly L-glutamate concentration, directly affects GABA yield up to an optimal threshold, beyond which inhibitory effects may occur. Supplementation with pyridoxal 5′-phosphate (PLP) or its precursors further enhances GABA synthesis by supporting GAD enzyme activity (Table 2).

Table 2. Impact of key fermentation parameters on GABA yield (synthesized from multiple studies)

Parameter	Typical range tested	Optimal range for GABA production	Effect on GAD system
Initial pH	3.0 – 7.0	4.5 – 5.5	Induces gad gene expression and enhances GAD enzyme activity.
Temperature	25 – 45 °C	30 – 37 °C	Supports optimal LAB growth and GAD enzyme kinetics.
Fermentation time	24 – 96 h	48 – 72 h	Enables sufficient biomass formation and extended GABA synthesis during the stationary phase.
Glutamate (MSG)	0.5 – 10%	2 – 5%	Direct precursor; moderate concentrations enhance GABA yield, while excessive levels may inhibit growth and GAD activity.
PLP (Vitamin B6)	0 – 200 µM	10 – 50 µM	Essential cofactor for GAD; supplementation can enhance enzyme activity, especially in strains with limited endogenous PLP.

Analytical Methods for Assessing GABA Production

Reliable quantification of GABA is essential for comparative evaluation of animal-associated LAB strains. The reviewed literature indicates that high-performance liquid chromatography (HPLC) is the most frequently applied analytical technique for GABA determination, providing high sensitivity and specificity. A representative HPLC chromatogram illustrating the separation of GABA and L-glutamate peaks in fermented samples is shown in Figure 2.

In addition to HPLC, thin-layer chromatography and enzymatic assay kits are commonly employed for preliminary screening of large numbers of animal-associated LAB isolates. These complementary methods enable efficient identification of high-performing GABA-producing

strains prior to detailed quantitative analysis. Collectively, the analytical approaches summarized in Figure 2 and related studies support the conclusion that substantial variability in GABA production exists among LAB strains, reflecting underlying microbial biodiversity linked to animal-associated environments.

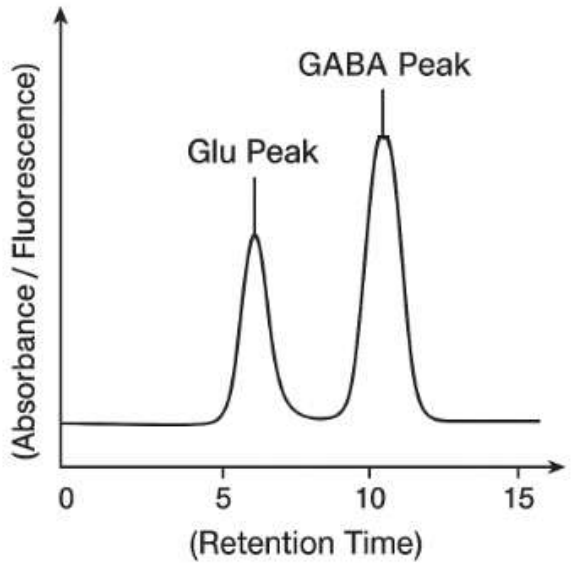

Figure 2. Representative HPLC Chromatogram of a Fermented Broth Sample Showing GABA and Glutamate Peaks.

Discussion

The synthesis of global research presented in this review demonstrates that lactic acid bacteria (LAB) represent an important functional component of animal-associated microbial biodiversity with considerable potential for gamma-aminobutyric acid (GABA) production. Rather than being a universal metabolic feature, GABA biosynthesis in LAB is a highly strain-specific trait shaped by ecological adaptation and evolutionary pressures associated with animal-related niches. These findings support earlier conclusions that LAB function not only as fermentation organisms but also as biologically significant contributors to animal and human health (Li & Cao, 2010).

The predominance of high GABA-producing species such as *Lactobacillus brevis* and *Lactobacillus buchneri* suggests that prolonged exposure to acidic and nutritionally variable environments has favored the selection of robust acid resistance mechanisms. Many of these species are frequently isolated from animal-origin fermented foods or animal gastrointestinal ecosystems, where low pH conditions impose strong selective pressure. As illustrated in **Table 1**, substantial interspecific and intraspecific variation in GABA yield reflects the underlying microbial biodiversity within animal-associated LAB populations. Similar observations have been reported for *L. brevis* OPK-3, a strain recognized for its exceptional GABA production capacity (Cho et al., 2007).

The close functional relationship between the glutamate decarboxylase (GAD) system and acid resistance provides important ecological insight into the biological role of GABA biosynthesis. GABA production should be interpreted as an adaptive survival strategy rather than merely a secondary metabolic output. The decarboxylation of glutamate consumes intracellular protons, thereby stabilizing cytoplasmic pH under acidic conditions common in animal gastrointestinal tracts and animal-derived fermentation systems (De Biase & Pennacchietti, 2012). This adaptive mechanism explains why slightly acidic environments, particularly around pH 5.0, consistently induce gad gene expression and enhance GABA accumulation, as summarized in Table 2.

Environmental and fermentation-related factors further modulate GABA production in animal-associated LAB. Optimal temperature ranges (30–37 °C), fermentation duration, and substrate availability interact to influence GAD enzyme activity and overall GABA yield. The importance of glutamate metabolism in bacterial stress responses has been emphasized by Feehily and Karatzas (2013), who demonstrated that glutamate-dependent systems play a central role in microbial survival under acid stress. These observations reinforce the concept that GABA synthesis is tightly linked to ecological fitness in animal-associated environments.

Variability in reported GABA yields among LAB strains, even within the same species, can be attributed to genetic diversity within the gad operon, including differences in promoter regions, gene copy number, and regulatory elements controlling gene expression. Additionally, methodological differences among studies, such as culture media composition and analytical techniques, complicate direct comparison of results. This variability underscores the necessity of standardized screening and evaluation protocols, particularly when assessing indigenous animal-associated LAB strains from underexplored regions such as Uzbekistan.

From an applied perspective, optimization of fermentation parameters is essential for translating biodiversity-driven discoveries into practical applications. The comparative trends summarized in Table 2 indicate that multivariate optimization strategies are preferable to one-factor-at-a-time approaches. Previous studies have demonstrated that statistical tools such as Response Surface Methodology can effectively enhance GABA yield by simultaneously optimizing interacting variables (Villegas et al., 2016). Such approaches are especially relevant for maximizing the functional potential of indigenous animal-associated LAB strains.

Beyond ecological screening and process optimization, metabolic engineering offers additional opportunities to improve GABA production. Targeted manipulation of gadB and gadC gene expression, optimization of pyridoxal 5′-phosphate availability, or reduction of competing metabolic pathways may lead to the development of high-performance LAB strains (Li & Cao, 2010). While genetically engineered strains are particularly suitable for pharmaceutical

applications, traditional selection and adaptive evolution remain valuable strategies for food-grade applications due to regulatory and safety considerations.

Finally, the valorization of animal-associated LAB biodiversity through functional food development represents a promising pathway for improving public health. Fermented animal-derived foods are recognized as important dietary sources of bioactive compounds, including GABA (Diana et al., 2014). The incorporation of GABA-producing LAB as starter or adjunct cultures in traditional Uzbek fermented products, such as katyk and kurut, offers a culturally relevant and scientifically grounded approach to developing functional foods. Given the documented antihypertensive and neurophysiological benefits of GABA, further validation through in vivo studies, initially in animal models and subsequently in human trials, is essential to confirm the health-promoting potential of these biodiversity-derived products.

Conclusion

This review highlights the biological and functional significance of animal-associated lactic acid bacteria (LAB) as natural producers of gamma-aminobutyric acid (GABA) and emphasizes their role within animal-related microbial biodiversity. The synthesis of available evidence demonstrates that GABA biosynthesis in LAB is a strain-specific and ecologically driven trait, closely linked to adaptation to acidic and stress-prone environments commonly found in animal gastrointestinal systems and animal-derived fermented foods. The presence of an efficient glutamate decarboxylase (GAD) system emerges as a key determinant underlying this functional capability.

Comparative analysis of published studies confirms that species such as *Lactobacillus brevis*, *L. paracasei*, and *L. buchneri* represent prominent GABA producers, although substantial interspecific and intraspecific variability exists. This variability reflects the broader microbial biodiversity associated with animals and underscores the importance of strain-level screening rather than species-level generalization. Environmental factors, including pH, temperature, fermentation duration, and substrate availability, further modulate GABA production, reinforcing the concept that GABA synthesis is an adaptive stress-response mechanism rather than a constitutive metabolic process.

From an ecological perspective, the findings support the view that animal-associated LAB contribute not only to fermentation processes but also to the functional resilience of animal-related microbial ecosystems. Their capacity to produce GABA links microbial survival strategies to the generation of bioactive compounds with recognized physiological benefits for animals and humans. From an applied standpoint, these characteristics position animal-associated LAB as promising candidates for the development of naturally enriched functional foods and nutraceuticals.

Importantly, this review identifies significant knowledge gaps regarding the animal-associated LAB biodiversity of Central Asia, particularly in Uzbekistan. Traditional fermented dairy products such as katyk and kurut represent underexplored reservoirs of indigenous LAB strains with potential for high GABA production. Systematic screening, standardized evaluation protocols, and multivariate optimization approaches are essential next steps to unlock this potential. Future research integrating biodiversity-driven strain selection, fermentation optimization, and in vivo validation in animal models will be critical for translating these microbial resources into health-promoting applications.

Overall, understanding the diversity, ecological adaptation, and functional traits of animal-associated lactic acid bacteria provides a valuable framework for bridging microbial ecology with applied biotechnology and supports the sustainable utilization of local animal-derived microbial biodiversity for improving animal and human health.

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