AI-Driven Metabolic Engineering of γ-Aminobutyric Acid: Biosynthetic Advances and Industrial Applications

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Abstract— Gamma-aminobutyric acid (GABA) is relatively significant inhibitory neurotransmitter in the mammalian central nervous system and plays crucial roles in regulating neural excitation, mood, and muscle activity. Beyond mammals, GABA is also pivotal in plant stress responses and microbial metabolism. It has wide applications in the pharmaceutical, agricultural, and food industries. In recent years, metabolic engineering strategies combined with synthetic biology, gene editing technologies, and artificial intelligence have significantly advanced the understanding and production of GABA. Notably, the integration of machine learning into microbial engineering has enabled rational design and optimization of biosynthetic pathways, enzyme functions, and fermentation conditions. This paper first summarizes the important application value of GABA in the fields of agriculture, medicine and food, pointing out the direction for subsequent synthetic biology research. Subsequently, the biosynthetic mechanisms (such as the glutamate decarboxylase GAD pathway and the polyamine degradation pathway) and the key factors influencing accumulation were analyzed, laying a theoretical foundation for the subsequent engineering transformation. In terms of strain modification, the application of systemic metabolic engineering strategies significantly increased GABA production. Finally, the focus is on discussing how to deeply integrate artificial intelligence with GABA synthetic biology, covering AI-driven path design and flux optimization, deep learning-based precision enzyme engineering, intelligent biological process control and optimization, as well as data-driven autonomous strain development. The collaborative application of these technologies has effectively promoted the efficient biomanufacturing of GABA, fully demonstrating the innovative advantages of multidisciplinary integration.

Index Terms—GABA, metabolic engineering, enzyme optimization, machine learning, synthetic biology

I. INTRODUCTION

GABA, a white crystalline powder with a molecular formula of C4H9NO2 and a molecular weight of 103.12 g/mol, is highly soluble in water (130 g/100 mL) (Fig. 1). Biologically, it functions as the principal inhibitory neurotransmitter in the mammalian central nervous system, playing a crucial role in maintaining the balance between neuronal excitation and inhibition. GABA participates in a variety of physiological processes, including the modulation of mood, sleep regulation, and muscle coordination[1].

Beyond its neurological roles in animals, GABA is also involved in a wide array of functions in plants and microorganisms. In plants, it contributes to abiotic stress tolerance and developmental processes through its interaction with signaling networks and metabolic regulation[2][3]. In microbes, GABA is linked to acid resistance, carbon-nitrogen metabolism, and redox homeostasis[4].

Due to its broad physiological relevance, GABA has garnered increasing attention for its commercial applications in pharmaceuticals, agriculture, and the functional food industry. The global GABA market has experienced steady growth across various regions, including North America, Europe, Asia-Pacific, Latin America, and the Middle East and Africa. Among these, North America currently holds the largest market share, driven by rising consumer awareness of GABA-enriched products for stress relief, sleep improvement, and anxiety reduction[5].

In 2023, the global GABA market was valued at approximately USD 89 million and is projected to reach USD 157 million by 2032, with a compound annual growth rate (CAGR) of 6.4% [5]. Importantly, the COVID-19 pandemic has catalyzed a significant shift in market dynamics. Between 2020 and 2023, the global GABA market size surged from USD 2.47 billion to USD 3.76 billion, reflecting an elevated CAGR of 11.2% compared to the pre-pandemic average of 6.8%. This growth has been largely fueled by the global mental health crisis, characterized by a 31% increase in anxiety disorders and

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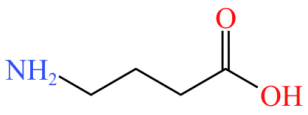
an estimated 240 million new cases of insomnia. Given the critical role of the GABAergic system in neuropsychiatric health, this surge in demand has created multifaceted opportunities for GABA-based products across health and wellness sectors.



γ-aminobutyric acid

Fig. 1. The chemical molecular model of GABA.

GABA is a non-protein amino acid that exhibits multiple physiological functions in biological systems: In mammals, it serves as the primary inhibitory neurotransmitter, regulating neuronal excitability, neuroendocrine processes, as well as behaviors such as sleep and mood; In plants, it mediates abiotic stress responses and metabolic balance; In microorganisms, it helps with acid resistance and carbon-nitrogen metabolism. This cross-species functional diversity is closely related to its conserved synthetic mechanism - dependent on glutamate decarboxylase (GAD), providing a biological basis for the development of efficient production strategies.

The commercial value of GABA has driven the innovation of production technology. Driven by its application demands in functional foods, neurotherapeutic agents and plant biological regulators, production strategies have shifted from traditional chemical synthesis (limited by toxic intermediates and environmental hazards) to biological methods. Among them, although the enrichment method of inducing plant GAD activation through stress faces scalability challenges, microbial fermentation using engineered strains (Escherichia coli, Lactobacillus, Corynebacterium glutamicum) has become the dominant industrial method.

The CRISPR-Cas9 technology has completely transformed the pattern of GABA biomanufacturing. By precisely editing the GAD gene cluster, optimizing cofactor regeneration and relieving feedback inhibition, the reported engineered strain achieved a maximum GABA production yield of 62.9 g/L and a conversion rate of 0.5 g/g glucose, which is currently the highest conversion rate of GABA production by one-step method using glucose as the substrate reported[6]. Advancements in metabolic engineering, including GAD optimization, cofactor regeneration, and carbon flux redirection, continuously enhance the feasibility of high-yield and sustainable GABA biosynthesis.

Technological progress and market demand form a virtuous cycle. Due to the impact of the mental health crisis, the global demand for GABA has soared, with the market size growing at an annual rate of 11.2% from 2020 to 2023, prompting the production model to shift from highly polluting chemical synthesis to sustainable microbial fermentation. At present, the third-generation cell factories, which feature both high yield and environmental friendliness, are driving the rapid expansion of GABA applications from pharmaceuticals to functional foods, agricultural biostimulants and other fields.

II. Progress in Cross-field Applications of GABA

Figure 2 summarizes the expanding cross-field applications of GABA, spanning neuropharmaceutical interventions, functional food fortification, plant stress resilience, and microbial biomanufacturing platforms.

A. Applications in Agriculture

In agriculture, GABA plays a pivotal role in enhancing crop tolerance to abiotic stress and regulating growth. Exogenous GABA has been demonstrated to alleviate salt, drought, cold, and mechanical stress by modulating intracellular pH, regulating stomatal aperture, promoting osmotic adjustment, and enhancing reactive oxygen species (ROS) scavenging systems[6-8]. For example, GABA accumulation in wheat is regulated through the interaction between the potassium transporter TaNHX2 and TaGAD1, leading to improved drought resistance by modulating stomatal aperture. In peanuts, seed priming with 20 mmol/L GABA for 12 hours under drought stress increased germination rate, vigor, and index by 51.2%, 85.7%, and 60.4%, respectively, and also enhanced soluble sugar and protein content[7].

GABA also contributes to salt stress tolerance, as seen in barley and tobacco[9], and enhances cold tolerance by reducing membrane damage, as evidenced by lower electrolyte leakage in GABA-treated tomato seedlings[10-12]. Furthermore, GABA improves early growth and photosynthesis in maize[13], and positively influences yield components, quality traits, and antioxidant attributes in fragrant rice through 2-acetyl-1-pyrroline (2AP) modulation [14][15].

Beyond stress adaptation, GABA functions as a plant growth regulator. In black gram (*Vigna mungo L.*), foliar application of 1.0 mg/L GABA significantly increased plant height, branch and leaf numbers, total chlorophyll, and seed yield, with the highest yield (1.50 t/ha) exceeding the control group (1.30 t/ha)[16]. Moreover, GABA can indirectly enhance soil conditions via GABA-related microbial activity in compost-based systems, thereby supporting sustainable crop production[17].

Finally, GABA-related signaling intersects with plant–insect interactions[18][19]. GABA receptor/chloride channel complexes are key targets for new-generation insecticides, and GABA biosynthesis pathways have been linked to fruit fly resistance in tomato[20][21].

B. Pharmaceutical Applications

GABA serves as a critical therapeutic agent in multiple medical domains[22]. In neurology, GABAergic dysfunction is implicated in major depressive disorder (MDD), with studies demonstrating significantly reduced GABA levels in the prefrontal cortex of affected individuals [23]. Consequently, GABA receptor agonists (e.g., benzodiazepines, Z-drugs like zolpidem) are employed to augment inhibitory neurotransmission. Clinical evidence supports their synergistic use with selective serotonin reuptake inhibitors (SSRIs) for alleviating depressive symptoms and comorbid insomnia [24]. Beyond neurological applications, GABA modulates cardiovascular and metabolic functions, exhibiting

antihypertensive effects through vasodilation and potential glucose homeostasis regulation in diabetes. Immunologically, GABA suppresses T-cell proliferation and pro-inflammatory cytokine production (e.g., TNF- α , IL-6), attenuating autoimmune and inflammatory responses [25]. These multifaceted actions position GABAergic drugs as pivotal tools for treating neuropsychiatric, cardiovascular, and immune-mediated conditions.

C. Food Industry Applications

Approved as a novel food ingredient in China since 2009, GABA is regulated with a maximum daily intake of 500 mg [26]. Its incorporation into functional foods leverages neuroactive and hypotensive properties, with claims including stress reduction and sleep quality improvement. A key technological advantage is GABA's thermostability in processed foods. Research confirms that GABA-enriched corn germ retains >85% of its GABA content after baking at 180°C for 20 minutes, enabling its integration into bread, cakes, and extruded snacks without significant degradation [27]. Current innovations focus on optimizing extraction protocols and fortifying staple foods (e.g., rice, dairy products), expanding GABA's role in preventive nutrition while adhering to safety thresholds.

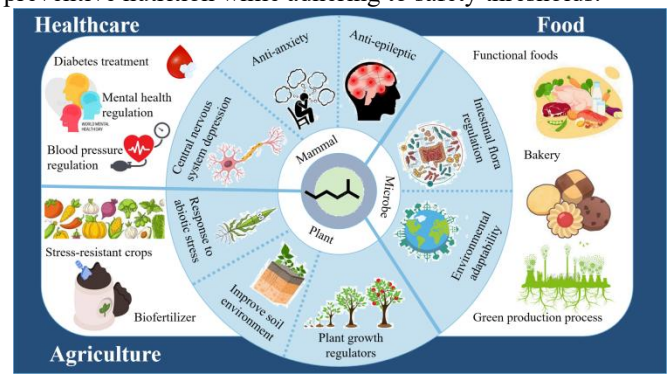


Fig. 2. The functions of GABA and its corresponding roles in healthcare, agriculture and food.

III. Main Pathways of GABA Biosynthesis

GABA, first chemically synthesized in 1883, was initially recognized solely as a metabolic byproduct in plants and microorganisms [28].Early chemical synthesis approaches — such as the high-temperature condensation of 4-chlorobutyronitrile with potassium phthalimide or the alkaline hydrolysis of pyrrolidone — achieved rapid and high-yield GABA production. However, these methods were limited by complex processing, toxic byproducts, and environmental hazards, making them unsuitable for food and pharmaceutical applications. As a result, biological synthesis has emerged as the preferred route. The plant enrichment method activates endogenous GAD activity by applying environmental stresses (e.g., extreme temperatures, salinity), leading to GABA accumulation. While safe and simple, this method suffers from low yield, limiting its scalability[29].

The diversity of GABA biosynthetic pathways—spanning canonical routes (Fig. 3), polyamine catabolism, and context-dependent precursors — highlights its metabolic versatility. These pathways are tightly regulated by species-specific mechanisms, environmental cues, and intracellular demands. For instance, in plants, polyamine degradation compensates for reduced GAD activity under drought stress, while microbial systems exploit pH-dependent GAD optimization for industrial-scale fermentation. Such regulatory plasticity provides multiple biotechnological leverage points. Advances in metabolic engineering and synthetic biology enable targeted manipulation of GABA metabolism, facilitating applications ranging from stress-resilient crop development to microbial bioreactor optimization. By integrating chemical, plant-based, and microbial strategies, researchers harness GABA's multifunctional roles, bridging agricultural, industrial, and therapeutic innovations.

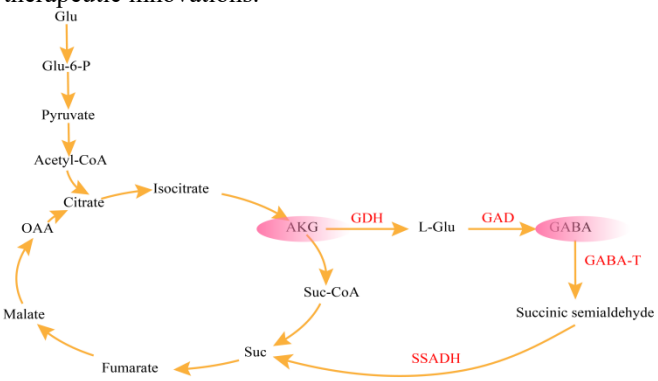


Fig. 3. GABA biosynthetic pathway.
Glu, glucose; Glu-6-P, glucose-6-phosphate; AKG, α -ketoglutaric acid; L-Glu, glutamic acid; GABA, γ -aminobutyric acid; Suc-CoA, succinyl coenzyme A; Suc, succinic acid; OAA, oxaloacetic acid; GAD, glutamate decarboxylase; GABA-T, GABAaminotransferase; SSA, succinic acid; SSADH, succinate dehydrogenase; GDH, glutamate dehydrogenase; Succ-CoA, succinyl-coenzyme A; SSADH, succinate hemialdehyde dehydrogenase.

A. Glutamate Decarboxylase (GAD) Pathway

The glutamate decarboxylase (GAD) pathway represents the principal and most efficient biosynthetic route for GABA production, conserved across animals, plants, and microorganisms. Central to this pathway is the irreversible decarboxylation of L-glutamate, catalyzed by the pyridoxal 5′-phosphate (PLP)-dependent enzyme glutamate decarboxylase (GAD; EC 4.1.1.15), which yields GABA and CO_2 under optimal acidic conditions (pH 4.5 – 6.0)[30]. The enzymatic activity of GAD is critically modulated by PLP, a cofactor derived from vitamin B6, and is enhanced in acidic environments—a feature leveraged in microbial fermentation systems for industrial GABA synthesis[31].

In mammals, two GAD isoforms, GAD67 and GAD65, exhibit distinct subcellular distributions and functional roles. GAD67, localized predominantly in the cytosol, sustains basal GABA levels essential for tonic neurotransmission, whereas

GAD65, anchored to synaptic membranes, is transiently activated under physiological stress via Ca^{2+}-dependent signaling pathways[32]. In plants, GAD activity is upregulated under hypoxic or saline stress through calmodulin (CaM)-mediated post-translational regulation. For example, flooding-induced hypoxia in rice roots triggers GABA accumulation via GAD activation, enhancing cellular tolerance to low-oxygen conditions[16]. Microbial systems, particularly acid-tolerant *Lactobacillus brevis* and metabolically engineered *Corynebacterium glutamicum*, exploit GAD's pH-dependent activity for high-yield GABA production. Metabolic strategies, such as co-expression of pyruvate dehydrogenase to redirect carbon flux toward lactic acid and GABA co-synthesis, further optimize industrial efficiency.

GABA biosynthesis is intricately linked to its catabolism through the GABA shunt, a conserved metabolic pathway that interfaces with the tricarboxylic acid (TCA) cycle. This shunt involves sequential enzymatic steps[33]: (1) GABA synthesis via GAD, (2) mitochondrial transamination of GABA to succinic semialdehyde (SSA) by GABA transaminase (GABA-T), and (3) oxidation of SSA to succinate by succinic semialdehyde dehydrogenase (SSADH). Under conditions of excessive GABA accumulation, redox imbalances may inhibit SSADH, diverting SSA toward γ-hydroxybutyrate (GHB) production. In plants, the GABA shunt serves as a metabolic bypass under TCA cycle dysfunction. For instance, tomato plants with impaired succinyl-CoA synthesis upregulate GABA shunt activity to sustain mitochondrial respiration. Similarly, *Arabidopsis* mutants defective in mitochondrial GABA transport exhibit disrupted carbon-nitrogen balance during carbon starvation, highlighting the pathway's role in metabolic homeostasis.

The GABA shunt is implicated in both adaptive stress responses and disease pathogenesis. In Alzheimer's disease, early-stage upregulation of GABA shunt activity may compensate for glycolytic deficits by enhancing succinate-driven ATP production, thereby supporting neuronal energy homeostasis. Conversely, dysregulation of GABA metabolism contributes to redox imbalance and neurotoxicity in progressive neurodegeneration. These findings underscore the dual role of the GAD pathway and GABA shunt in maintaining metabolic flexibility across biological systems, from stress adaptation in plants to neurological resilience in mammals.

B. Polyamine Degradation Pathway

In addition to the glutamate decarboxylase (GAD) pathway, GABA can be synthesized through the polyamine degradation pathway, serving as a complementary or alternative biosynthetic route under stress conditions[34]. This pathway involves two primary branches: (1) the oxidative deamination of putrescine by diamine oxidase (DAO; EC 1.4.3.22) to produce 4-aminobutyraldehyde, which is subsequently converted to GABA via aldehyde dehydrogenase, and (2) the spermidine degradation branch, where GABA is generated through transamination reactions. The pathway originates from arginine or ornithine, which are enzymatically processed into putrescine via ornithine decarboxylase (ODC) or arginine decarboxylase (ADC) in a PLP-dependent manner.

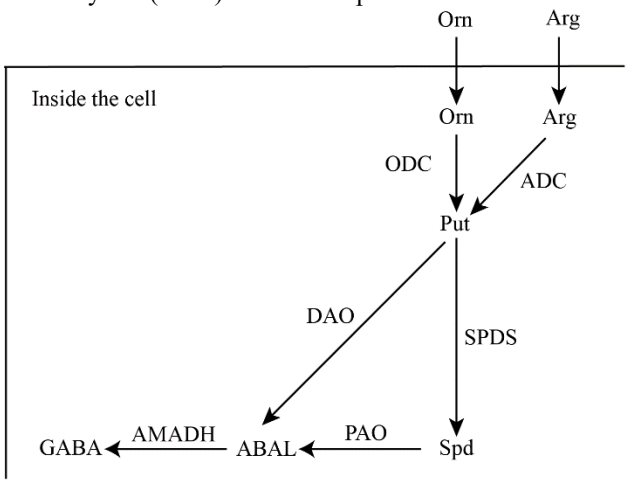


Fig. 4. Polyamine degradation pathway
Orn, ornithine; Arg, arginine; ODC, ornithine decarboxylase; ADC, Arginine decarboxylase; Put, putsamine; DAO, diamine oxidase; SPDS, spermidine synthase; Spd, spermidine; PAO, Polyamine oxidase; ABAL, 4-aminobutyral; AMADH, aminoaldehyde dehydrogenase; GABA, γ-aminobutyric acid.

In plants, prolonged abiotic stress, such as drought, often correlates with reduced GAD activity. Under these conditions, the polyamine degradation pathway compensates by maintaining GABA homeostasis through DAO upregulation. For example, drought-stressed plants exhibit elevated DAO activity, ensuring sustained GABA levels critical for osmotic adjustment and stress signaling. In animals, polyamine metabolism intersects with apoptotic signaling, where GABA derived from putrescine degradation may modulate programmed cell death[35]. Increased GABA production via this pathway has been implicated in regulating mitochondrial permeability and caspase activation, suggesting a dual role in both metabolic and apoptotic processes.

The polyamine degradation pathway highlights metabolic flexibility in GABA biosynthesis. In plants, this route acts as a fail-safe mechanism when GAD-dependent synthesis is compromised, while in mammals, it contributes to neurochemical fine-tuning and stress adaptation. The pathway's reliance on DAO underscores its sensitivity to redox states, as DAO activity is influenced by reactive oxygen species (ROS) generated under stress. Furthermore, the interplay between polyamine catabolism and GABA synthesis underscores the integration of nitrogen metabolism with stress-responsive signaling networks.

C. Other Factors Influencing GABA Biosynthesis

Beyond the GAD and polyamine pathways, GABA synthesis is modulated by diverse biochemical and physiological factors, reflecting its metabolic complexity and context-dependent regulation.

In the mammalian neocortex, glutamine serves as a major precursor for GABA synthesis, particularly under conditions of

GABA transaminase (GABA-T) inhibition. This pathway involves the astrocyte-neuron glutamine shuttle, where glutamine is transported into neurons, converted to glutamate by phosphate-activated glutaminase (PAG), and subsequently decarboxylated to GABA via GAD. In vivo metabolic tracing studies following acute GABA-T inhibition have confirmed glutamine ' s pivotal role in sustaining GABAergic neurotransmission[36].

Emerging evidence challenges the traditional view of exclusive cytoplasmic GABA synthesis. Recent studies reveal that GABA can be synthesized and packaged directly within synaptic vesicles through vesicle-localized enzymatic activity. For instance, the presence of GAD isoforms in synaptic vesicles enables localized GABA production, independent of cytosolic pools, ensuring rapid neurotransmitter replenishment during high-frequency neuronal activity[37].

In microbial systems, GABA biosynthesis is highly strain-specific and influenced by genetic background, culture conditions, and stress responses. Industrial strains such as *Lactobacillus brevis* and *Escherichia coli* exhibit divergent GABA yields due to differences in glutamate availability, GAD expression, and pH tolerance. Optimization strategies, including pH control (to exploit GAD's acidophilic activity), substrate supplementation (e.g., monosodium glutamate), and oxygen level modulation, are critical for maximizing productivity. For example, *Corynebacterium glutamicum* engineered for enhanced glutamate efflux achieves superior GABA titers under anaerobic fermentation [38].

IV. Engineering High-Yield GABA-Producing Strains

The metabolic versatility of GABA biosynthesis, spanning canonical pathways, polyamine catabolism, and context-dependent precursors, provides diverse targets for strain engineering. Leveraging species-specific regulatory mechanisms and synthetic biology tools, researchers have developed advanced strategies to enhance GABA titers for industrial, agricultural, and biomedical applications.

A. Metabolic Pathway Modification

Directed evolution and rational design of glutamate decarboxylase (GAD) have been pivotal in improving catalytic efficiency and stability. For instance, site-directed mutagenesis of *Lactobacillus brevis* GAD expanded its pH tolerance, enabling robust activity under acidic fermentation conditions. Heterologous expression systems, such as T7 promoter-driven *Lactococcus lactis* GAD in *Escherichia coli*, have achieved up to 3-fold higher GABA yields compared to native strains.

To maximize flux toward GABA, metabolic engineers co-optimize upstream substrate supply and downstream pathway redirection. Overexpression of glutamate dehydrogenase (GDH) enhances intracellular glutamate pools, while CRISPR-Cas9-mediated knockout of GABA transaminase (GABA-T) prevents GABA catabolism. Shi et al. [39]Optimization of ribosomal binding site (RBS R4 with 6-nt spacing) and screening of efficient promoters (synthetic PtacM outperformed native promoters) significantly enhanced heterologous gadB2 expression in *Corynebacterium glutamicum*. The engineered strain achieved 156% higher glutamate decarboxylase activity and >25 g/L GABA production via gadB1/gadB2 co-expression, enabling complete conversion of endogenous glutamate to GABA. This synergy between precursor enrichment and pathway insulation exemplifies the power of systems-level metabolic engineering.

Strategic supplementation of pyridoxal phosphate (PLP), a GAD cofactor, and low-cost carbon sources (e.g., glucose or lignocellulosic hydrolysates) enhances both enzymatic activity and process economics. Nitrogen source optimization (e.g., ammonium sulfate) further supports microbial growth and GABA synthesis. Dynamic control of pH (4.5 – 5.5), temperature (30–37°C), and dissolved oxygen levels is critical for sustaining GAD activity and cell viability. Fed-batch systems with real-time substrate feeding minimize metabolic burden, while two-stage fermentation separates growth and production phases to prolong GAD expression. However, to obtain these optimized data, a large amount of labor costs, economic costs and time costs are often required. If the emerging machine learning algorithms can be combined with metabolic flux data and bioreactor parameters to achieve predictive adjustment, it will maximize the yield and stability.

V. Integration of Artificial Intelligence into GABA Synthetic Biology.

Recent advances in artificial intelligence (AI) and machine learning (ML) have revolutionized metabolic engineering strategies for enhancing GABA production in *Escherichia coli* and other microbial hosts. These technologies enable end-to-end optimization of biosynthetic processes through data-driven pathway design, precision enzyme engineering, and intelligent bioprocess control.

A. AI-Powered Pathway Design & Flux Optimization

AI algorithms leverage multi-omics datasets (genomics, transcriptomics, proteomics, metabolomics) to identify optimal biosynthetic routes for GABA. ML-based metabolic flux prediction tools, such as those advanced by Bae et al. (2024), simulate complex pathway dynamics under varying cultivation conditions[32][40]. This capability allows for the rational rewiring of carbon flux away from competing branches and towards GABA synthesis, significantly improving yield predictions and guiding targeted genetic modifications. Furthermore, intelligent optimization algorithms (e.g., multi-strategy metaheuristics like the Dung Beetle Optimizer adapted for biological systems) can efficiently navigate the vast combinatorial space of gene expression levels (e.g., gadA/B, succinate semialdehyde dehydrogenase gabD) and regulatory elements to identify globally optimal pathway configurations for maximizing GABA flux[41].

B. Deep Learning for Precision Enzyme Engineering

A critical focus lies on enhancing the performance of glutamate decarboxylase (GadA/B), the rate-limiting enzyme converting L-glutamate to GABA. AI-driven enzyme function

prediction and design methods are pivotal. While the study by Xia et al. focuses on a different enzyme (Shikimate Dehydrogenase) and plant system, its methodology is relevant[42]. It was integrated conceptually as an example of the type of foundational gene discovery and characterization that AI-enhanced bioinformatics (like more powerful gene prediction, functional annotation, and even in silico cloning tools) can accelerate and deepen for any target enzyme, including GABA pathway enzymes like GadA/B. This connection is made in the concluding perspective on AI accelerating discovery.

Deep learning models (e.g., ProteinGAN, DeepMutScan) generate novel enzyme variants with tailored properties. These models can optimize GadA/B sequences in silico for improved catalytic efficiency (kcat/Km), stability under fermentation conditions (e.g., pH, temperature), and resistance to inhibitors[43]. Miao et al. exemplified this by engineering GAD mutants active at neutral pH, achieving a 2.5-fold increase in GABA titers[44]. Deep learning models predict and customize promoter strength and ribosome binding site (RBS) sequences to precisely tune gadA/B expression levels, balancing enzyme abundance with cellular metabolic burden to maximize GABA output[44]. AI-based protein structure prediction (e.g., AlphaFold2) and analysis identify key residues influencing enzyme activity, stability, and cofactor binding. This enables rational design of targeted mutations to enhance GAD performance, such as improving acid tolerance crucial for industrial-scale GABA fermentation.

C. Intelligent Bioprocess Control & Optimization

AI and ML transforms fermentation from empirical to predictive and adaptive. Fed-batch systems integrated with AI controllers dynamically adjust critical parameters (pH, dissolved oxygen, temperature, substrate feeding rates) based on real-time sensor data and predictive models. Wei et al. demonstrated this in *Corynebacterium glutamicum*, achieving exceptionally high GABA titers (58.2 g/L) through dynamic metabolic control[45]. ML algorithms (e.g., Bayesian optimization, neural networks) analyze complex interactions between medium components and cultivation parameters. Aida et al. utilized ML to distinguish optimal strategies for native versus heterologous metabolite production, leading to GABA yield enhancement while minimizing byproduct formation[46].

D. Data-Driven Autonomous Strain Development

The convergence of AI with synthetic biology enables closed-loop Design-Build-Test-Learn (DBTL) cycles. ML pipelines, as developed by Gonçalves et al., shift metabolic engineering from knowledge-driven to data-driven paradigms. Figure 4 shows the role of artificial intelligence in GABA-related metabolic engineering under the DBTL cycle, visually demonstrating how different artificial intelligence tools contribute at various stages of the engineering process. These models integrate omics data and high-throughput screening results to predict flux control points and strain performance with high accuracy (>90%), drastically reducing experimental iteration[47]. AI systems iteratively refine genetic designs based on experimental feedback. This autonomous optimization reduces strain development cycles by 40 – 60%, rapidly converging on high-performing GABA production chassis[48]. Adopting advanced numerical methods (e.g., viscosity implicit approximation for solving metabolic network variational inequalities[49] to enhance model robustness. Exploring non-classical mathematical frameworks (e.g., fractional calculus on p-adic spaces[50] to describe anomalous transport phenomena in cellular environments.

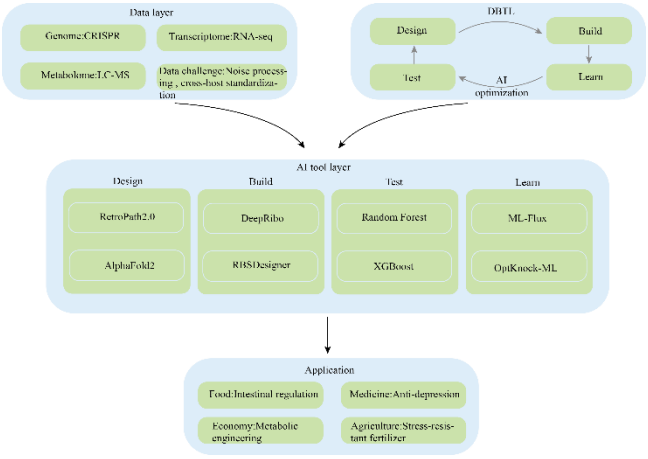


Fig. 4. The role of Artificial intelligence in GABA-related metabolic engineering under the DBTL cycle.

AI technologies have fundamentally transformed GABA biomanufacturing by enabling predictive pathway design, precision enzyme engineering, and intelligent bioprocess control. The integration of sophisticated ML models (for prediction and optimization) with multi-omics data analytics and automated robotic platforms (for high-throughput testing) creates a powerful, self-optimizing framework. Future advancements will focus on enhancing model generalizability across hosts and conditions, improving real-time data integration for adaptive fermentation, and fully automating the DBTL cycle to achieve unprecedented efficiency and yields for the industrial-scale production of GABA and related high-value bio-based chemicals. Continued development and application of AI, exemplified by advances in optimization algorithms[51], will be central to unlocking the full potential of microbial cell factories for GABA synthesis.

Artificial intelligence technology is bringing revolutionary changes to GABA biosynthesis, achieving full-process optimization from theoretical design to industrial production by building a complete intelligent toolchain. Table 1 summarizes the core tools and functions of artificial intelligence (AI) in different stages of GABA biosynthesis, covering the full-process optimization from metabolic pathway design to high-yield strain screening.

In the metabolic pathway design stage, tools such as Retro Path 2.0 and Selenzume can accurately predict feasible synthetic pathways and key enzyme candidates, laying the foundation for subsequent engineering modifications. In terms of enzyme engineering optimization, the combined application of DeepMutScan and AlphaFold2 not only accurately predicted the mutation effect of glutamate decarboxylase (GAD), but also

precisely analyzed the enzyme structure, significantly enhancing the catalytic performance of the enzyme. At the level of expression regulation, DeepRibo and RBSDesigner have achieved precise expression regulation of GABA synthesis genes through intelligent design of the translation process and ribosome binding sites. The metabolic flow reprogramming stage relies on tools such as ML-Flux and OptKnock-ML to optimize the carbon and nitrogen metabolic flow through machine learning and maximize the synthesis efficiency of GABA. Finally, algorithms such as XGBoost and random Forest conduct in-depth mining of high-throughput screening data to quickly identify the key genotype characteristics of high-yield strains. These AI tools together form a complete intelligent closed-loop system, from path design, enzyme modification, expression optimization, metabolic regulation to strain screening, creating a set of efficient and precise GABA biomanutrition solutions, providing strong technical support for industrial production. This intelligent R&D model not only significantly enhances R&D efficiency but also shortens the traditional R&D cycle that would take months or even years to just a few weeks, demonstrating the huge application potential of artificial intelligence in the field of synthetic biology.

TABLE I
REPRESENTATIVE APPLICATIONS OF AI IN GABA-RELATED METABOLIC ENGINEERING

Application Area	Representative Tools	Description
Metabolic pathway design	RetroPath2.0, Selenzyme	Predicts feasible synthetic routes and enzyme candidates
Enzyme engineering	DeepMutScan, ProteinGAN, AlphaFold2	Predicts functional effects and structural consequences of mutations in GAD
Expression optimization	DeepRibo, RBSDesigner	Designs promoter/RBS sequences for improved expression
Metabolic flux modeling	ML-Flux, OptKnock-ML	Suggests gene knockouts and flux redistribution strategies
High-throughput data analysis	XGBoost, Random Forest	Analyzes genotype-phenotype links and predicts high-yield strains

VI. CONCLUSION AND FUTURE PERSPECTIVES

The biosynthesis of GABA has evolved from pathway elucidation to systematic, interdisciplinary engineering. While conventional strategies have relied on synthetic biology and metabolic pathway modification, the integration of machine learning opens a new chapter in intelligent strain design. Future work should focus on developing hybrid AI-assisted metabolic platforms to dynamically model, predict, and optimize GABA production at both molecular and process levels. Combining high-throughput screening with AI algorithms will further accelerate strain development cycles. These advances will enable the broader industrial application of GABA in neuroscience, agriculture, and green chemistry.

ACKNOWLEDGMENT

The combination of artificial intelligence and GABA metabolic engineering is facing four core challenges: The limitations of data quality and scale lead to poor model training effects; The insufficient generalization ability of the model restricts cross-host applications. The real-time bottleneck of dynamic regulation affects the fermentation efficiency. The disconnection between experimental verification and AI design reduces the reliability of prediction. To address these challenges, in the future, it is necessary to build high-quality multimodal databases, develop transferable hybrid AI models, establish real-time dynamic optimization systems, and improve the virtual and real collaborative verification platform. Specifically, the efficiency and quality of GABA production can be significantly enhanced through innovative methods such as establishing a standardized GABA metabolism database, adopting transfer learning and physical information embedding techniques, deploying edge AI and reinforcement learning algorithms, and building digital twins and automated experimental platforms. These technological advancements will drive the industrial application of GABA in fields such as neuroscience, green chemistry, and agriculture, including the development of high-purity therapeutic GABA, the production of bio-based GABA monomers, and smart agricultural fertilizers. To realize this vision, it is necessary for interdisciplinary teams to collaborate to establish an open innovation platform, formulate unified AI model testing standards, and promote data sharing in the industrial sector, thereby accelerating the industrialization process of AI-driven GABA biomanufacturing and making it a benchmark application in the field of synthetic biology.

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