High efficiency biosynthesis of gamma-aminobutyric acid by a novel GABA- producing strain, *Enterococcus avium*

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Gamma-aminobutyric acid (GABA), also known as 4-aminobutyric acid, is a non-protein amino acid. Discovered in the brain in 1950 and recognized as an inhibitory neurotransmitter in 1967, GABA plays a crucial role in various physiological functions. Its functions include sedation, lowering blood pressure (Zhou et al., 2023), promoting growth hormone secretion, treating epilepsy, controlling asthma, promoting reproduction (Silva et al., 2019), preventing dementia (Burbaeva et al., 2014), combating obesity, and improving visual cortex function. Moreover, GABA and GABA receptors are involved in regulating gastrointestinal motility and inflammation (Hinton et al., 2020). Given its potential as a bioactive ingredient in food and pharmaceuticals, research on GABA's applications in these sectors has garnered significant attention.

Traditional methods of GABA synthesis include chemical synthesis, plant enrichment, and microbial fermentation. However, extracting GABA is challenging due to its relatively low level (Ramesh et al., 2017). Chemical synthesis is expensive and not environmentally friendly. Microbial fermentation is considered the most efficient and safe method for GABA synthesis (Cui et al., 2020). Many fungi, bacteria, yeasts, and molds can produce GABA (Lee et al., 2017). Among them, lactic acid bacteria have gained popularity and are suitable for GABA production due to their food-grade potential. Researchers have successfully obtained a strain of lactic acid bacteria with high GABA synthesis. By optimizing the fermentation conditions and medium components, they have developed an effective fermentation strategy for GABA production. These findings are of significant importance for the industrial production of GABA and the development of functional food.

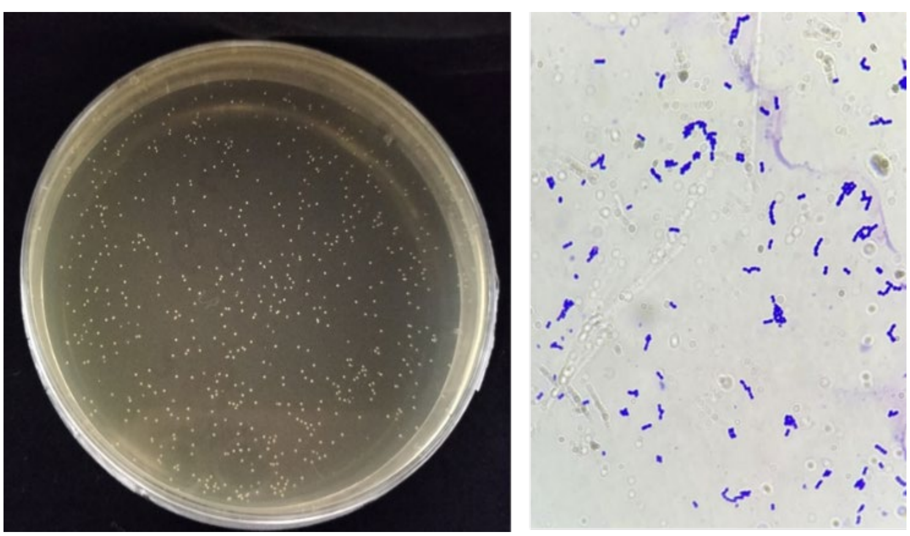

Figure 1 Colony morphology and microscopic examination results of GL1

GABA-producing bacteria primarily use glutamate as a substrate, which undergoes decarboxylation by glutamate decarboxylase to produce GABA. However, it is possible to find a strain of lactic acid bacteria capable of synthesizing GABA without glutamate. In this study, *Baijiu Daqu*, a source of high-quality lactic acid bacteria, was employed for screening purposes. The efficiency of screening was significantly enhanced by utilizing a pH-guided approach. A novel GABA-producing bacterium, *Enterococcus avium* GL1, was identified. *E. avium* GL1 colonies exhibit specific characteristics such as being small, milky white, moist, Gram-positive, and round in shape (Figure 1).

E. avium GL1 produces 16 g/L of GABA by using glutamate sodium as a substrate. The strain can still synthesize 9.8 g/L of GABA even without glutamate sodium. This indicates the potential of *E. avium* GL1 to synthesize GABA independently. To further enhance the GABA synthesis capability of *E. avium* GL1, optimization efforts were carried out for the carbon source, nitrogen source, temperature, and pH of the culture medium. The final approach involved batch fermentation with a feeding strategy, resulting in an impressive GABA production of 208 g/L. This novel strain, *E. avium* GL1, exhibits promising probiotic properties and can find applications in the food and pharmaceutical industries.

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