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BIOSYNTHESIS OF GAMMA-AMINOBUTYRIC ACID USING LACTIC BACTERIA - A CASE STUDY ON VIETNAMESE RICE

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Abstract

Gamma amino butyric acid GABA) is the chief inhibitory neurotransmitter in the developmentally mature mammalian central nervous system. Its principal role is to reduce neuronal excitability throughout the nervous system. Therefore, GABA has such important physiological functions, many studies focus on the development of GABA into dietary supplements. Currently, GABA was produced by different ways, such as extraction from grains, determination of the optimal conditions for rice grain to germinate, or fermentation by microorganisms. In which, lactic acid bacteria have been applied and met high efficiency in the process of biosynthetic fermentation of large amounts of GABA in the industrial production. A case study on Vietnamese rice bran will be introduced in this paper consisting of the effect of different fermentation conditions using Lactobacillus brevis on GABA concentration in red brown rice; isolation, classification of lactic bacteria from Vietnamese traditionally

Key words: gamma amino butyric acid (GABA), lactic acid bacteria, fermentation, rice bran, red brown rice-tea.

fermented foodstuffs and assessment of their ability to proliferate GABA. Based on the results of those studies, a new type of tea containing GABA named "GABA Rice-Tea" was produced by mixing red brown rice with oolong tea leaves and used as a functional food in Vietnam.

1. Introduction

Gamma-Aminobutyric acid, or γ-aminobutyric acid, is the chief inhibitory neurotransmitter in the developmentally mature mammalian central nervous system. Its principal role is to reduce neuronal excitability throughout the nervous system.

GABA is sold as a dietary supplement in many countries. It has been traditionally thought that exogenous GABA doesn't cross the blood-brain barrier, however data obtained from more current research indicates that it may be possible. In 1883, GABA was first synthesized, and it was first known only as a plant and microbe metabolic product. In 1950, GABA was discovered as an integral part of the mammalian central nervous system (Roth et al., 2003). In 1959, it was shown that at an inhibitory synapse on crayfish muscle fibers GABA acts like stimulation of the inhibitory nerve. Both inhibition by nerve stimulation and by applied GABA are blocked by picrotoxin. In addition to the brain, GABA is also present in B cells of the pancreas, ovaries, testes, and gastrointestinal tract. GABA has important functions in the nervous system (Abe et al., 1995). The action of GABA together with glutamate and aspartate performs most of its action through the synaptic cleft in the central nervous system (Tsai et al., 2006; Ueda et al., 2007).

GABA ensures the maintenance of the normal functioning of the brain, especially the neurons. GABA plays a major role in reducing neuronal activity and inhibiting the propagation of signal transduction cells. GABA prevents stress and anxiety signals from reaching the central nervous system by occupying or controlling the receptor regions of these cells (Jin et al., 2013). This transmission inhibitor is commonly known as a natural sedative produced by the body to help relax the nerves and have a good night's sleep. Up to now, many scientific studies have published that GABA helps people have good and deep sleep, improves the ability to temporarily lose self-control, persistent pain, attention deficit hyperactivity disorder or depression, premenstrual state of mind (Tsai et al., 2006).

GABA is a four-carbon free amino acid (the carboxyl group deprotonated and the amino group protonated) that is widely present in bacteria, plants and vertebrates. In plants and bacteria it plays a metabolic role in the Krebs cycle, and in vertebrates it acts as a potent neural signal transmitter. GABA is primarily formed by the irreversible ?-decarboxylation reaction of L-glutamic

acid or its salts, catalysed by glutamic acid decarboxylase enzyme (GAD). Its conformation depends on its environment. In the gas phase, a highly folded conformation is strongly favored due to the electrostatic attraction between the two functional groups. The stabilization is about 50 kcal/mol, according to quantum chemistry calculations. In the solid state, an extended conformation is found, with a trans conformation at the amino end and a gauche conformation at the carboxyl end. This is due to the packing interactions with the neighboring molecules. In solution, five different conformations, some folded and some extended, are found as a result of solvation effects. The conformational flexibility of GABA is important for its biological function, as it has been found to bind to different receptors with different conformations. Many GABA analogues with pharmaceutical applications have more rigid structures in order to control the binding better. It is this ability to exist in many different structural forms that gives GABA many important biological functions (Kayahara et al., 2001; Jin et al., 2013).

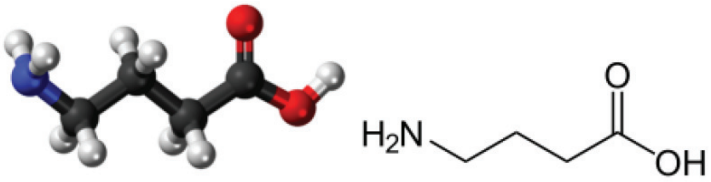

Figure 1. Molecular structure of GABA

GABA is synthesized from different sources, microorganisms are considered to have indirectly produced GABA (Dhakal et al., 2012). Bacteria, yeasts, and molds have also been identified to be able to biosynthesize GABA through several control methods. Some fungi also contain certain levels of GABA such as Aspergillus nidula (A. nidula), Aspergillus niger (A. niger) (Kleinrok et al., 1998), Aspergillus oryzae (A. oryzae) isolated from peas (Nguyen Minh Thong, 2015). Currently, production of GABA from bacteria trends to research, especially for LAB (Lactic acid bacteria).

GABA products from LAB have high content, safe for users. LAB is able to biosynthesize GABA mostly belonging to genera Lactobacillus, Lactococcus and isolated from traditional fermented foods such as kimchi, cheese, yogurt (Kim et al., 2009). The potential for GABA production of LAB was studied from LAB species and strains isolated from some fermented foods capable of GABA biosynthesis and has been studied extensively in recent years including yogurt, cheese (Park and Oh, 2007; Rizzello et al., 2008), kimchi (Lu et al., 2008; Park and Oh, 2007), sourdough (Rizzello et al., 2008) and paocai (Li et al., 2008).

2. Discussion

GABA is primarily formed by the irreversible α-decarboxylation reaction of L-glutamic acid or its salts, catalysed by glutamic acid decarboxylase enzyme whose biochemical properties have been characterized. This enzyme has been found in bacteria such as LAB (Li et al., 2008; Yang et al., 2008), Streptococcus, Aspergillus (Kato, Furukawa, & Hara, 2002) and molds (Kono and Himeno, 2000), yeasts (Masuda et al., 2008); in plants such as tea (Zhao et al., 2011), tomato (Yoshimura et al., 2010), soybean (Serraj et al., 1998), mulberry leaf (Yang, Jhou, & Tseng, 2012), germinated brown rice (Dai-xin, Lu, Lan, Li-te, & Yong-Qiang, 2008) and petunia. Lactic acid bacteria are an important group of Gram-positive bacteria that produce GAD through fermentation.

Glutamate decarboxylase or glutamic acid decarboxylase (GAD) is an enzyme that catalyzes the decarboxylation of glutamate to GABA and CO2. GAD uses pyridoxal phosphate (PLP) as a cofactor (Waagepetersen S., 2004). The reaction proceeds as follows:

$$HOOC - CH_2 - CH_2 - CH(NH_2) - COOH \rightarrow CO_2 + HOOC - CH_2 - CH_2 - CH_2NH_2$$

In mammals, GAD exists in two isoforms with molecular weights of 67 and 65 kDa (GAD67 and GAD65), which are encoded by two different genes on different chromosomes (GAD1 and GAD2 genes, chromosomes 2 and 10 in humans, respectively) (Peter T. Bosma et al., 1999). GAD67 and GAD65 are expressed in the brain where GABA is used as a neurotransmitter, and they are also expressed in the insulin-producing ?-cells of the pancreas, in varying ratios depending upon the species. Together, these two enzymes maintain the major physiological supply of GABA in mammals,though it may also be synthesized from putrescine in the enteric nervous system, brain, and elsewhere by the actions of diamine oxidase and aldehyde dehydrogenase. GAD67 and GAD65 are also regulated differently post-translationally. Both GAD65 and GAD67 are regulated via phosphorylation of a dynamic catalytic loop, but the regulation of these isoforms differs; GAD65 is activated by phosphorylation while GAD67 is inhibited by phosphorylation. GAD67 is predominantly found activated, whereas GAD65 is predominantly found inactivated. GAD67 is phosphorylated at threonine 91 by protein kinase A (PKA), while GAD65 is phosphorylated, and therefore regulated by, protein kinase C (PKC). Both GAD67 and GAD65 are also regulated post-translationally by Pyridoxal 5'-phosphate (PLP); GAD is activated when bound to PLP and inactive when not bound to PLP. Majority of GAD67 is bound to PLP at any given time, whereas GAD65 binds PLP when GABA is needed for neurotransmission. This reflects the functional properties of the two isoforms; GAD67 must be active at all times for normal cellular functioning, and is therefore constantly activated

by PLP, while GAD65 must only be activated when GABA neurotransmission occurs, and is therefore regulated according to the synaptic environment.

2.2. GABA production using lactic acid bacteria

It is difficult to extract GABA from microorganisms because of the low content in natural biological tissues, and chemical synthesis has been rejected because of the corrosive reactants that are used. However, various studies have reported GABA producing ability by lactic acid bacteria species/subspecies and the presence of GAD activity in their cells. Vast concentrations of gamma-aminobutyric acid production by LAB have also been shown.

LAB's high GABA production is related to the activity of the GAD enzyme in the cells. The concentration of glutamic acid in the food matrix should be high enough. Accordingly, GABA-producing LAB can be used to develop fermented healthoriented food.

2.2.1. Different source of synthetic materials GABA

Natural GABA was first discovered in potatoes, then small amounts of GABA were identified in some vegetables. Pradeep et al (2011) suggested that GABA occurs in germinating seeds and legumes.

Study on synthesis of GABA at 5.42 mg/ml from black soybean milk using *Lactobacillus brevis* FPA 3709 isolated from fish intestine for fermentation (Ko et al., 2013). Hasegawa et al. (2018) used the date residue after processing to ferment GABA biosynthesis by *Lactobacillus brevis.* Unused production by-products were also utilized in a study by Kei-Anne Baritugo (2018) using *Corynebacterium glutamicum* as a host to enhance GABA production from glucose and xylose as a biocarbon source. This study uses mutant *Escherichia coli gadB* with wide pH activity and E. coli xylAB gene expressed under the control of a synthetic H36 promoter. GABA 12.54 ± 0.07 g/L is generated by the recombinant C. *glutamicum gene* H36GD1852. GABA is synthesized by many raw materials and using lactic acid bacteria that have been studied for the ability to synthesize GABA. Kook (2010) used *Lactobacillus* to ferment rice bran and gave a GABA fermentative yield of 660 mM in the fermentation solution from 1 kg of rice bran added 10 liters of water and 12% monosodium glutamate equivalent to 679. g/kg rice bran.

2.2.2. Classification and evaluation of gamma-aminobutyric acid producing lactic acid bacteria isolated from traditionally fermented foods in Vietnam

Lactic acid bacteria play important roles in processing and preservating fermented food in Vietnam. From 102 bacteria isolated from "nem chua" and "kim chi" 31 strains of lactic acid bacteria were selected. Bacteria cells whose colonies were round, convex, and white were immobilized, spherical or rod-shaped, positive Gram organized in pairs or beads, those strains could not

Table 1: Some of studies on GABA biosynthesis from lactic acid bacteria

Materials	Content	Authors
Brown rice	production of v-aminobutyric acid using rice	Ohtsubo et al., 2000; Karladee et al., 2012;
Shochu rice	Production of gamma-aminobutyric acid using *Lactobacillus brevis*	Yokoyama et al., 2002
Soybean	Production of gamma-aminobutyric acid using lactic acid bacteria and germinated soybean extract	Park et al., 2007; Tsai et al., 2006; Yang et al., 2016
Black soybean milk	Gamma-amino butyric acid production in black soybean milk by *Lactobacillus brevis*	Ko et al., 2013
Black raspberry juice	Production of γ-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis*	Kim J. Y. et al., 2009
Grape	Synthesis of gamma-aminobutyric acid (GABA) by *Lactobacillus plantarum*	Cagno et al., 2009
Bran rice	*Using Lactobacillus* to enrich GABA	Kook, 2010
Red seaweed	Enhancement of γ-aminobutyric acid in a fermented red seaweed beverage by starter culture *Lactobacillus plantarum*	Ratanaburee et al., 2011
Date residue	Gamma-aminobutyric acid fermentation with date residue by a lactic acid bacterium, *Lactobacillus brevis*	Hasegawa et al., 2018

perform oxidase and catalase activities. Among which the 3 were isolated produced GABA high concentration and they were classified by PCR (Polymerase Chain Reaction) and 16S rDNA sequences *Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus pentocus* with a similarity of 99% -100%. The GABA content of *Lactobacillus brevis* was 1261.15 mg / l, *Lactobacillus plantarum* was 959.23 mg / l and *Lactobacillus pentosus* was 506.29 mg / l (Ho Thi Ngoc Tram et al., 2021).

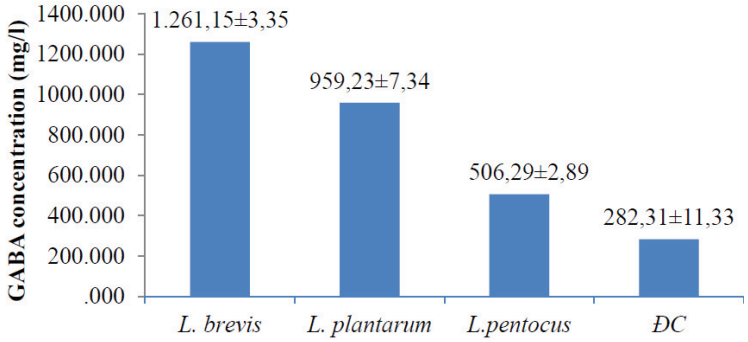

Figure 2: GABA content graph of GABA biosynthetic lactic acid bacteria strain; in which DC is the control: fermentation medium without lactic acid bacteria.

2.2.3. Influence of different fermentation conditions on GABA biosynthesis with Lactobacillus brevis supplemented in Huyet Rong rice of Vietnam

Gamma-aminobutyric acid (GABA) is an important amino acid in human body because of its special bioactivity in reducing blood pressure, improving brain function, enhancing immunity, and postponing intelligence degradation. Therefore, a lot of scientists have researched on the existence and quantification of GABA from many kinds of food. With regards to Huyet Rong (HR), a high quality special rice variety of Vietnam, was studied on GABA biosynthesis process with *Lactobacillus brevis* supplied and different fermentation conditions as follows: at first, HR brown rice grains were soaked in fresh water for 6 hours, then pulverized. After that, *Lactobacillus brevis* isolated from fermented traditional Vietnamese foods was cultivated in MRS broth containing 1% MSG. 01 (one) % of the enrichment broth of *Lactobacillus brevis* was added into the HR solution (HR powder + distilled water). Then, GABA was produced by incubating at different pH (5, 6, 7), temperatures (30^{o}C, 35^{o}C, and 40^{o}C) with incubation duration (18 hours, 24 hours, and 30 hours) under natural conditions with and without lids, and *Lactobacillus brevis* supplemented. The

results showed that GABA content in HR was highest after soaked for 24 hours at 35^0C. The GABA content obviously was increased with *Lactobacillus brevis* supplement (1451.4 μg/20g) higher two times than that of the control (Ho Thi Ngoc Tram et al., 2019).

Huyet Rong Brown rice (HRB) provided by KVIP-KFRI international project located in Can Tho Mekong River Delta that was soaked in distilled water with ratio 1:2 (w/v) at room temperature for 6 hours to meet the equilibrium moisture content (An et al., 2016), then let be dried at room temperature for 5-7 minutes before grinded at 28.000 rpm (QE-2000, Vietnam) for 30 seconds to fine powder.

Lactobacillus brevis isolated from fermented traditional foods and classified and supplied by Department of Food Microbiology of Faculty of Food Science and Technology-Nong Lam University. The Bacterium was inoculated into MRS broth (De Man, Rogosa, and Sharpe) (10ml) added with 1% MSG (mono sodium glutamate), and then incubated at 37^0C for 48 hours.

In this study, the GABA production was carried out at incubation temperatures of 30, 35 and 40oC. Different incubation time (18, 24, 36h) at the same temperature showed non - significantly different GABA content ($p > 0.05$) at 30 oC, however, a while at 35^oC and 40^oC, concentration of GABA was significantly different. GABA content was affected by the soaking temperature. The GABA contents were significantly different ($p < 0.05$), the most influential factors in the GABA production process were Lactobacillus brevis at 35^oC for 24 hours incubation. As a result, although the levels of GABA produced in natural conditions of HRR are relatively high, GABA production using Lactobacillus got highest value.

Table 2: Effect of incubation conditions on GABA content

Temperature and time (35°C, 24 hrs)	**GABA concentration (µg/20g)**
Aerobic	$1191{,}8 \pm 12{,}4^{C}$
Anaerobic	$1384{,}2 \pm 7{,}3^{B}$
Lactobacillus brevis	$1451{,}4 \pm 34{,}5^{A}$

3. CONCLUSION

The pharmaceutical industry is associated with the food industry in a modern framework that promotes the inclusion of bioactive molecules in food to help control some diseases. In fact, the development of functional foods has been a key field of nutritional researches in economically powerful countries, as a result of the experience gained in recent decades. However, there is a current perception that primarily bioactive compounds are obtained synthetically. Therefore, the extraction of bioactive molecules from natural sources is important in the field of discovering new compounds. GABA research has been intensified in recent years, with numerous scientific studies clinically demonstrating its benefits in physiological disorders, and particularly in hypertension, as GABA contributes efficiently to the regulation and stability of blood pressure. The generation of GABA from glutamic acid or its salt in probiotic cells and other natural resources such as plants or fungi is added value to the food industry, because of the notable increase in interest in natural and organic foods.

Industrial scale production of GABA from other sources may be economical with lactic acid bacteria production. High performance production, optimization through different biotechnological techniques, and the discovery of new high-GABA producing strains will remain a focus of interest in research into GABA as a health-related novel biological active compound.

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