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Study on conditions for gama buryric acid (GABA) production in *Lactobacillus fermentum* 101 isolated from human

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Abstract

Lactic acid bacteria play a vital role in biosynthesis of γ - aminobutyric acid (GABA) in the presence of glutamic acid - major substrate for the process. In the present study, Lactobacillus fermentum (L. fermentum) isolated in various sources in Vietnam that were screened for bacteria strains with high-efficiency in GABA formation. In the study, L. fermentum was cultured in MRS broth containing 25 mg/mL MSG, at pH of 6.5 and incubated at the optimal time (37ºC, for 24, 48, 72 h). After extraction and thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis, L. fermentum 101 showed the GABA yield about 1.34 mg/g dried supernatant, suggesting that L. fermentum 101 was found to be a promising GABA producer for food and pharmaceutical field).

Keywords: Gamma-aminobutyric acid (GABA), Glutamate, Lactobacillus fermentum, optimization.

Introduction

Gamma (γ) - aminobutyric acid (GABA) is a non-protein amino acid that is widely distributed in microorganisms, plants and animals. GABA has potential as a bioactive component with variety of physiological functions and thus a great potential in application of pharmaceuticals and functional foods (Dhakal et al., 2012). It is considered to be the major inhibitory neurotransmitter in the central nervous system (CNS). GABA is the product of decarboxylation of glutamate via l-glutamic acid decarboxylase (GAD), of glutamate and is thus an amino acid-derived neurotransmitter (Shi and Li, 2011). Interestingly, GABA is derived from the same source as the brain's most common excitatory neurotransmitter, glutamate, and sometimes acts in opposition to glutamate to achieve a balance between excitation and inhibition at the synaptic level. Hence, consumption of food enriched GABA can improve memory and the learning abilities as well as control pain and anxiety. In addition, GABA can inhibit the growth of cancer cell and shows anti-diabetic and antihypertensive effects in humans (Schuller et al., 2008). However, the direction of supplementing of chemical GABA to food is considered unsafe and unnatural. Thus, it is very necessary to come up with a natural method that can produce and increase GABA.

There have been many attempts to synthesize GABA chemically or biologically because of the beneficial functions of GABA and the increasing commercial demand (Dhakal et al., 2012). Lactic acid bacteria (LAB) are the safe microorganisms that were proved as GABA producing sources (Haixing et al., 2010; Li et al. 2010; Park and Oh 2007; Yokoyama et al. 2002; Li et al., 2010).

Biosynthetic methods of GABA may be much more promising than chemical synthesis methods since they have a simple reaction procedure, high catalytic efficiency, mild reaction condition and environmental compatibility. The biosynthesis of GABA is one step reaction of decarboxylating glutamate to GABA, catalyzed by glutamate decarboxylase (GAD). From that, the role of *L. fermentum* will be understood more in GABA in human as well as create a source for pharmaceutical and food industry.

Materials and methods

Bacterial strain, media and culture conditions

L. fermentum strains were obtained from faces, saliva and vagina of people inhabiting the central region of Vietnam. It was evaluated for their ability to produce GABA. Lactobacilli de Man, Rogosa, Sharpe (MRS) broth was autoclaved. The isolated bacteria were cultured in 5 mL MRS medium (De Man et al., 1960), followed by incubation at 37°C for 24 hours with pH was maintained at 6.5.

Optimization of conditions for CLA production

The morphology of the strain was observed under oil immersion (×1,000, magnification) at light microscope level (Olympus CX21 BIM-SET6, Japan). A Gram staining test (Nugent et al., 1991) was implemented to investigate the characteristics of the strains.

Cultivation of GABA-Producing Microorganism

Glutamic acid was dissolved in distilled water aseptically and then added in sterilized MRS broth to obtain the final concentration at 25 mg/mL for 24, 48, 72 hours at 37°C to screen for the GABA-producing microorganisms. After cultivation, the culture broth was centrifuged at 13000 rpm, 4°C for 10 min, and the resultant supernatants was used for analysis of GABA presence.

Extraction of GABA

After cultivation, 5 mL of culture broth was centrifuged at 13000 rpm, 4°C for 10 min, and the resultant supernatant was freeze-dried to evaporate all water and then extracted with chloroform, methanol and ethyl acetate. Each of the samples (100 mg) was dissolved with 1 mL of chloroform and shaken thoroughly. After centrifugation at 13000 rpm in 10 min. Chloroform layer was discarded, the liquid portion was removed totally by pipetting and evaporation. Dried extract was continually washed with chloroform, methanol and finally ethyl acetate to obtain partially pure GABA. GABA was diluted in distilled water for analysis.

Qualitative analysis by Thin Layer Chromatography (TLC)

Presence of GABA in this supernatant was first analyzed by using TLC. One microliter of supernatant was spotted onto the TLC plates. Mobile phase was a mixture including methanol: chloroform: distilled water (5:2:2 in v/v) mixture.

The Rf value was calculated as follows:

Rf = migration distance by component/migration distance by solvent.

Cultures of bacterial strains showing the same Rf value as the authentic standard GABA.

Qualitative analysis by High Performance Liquid Chromatography HPLC

According to GABA quantitation of Min et al., 2011 and Hayat et al. 2015 with modification, GABA content was determined by a Shimadzu LC-8A preparative HPLC system (Shimadzu Corporation, Tokyo, Japan) equipped with an YMC C18 reverse-phase column with 5 μm diameter, 250 mm length and 4.6 mm internal diameter. Later, the sample was diluted and subjected to HPLC analysis. The injection volume was 20 μL with a flow rate of 0.6 mL/min. The HPLC mobile phase A was 1% trifluoroacetic acid (TFA). The pH of the mobile phase A

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was adjusted to 2.8 using triethylamine (TEA). HPLC mobile phase B was a mixture of isopropanol: acetonitrile. All mobile phases were passed through a 0.22 µm membrane filter. The column temperature was set up at 25 °C; sample injection volume was 20 µL and the compound was detected through a UV detector at 250 nm. The presence of GABA was confirmed by comparing the retention time of sample with the corresponding GABA standard. The amount of GABA was calculated by comparing the peak area with the corresponding standard GABA.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software version 20.0 and Microsoft Excel was employed. Values of $P < 0.05$ refer to significant differences among experiments.

Results and discussion

Screening of GABA-Producing *L. fermentum* using TLC

After checking GABA production on TLC, *L. fermentum* 101 exhibited strong spots for GABA production when MSG presenting in growth medium. The Rf value of *L. fermentum* 101 was nearly the same as the authentic GABA production (Fig.1).

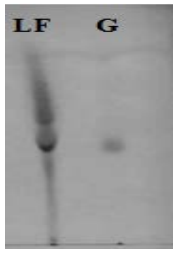

Fig. 1: Thin Layer chromatography analysis of GABA production by *L. fermentum* 101 after 72-hour cultivation with MSG. (G): standard GABA; (LF): *L. fermentum* 101

Optimal condition for GABA production

The time for cell growth and GABA production of bacterial strain was optimized by cultivation in 5 mL of MRS medium containing 1.5 mL MSG at 37°C for up to 72 h. Cell growth reached the stationary phase after 24 h of cultivation, whereas GABA production, based on TLC plates, dramatically increased upon 72 h of cultivation by showing a stronger spot than that of 24 and 48 – hour cultivation. The dried supernatant collected after 72 h from broth containing 25 mg/mL MSG were analyzed and confirmed further by HPLC analysis

Quantitative analysis of GABA production using HPLC

GABA formation was quantitatively confirmed using HPLC-UV. The data of retention time in case of *L. fermentum* 101 was approximately 21.300 min in samples and insignificantly different as compared to that of authentic GABA, 21.326 min (Table 1). The retention time pointed that GABA was produced in this cultures. Moreover, peak area of interest GABA was proportional to concentration of GABA in ppm unit. GABA produced by total five strains of *L. fermentum* 101 was identical in terms of both the retention time and mass spectrum of HPLC analysis by a comparison with an authentic GABA.

Table 1: Retention time of LAB in comparison with standard GABA

Lactic Acid Bacteria	Retention time
Lactobacillus fermentum	21.303
GABA standard	21.326

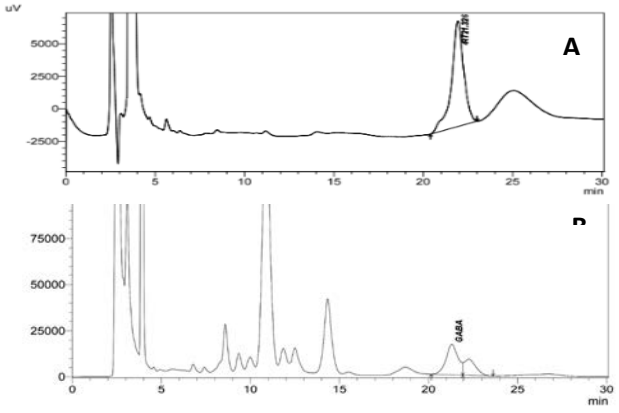

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Fig. 2: HPLC spectra of standard GABA (A), *Lactobacillus fermentum* (B)

After qualitative analysis of GABA production, data showed that *L. fermentum* 101 had GABA-producing ability with high-efficiency. The GABA yields in freeze-dried samples (170 mg) of *L. fermentum* 101 was 0.2278 mg after 72 h, respectively. The reasons why 72 h incubation was used to identify and quantify GABA because 24 h and 48 h periods were not enough time to produce high GABA. By TLC analysis, there was observation that the extract of *L. fermentum* showed the traces having similar Rf to standard GABA, suggesting GABA produced extracellularly. The peaks appeared at the retention time that was similar to standard, showing GABA produced in the cultures. The other peaks appeared in the chromatogram because there were contaminants in samples. However, in the chromatogram, retention time of GABA produced in extracts should similar to standard GABA, suggesting the expected GABA producing ability in *L. fermentum* 101.

Conclusion

L. fermentum 101 was evaluated for ability to produce GABA at largest amount in 72 h condition in MRS contain MSG (25mg/mL). Detection of GABA-producing *L. fermentum* originated from human will give more convenient in pharmaceutical and food development with side effect limitation for human.

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References

1. Dhakal, R., Bajpai, V.K. & Baek, K.H. Production of gaba (γ-aminobutyric acid) by microorganisms: A review. Braz J Microbiol., 43, 2012, pp. 1230–1241.
2. Shi, F., & Li, Y. Synthesis of γ-aminobutyric acid by expressing *Lactobacillus brevis*-derived glutamate decarboxylase in the *Corynebacterium glutamicum* strain ATCC 13032. *Biotechnology Letters,* 33(12), 2011, pp. 2469-2474.
3. Schuller, H.M., Al-Wadei, H.A. & Majidi, M. GABA B receptor is a novel drug target for pancreatic cancer. *Cancer,* 112(4), 2008, pp. 767-78.
4. Haixing, L., Ting, Q., Guidong, H. & Yusheng C. Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. *Microb Cell Fact*, 9, 2010. doi: 10.1186/1475-2859-9-85.
5. Park, K.B. & Oh, S.H. Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresour Technology*, 98, 2007, pp. 1675–1679.
6. Yokoyama, S, Hiramatsu, J-I and Hayakawa, K. Production of gamma-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO-12005. *J Biosci Bioeng*, 93, 2002, pp.95–97.
7. De man, J.C., Rogosa, M. & Sharpe, M.E. A medium for the cultivation of lactobacilli. *J Appl Bacteriol*, 23, 1960, pp.130-135.
8. Nugent, R.P., Krohn, M.A. & Hillier, S.L. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol*, 29(2), 1991, pp. 297-301.
9. Hayat, A., Jahangir, T. M., Khuhawar, M. Y., Alamgir, M., Hussain, Z., Haq, F. U., & Musharraf, S. G. HPLC determination of gamma amino butyric acid (GABA) and some biogenic amines (BAs) in controlled, germinated, and fermented brown rice by pre-column derivatization. *J Cereal Sci*, 64, 2015, pp. 56-62.

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10. Li, H, Qiu, T, Huang, G and Cao, Y. Production of gamma-aminobutyric acid by *Lactobacillus brevis* NCL912 using fed-batch fermentation. *Microb Cell Fact*, 9, 2010, pp. 85.
11. Ming Z, Yan M, Zhen-zhen W, Wen-xia Y, Ya-li L, Chun-hua Z, Xiao-ting X, and Hong-jie Z. Determination and Comparison of γ-Aminobutyric Acid (GABA) Content in Pu-erh and Other Types of Chinese Tea. *J Agric Food Chem*, 59 (8), 2011, pp 3641–3648.

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