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Novel, Practical and Cheap Source for Isolating Beneficial γ-Aminobutyric Acid-Producing *Leuconostoc* NC5 Bacteria

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Abstract: GABA is well known for its physiological functions as antioxidant, diuresis, tranquilizer, anti-hypertensive, epilepsy treatment and diabetic prevention. Most of the fermented food products are known and proven for its high content GABA producer, which contributes the food as potential functional foods. Among 5 positive isolates from the Malaysian commercial fermented shrimp product (*cincaluk*), only one strain of bacteria showed the highest GABA-producing activity. *Leuconostoc* NC5 showed the highest production ability of both extracellular and intracellular GABA content (2.84±0.19 mM, respectively) with GAD activity at 1.93±0.37 unit. The effect of cultivation time, temperature, pH and different concentration of glutamate, carbon source, nitrogen source and Pyridoxal-5'-Phosphate (PLP) on the production of GABA was investigated. This study concludes that the optimum conditions for GABA production were recorded at the cultivation temperature of 37°C, pH 5.0 and cultivation time at 168 h. Furthermore, the addition of PLP and nitrogen source to the culture medium significantly increased GABA production by 30-fold. Thus, *Leuconostoc* NC5 can be a potential starter culture for GABA-enriched functional foods. This strain showed a potential of being starter culture for the production of fermented functional foods containing GABA.

Key words: γ-aminobutyric acid, GAD activity, lactic acid bacteria, *Leuconostoc* sp., fermented shrimp product, fermentation

INTRODUCTION

Cincaluk is a fermented shrimp product, produced by fermenting of small shrimp called *Acetes* or locally known as *Udang geragau*, *Udang bubok* or *Udang gari*, which is widely consumed in most Asian countries. *Cincaluk* is a delicacy, known for its fishy flavor, pungent odor, salty taste and pale pink appearance. This product is normally prepared by washing and soaking shrimps in seawater and mixed with 20-25% salt and 6% cooked-ground rice from the wet weight of the shrimp. The mixture is fermented for 20-30 days until it matures and turns pink.

Lactic Acid Bacteria (LAB), a group of related bacteria produce lactic acid as a result of carbohydrate fermentation. Several LAB have been previously isolated and identified from shrimp fermented products such as *Leuconostoc mesenteroides* subsp. mesenteroides, *Streptococcus faecalis*, *Pediococcus halophilus* (Tanasupawat and Komagata, 1998), *Tetragenococcus* (Kobayashi *et al*., 2003) and *Leuconostoc durionis* sp. nov (Leisner *et al*., 2005). The primary function of LAB in fermentation system is to convert carbohydrates to several desired metabolites such as alcohol, acetic acid, lactic acid and carbon dioxide. Moreover, LAB are capable of producing: amino acids and peptides as a result of proteolysis; lactate, bacteriocin and γ-aminobutyric acid (GABA) as secondary metabolites (Stiles, 1994; Nomura *et al*., 1998).

GABA, a non-protein amino acid with the molecular formula, $C_4H_9NO_2$ is known as one of the major inhibitory neurotransmitters in the sympathetic nervous system and has a critical role in the regulation of neural activity. Due to its exceptional physiological functions, several GABA enriched functional foods have been manufactured, such as GABA-enriched tea (Tsushida and Murai, 1987). GABA-enriched rice-germ (Saikusa *et al*., 1994), Japanese distilled alcohol-less and fermented fish (*funa-sushi*) (Yokohama *et al*., 2002; Komatsuzaki *et al*., 2005), *tempeh* like fermented soybean extract (Watanabe *et al*., 2006), Korean fermented vegetable (*Kimchi*) (Choi *et al*., 2006), yogurt and germinated soybean extract (Park and Oh, 2007). Recently, Yang *et al*. (2008) have proven that GABA production can be optimized and increased through submerged fermentation using novel strain.

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GABA is synthesized by Glutamate Decarboxylase (GAD) (EC 4.1.1.15), a pyridoxal-5'-phosphate dependent enzyme, catalyzes the irreversibleα-decarboxylation of L-glutamate into GABA. Several studies have reported that GAD is widely distributed in the environment mostly in plants materials (Tsushida and Murai, 1987) and crops (Johnson *et al*., 1997). In mold and bacteria, high GAD activity have been reported to be found in *Aspergillus oryzae* (Tsuchiya *et al*., 2003) and *Lactobacillus brevis* (Komatsuzaki *et al*., 2008). It is very crucial for us to find alternative for GABA producer as a new source or GABA-enriched functional foods production. This can be achieved by screening and isolating a novel *Leuconostoc* strain, which can produce high concentration of GABA as well as enhancing GABA product.

MATERIALS AND METHODS

Fermented shrimp products were purchased from a local grocery store in Kuala Lumpur, Malaysia. Man Rogosa Sharp (MRS) medium (Becton, Dickinson and Company Sparks, USA) was used for LAB strains growth and maintainance.

Isolation of LAB: Samples were homogenized in 0.85% NaCl (without shaking) and incubated at 37°C for 48 h, anaerobically. To distinguish the acid producing bacteria from other bacteria, 1% $CaCO_3$ was added into MRS agar containing 2, 6.5 and 10% of NaCl, respectively. Incubation was conducted anaerobically for 48 h at different temperature of 15, 30 and 45°C. Colonies with clear zone around them were selected. About 1% $CaCO_3$ was added to the mixed culture and spread evenly onto the surface of MRS agar plates. Colonies were initially examined for Gram staining, catalase production, gas production, oxidase and motility. The selected strains were then stored at -20°C in 20% of glycerol (Chen *et al*., 2006).

Screening of GABA-producing LAB: All strains from the sample were screened for their GABA-producing ability. They were grown in MRS medium containing 50 mM of glutamate for 168 h at 37°C, anaerobically. GABA content in the supernatants was determined and quantified by High Performance Liquid Chromatography (HPLC) (Shimadzu Asia Pte).

GABA analysis: The extracellular GABA concentration in the culture medium was determined. The isolated bacteria were inoculated into MRS broth and separated from the cells by centrifugation at 8,000 g, for 15 min at 4°C. Twenty micoliter of the supernatant were diluted at 1:100 ratio in 7% (v v^{-1}) acetic acid. The diluted sample was then centrifuged at 8,000 g, for 15 min at 4°C and the supernatant was filtered using 0.22 μm Whatman syringe filter (Whatman, UK) for further analysis.

The intracellular GABA content was determined as well. The cells were washed twice with Phosphate Buffer Saline (PBS) at pH 7.0 (Sigma, USA) and the homogenate was centrifuged at 8,000×g, for 15 min at 4°C. The cells were suspended in 0.5 mL of 75% (v v^{-1}) ethanol and vigorously mixed for 1 min at room temperature. The homogenate was then centrifuged at 8,000×g, for 15 min at 4°C and the supernatant was filtered using 0.22 μm Whatman syringe filter for further analysis. The extracellular and intracellular GABA contents were used in triplicate in each independent experiment.

One hundred μL aliquot of the extracellular GABA or intracellular GABA were derivatized to Phenylthiocarbamyl-GABA (PTC-GABA) and analyzed by HPLC according to Rossetti and Lombard (1996) with minor modifications. The dried residue containing PTC-GABA was dissolved in 100 μL of mobile phase, consisting of a mixture of 80% solution A (aqueous solution of 13.608 g sodium acetate-3-hydrate, 0.5 mL triethylamine, 0.7 mL acetic acid and 5.0 mL acetonitrile in 1000 mL deionized water) and 20% solution B (acetonitrile-water (60:40)) adjusted to pH 5.8. Isocratic HPLC separation was performed using HPLC (Shimadzu Asia Pte.) with UV set of 254 nm and PDA detector. The used column was purospher STAR RP-18 LiChroCART (250-4.6 Merck, Darmstadt, Germany) and the column temperature was set at 35°C. The flow rate for the mobile phase of isocratic elution was 1.0 mL min^{-1}.

Assay for GAD activity of the cells: In order to determine, the GAD activity of the cells, the method described by Komatsuzaki *et al*. (2005) was conducted with minor modifications. The GABA-producing LAB strains were cultivated in 15 mL of MRS medium containing 100 mM of glutamate for 4 days at 37°C. The cells were collected from centrifugation at 8,000 g, for 15 min at 4°C and washed twice with PBS (pH 7.0). The cells were suspended in 20 mM of sodium phosphate buffer (pH 7.0) and left for 10 min at room temperature. The cells in suspension were disrupted by shaking vigorously for 1 min at 4°C. The crude extr acts were obtained after being centrifuged at 10,000 g for 15 min at 4°C. The crude extracts were then incubated with 50 mM glutamate and 0.2 mM of Pyridoxal-5-Phosphate (PLP) in 200 mM sodium phosphate buffer (pH 5.6) at 37°C for 30 min. The enzyme reaction was terminated by adding 3 mL of absolute ethanol at -20°C. The suspension was centrifuged at 8,000 g for 15 min at 4°C and filtered using 0.22 μm Whatman syringe filter. The

GABA content in the suspension was then determined using HPLC. One unit of enzymes activity was defined as the amount of enzyme that produced 1 μmoL of GABA in 1 min. The assay for GAD activity was done in triplicate.

The effect of cultivation time, temperature and initial pH on the extracellular and intracellular GABA contents: To determine the optimal conditions for production of extracellular and intracellular GABA, strains were cultivated in a culture medium using the following conditions; time (24, 48, 96, 120, 144, 168 and 174 h), temperatures (32, 37 and 42°C) and pH (3.5, 4.0, 5.0, 5.5 and 6.0) under anaerobic condition.

The effect of cultivation time, temperature and initial pH on GAD activity by the strain: To determine the optimum cultivation time, temperature and initial pH for GABA production, GAD activities under various cultivation temperatures (32, 37 and 42°C) and various pH (3.5, 4.0, 5.0, 5.5 and 6.0) were determined at 24, 48, 96, 120, 144, 168 and 174 h.

The effect of glutamate concentrations, addition of PLP, carbon and nitrogen sources to culture medium on GABA production: The effect of different concentrations of glutamate (100, 150, 200 and 250 mM), PLP (5, 10 and 50 mM) and carbon and nitrogen source on the GABA production was investigated. The effect of carbon and nitrogen source was carried out by adding 50 mM glutamate and 100 and 50 mM glutamate and 10 mM ammonium sulphate into MRS medium at optimum conditions (37°C, pH 5.0 and 168 h).

Determination of cell viability: Diluted sample (0.1 mL) was collected after 48 h incubation, smeared on modified MRS plate count agar and incubated anaerobically for 48 h at 37°C. Cell viability was expressed as Colony Forming Units/mL (CFU mL^{-1}).

Statistical analysis: Data were analyzed for statistical significance using single factorial ANOVA and factorial design SAS version 6.12 as mean±SD from three independent experiments with $p<0.05$.

RESULTS AND DISCUSSION

Screening and isolation of GABA-producing LAB: To screen LAB strains that produce GABA at high concentration in culture medium, 50 strains cultivated in MRS medium containing 50 mM of glutamate and measured GABA concentrations in the culture supernatants. The morphological and biochemical characteristics of the 50 isolated strains are shown in Table 1. All the positive isolates were gram positive cocci, non-motile, produce gas, catalase and oxidase negative and all the isolates grew well at 30°C compared to 15 or 45°C. The isolates were able to grow well in a medium containing 6.5 and 10% of salt, but compared to 2% salt-containing medium. This exhibits that the isolated bacteria were halophilic. Apparently, a new study has been done to isolate halophilic bacteria from the environment and can be divided into 2 groups; halophilic bacteria that grew in 1-20% concentration of NaCl were assigned as moderate halophilic and isolates that grew in 5-35% were assigned as extreme halophilic bacteria (Tarawneh *et al*., 2008). Other studies were done on the isolation of halophilic-LAB from various fermented foods, such as fermented fish, terasi (shrimp paste), fresh goat's milk, dochi (fermented black beans). Fresh goat's milk reported that most halophilic bacteria can survive in a medium containing a range of 3.2-18% of salt concentration (Tanasupawat and Komagata, 1998; Kobayashi *et al*., 2003; Chen *et al*., 2006; Mohd Adnan and Tan, 2007).

Table 1: Morphological and biochemical characteristics of LAB isolated from cincaluk

	Isolates				
Characteristics	C1	C2	C3	C4	C5
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci
Gram stain reaction	G+	G+	G+	G+	G+
Catalase activity	-	-	-	-	-
Gas production	+	+	+	+	+
Oxidase	-	-	-	-	-
Motility	-	-	-	-	-
Growth at temperature (°C)					
15	-	-	-	-	-
30	+	+	+	+	+
45	-	-	-	-	-
Growth at salt concentration (%)					
2	-	-	-	-	-
6.5	+	+	+	+	+
10	+	+	+	+	+
GABA concentration (mM)	1.5±0.07	0.4±0.91	2.1±0.16	1.2±0.13	2.84±0.19
GAD activity (unit)	1.1±0.08	0.6±0.04	1.3±0.2	0.9±0.11	1.93±0.37

+: Positive reaction; -: Negative reaction; ND: Not Detected; G+: Gram positive bacteria

Total GABA produced by the isolated LAB from *cincaluk* and their GAD activity are shown in Table 1. The result shows that out of 5 positive strains, NC5 produced the highest concentration of GABA at 2.84±0.19 mM compared to other isolates with GAD activity recorded at 1.93 mM. The isolate was identified through biochemical test and gram staining and proven to be part of the *Leuconstoc* sp. This isolate was selected for further analysis and optimization on GABA production and GAD activity.

The effect of cultivation time, temperature and initial pH on extracellular and intracellular GABA content: GABA production by NC5 strain at various cultivation times and temperatures were carried out. As shown in Fig. 1, the

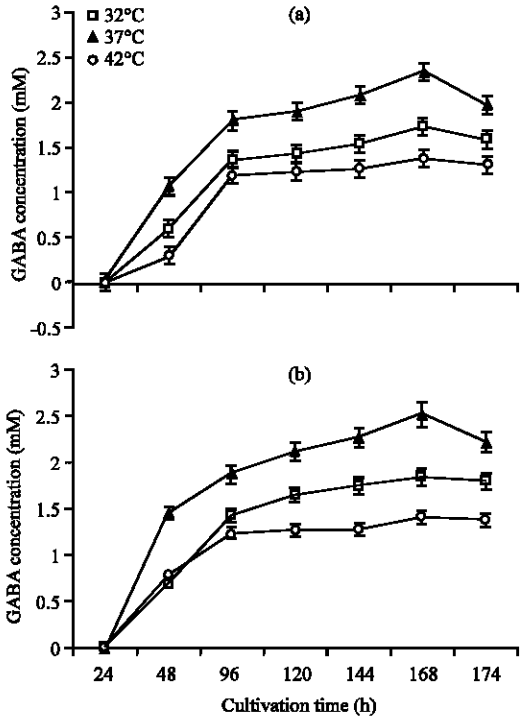

Fig. 1: The effect of cultivation time and temperature on GABA production by IC5. Extracellular (a) and intracellular (b) GABA content were investigated at various cultivation times and temperatures in MRS medium containing 50 mM glutamate. Data were expressed as mean±SD from three independent experiments

highest GABA production was at 168 h and at 37°C. At 37°C, the concentrations of GABA produced were reported at 2.3±0.16 mM extracellular and 2.5±0.71 mM intracellular. However, GABA production was the lowest at 42°C (Fig. 1a and b), compared to 32°C. The results also, showed that the NC5 strain produced intracellular GABA concentration slightly higher than extracellular GABA. These results are in contrast with those of Komatsuzaki *et al.* (2005) and Yang, *et al.* (2008), who reported that the extracellular GABA content was extremely higher than the intracellular GABA content. These two findings indicate that GABA is synthesized in the cytoplasm and secreted into the culture medium. However in this study the intracellular and the extracellular GABA content were not significantly different. This might suggest that the intracellular GABA has been synthesized prior to its secretion into the culture medium due to the presence of responsible enzyme. Yet, the exact molecular mechanism is still ambiguous.

Figure 2 shows that GABA was synthesized when the pH of the culture medium was at acidic range (pH 3.5-5.5).

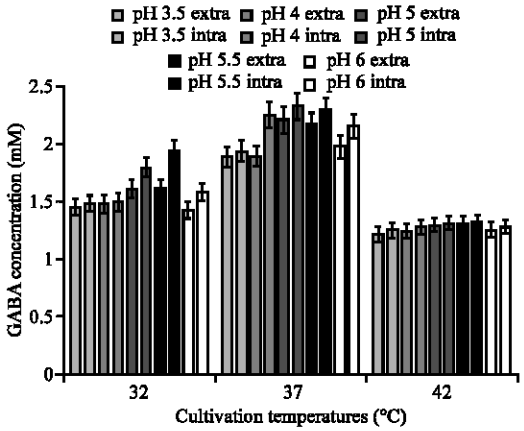

Fig. 2: The effect of cultivation temperatures and pH on GABA production by IC5 were investigated at various cultivation temperatures and pH in MRS medium containing 50 mM glutamate. Symbols: extra: extracellular and intra: intracellular. Data were expressed as mean±SD from three independent experiments

Their synthesis started to decrease close to pH 6.0. These results indicated that pH 5.0 was the optimal pH for extracellular and intracellular GABA production at 2.2±0.23 mM zand 2.3±0.21 mM, respectively.

The effect of cultivation time, temperature and initial pH on GAD activity: GABA is synthesized by α-decarboxylation of L-glutamate, catalyzed by GAD dependent PLP (E 4.1.1.15). The higher the GAD activity, the higher the amount of GABA produced. To determine the optimal medium conditions for GABA production, GAD activity under various cultivation times, temperatures and pH was measured for *Leuconostoc* NC5. Figure 3 clearly shows that, GAD activity increased at 37°C and with increased in cultivation times, up to 168 h after, which the GAD activity decreased. This result was similar to previous study that has done (Komatsuzaki *et al.*, 2005). It can be concluded that the optimal temperature and cultivation time for high GAD activity is at 37°C and cultivated at 168 h with the highest GABA production at 5.9±0.41 mM.

To determine the optimal culture medium conditions for GABA production, GAD activity under various pH and the optimal temperatures were carried out. As shown in Fig. 4, the optimal pH of GAD activity was at pH 5.0 and at cultivation temperature of 37°C, commenting the highest GABA production is at 4.4±0.51 mM. These results suggested that GABA production can be effectively improved by maintaining cellular pH of the medium to acidic conditions, it will facilitate the cell survival, because GAD must consume an H^+ ion for GABA production (Yang *et al.*, 2008).

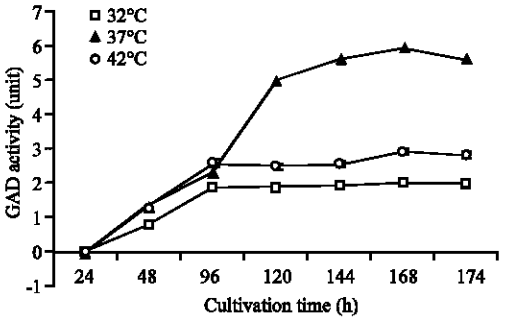

Fig. 3: The effect of cultivation time and temperature on GAD activity for GABA production by IC5. GABA content was investigated at various cultivation temperatures and time in MRS medium containing 50 mM glutamate. Data were expressed as mean±SD from three independent experiments

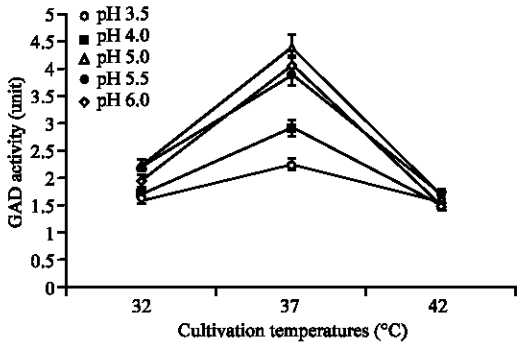

Fig. 4: The effect of cultivation temperature and pH on GAD activity for GABA production by IC5. GABA content was investigated at various cultivation temperatures and pH in MRS medium containing 50 mM glutamate. Data were expressed as mean±SD from three independent experiments

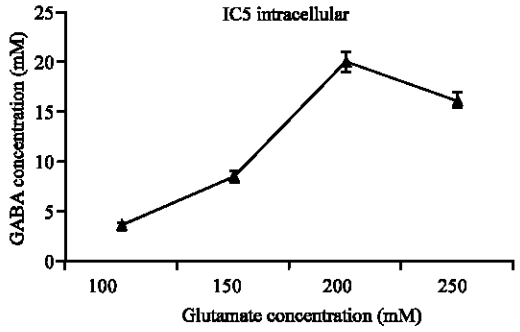

Fig. 5: The effect of glutamate concentration for GABA production. IC5 was cultivated to culture medium containing various concentration of glutamate at pH 5.0, 37°C for 168 h. Data were expressed as mean±SD from three independent experiments

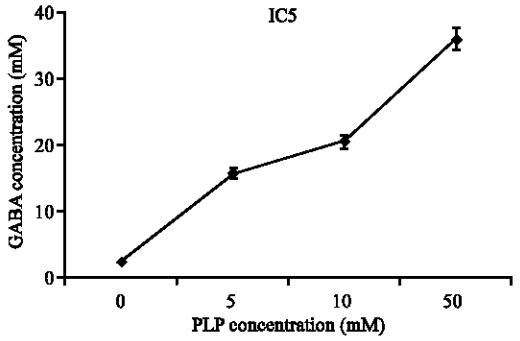

Fig. 6: The effect of addition of PLP on GABA production by IC5 grown in MRS medium containing 200 mM of glutamate at pH 5.0, 37°C for 168 h. Data were expressed as mean±SD from three independent experiments

The effect of glutamate concentrations and PLP addition to culture medium on GABA production: The highest production of GABA was achieved at 20.0±0.03 mM in 200 mM of glutamate containing culture medium during the cultivation. However, when the concentration of glutamate exceeded 200 mM, GABA production decreased (Fig. 5). These results suggested that the increment of GABA concentration depends on the addition of glutamate in the culture medium. Therefore, the glutamate concentration of 200 mM was the optimum for GABA production by *Leuconostoc* NC5.

The GABA production reached the highest level 35.7±0.01 mM, when 50 mM of PLP concentration were added under cultivation conditions at pH 5.0, 37°C and incubated for 168 h in 200 mM of glutamate (Fig. 6). The results of the current study provided additional evidence on what was previously revealed that the activity of GAD increases by adding Pyridoxal 5'-Phosphate (PLP) (Komatsuzaki *et al*., 2005) and PLP might act as a necessary coenzyme of GAD (Sandmeier *et al*., 1994).

The effect of medium additives on GABA production: Five percent of glutamate converted to GABA, when 10 mM ammonium sulphate was added to the medium. The results showed that the addition of 10 mM ammonium sulphate significantly increased the GABA production to 12.3±0.06 mM, even though no dose dependent data was observed (Fig. 7). The addition of 1% glucose as a carbon source shows no increment in the GABA production. The productivity of microbial metabolites can be improved by manipulating the physical and nutritional factors of the microbial growth. Although, glutamate is known as flavor

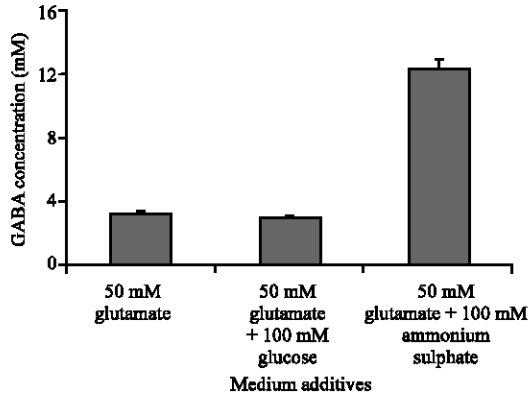

Fig. 7: The effect of medium additives on GABA production by IC5 during cultivation conditions. Data were expressed as mean±SD from three independent experiments

enhancer in foods, glutamate is associated with human body disorders like stroke, autism and Alzheimer's disease because of its excitatory neurotransmitter effect in mammalian nervous system. Hence, it is very crucial to minimize the usage of glutamate in food industry for human consumption.

GABA-producing LAB strains proven to have unique fermentation profiles such as acid production and flavor formation ability were screened for use as starters for various fermented foods. This helps to improve the nutritional and functional properties of the fermented foods as it is evident that GABA can alleviate blood pressure in spontaneously hypertensive and normotensive Wistar-Kyoto rats (Hayakawa *et al*., 2004) and functions as neurotransmission, diuretic effects and analgesics (Ueno, 2000). Therefore, the effects of GABA on human health have become a growing interest in food production.

CONCLUSION

From this study, we can conclude that the GABA production during cultivation of the isolated strain can be improved by optimizing the growth and cultivation condition as well as its available nutrient. It was found that GABA is produced under acidic conditions (pH 5.0), which contribute towards cells survival-due to the GAD consumption of H^+ ion. Therefore, GABA-producing LAB are capable of growing and surviving in the human gastrointestinal system (acidic condition) and exerting a potential probiotics effects. Although, *Leuconostoc* sp. has been widely used in dairy and vegetables fermentation, this finding can be considered as the first coverage on Malaysian's fermented product.

Application of the successfully isolated strain into other food matrix has led to a growing interest among food manufacturers to food technology to improve nutrition in functional foods for the industry. As *Leuconostoc* NC 5 strain can survive in different sources of foods and extreme conditions such as high salt concentration and various pHs, this starter culture can be also be incorporated into canned foods and other food productions.

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REFERENCES

Chen, Y.S., F. Yanagida and J.S. Hsu, 2006. Isolation and characterization of lactic acid bacteria from *dochi* (fermented black beans), a traditional fermented food in Taiwan. Lett. Applied Microbiol., 14: 76-81. DOI:10.1111/j.1472-765X.2006.01922.x. PMID: 16869910.

Choi, S.I., J.W. Lee, S.M. Park, M.Y. Lee, G.E. Ji, M.S. Park and T.R. Heo, 2006. Improvement of γ-Aminobutyric Acid (GABA) production using cell entrapment of *Lactobacillus brevis* GABA 057. J. Microbial. Biotechnol., 16: 562-568. http://www.scopus.com/record/display.url?view=basic&eid=2-s2.0-33646567772&origin=resultslist&sort=plf-f&src=s&sid=kwQum5lZ-H2betsFIHT6im-%3a150&sot=q&sdt=b&sl=105&s=TITLE-ABS-KEY-AUTH%28improvement+of+GABA+production+using+cell+entrapment+of+lactobacillus+brevis+GABA+057%29&relpos=0&relpos=0.

Hayakawa, K., M. Kimura, K. Kasaha, K. Matsumoto, H. Sansawa and Y. Yamori, 2004. Effect of γ-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. Br. J. Nutr., 92: 411-417. DOI: 10.1079/BJN20041221. PMID: 154696444. http://journals.cambridge.org/download.php?file=%2FBJN%2FBJN92_03%2FS0007114504001850a.pdf&code=8d0b3d00837e015a968140bc4db80943.

Johnson, B.S., N.K. Singh, J.H. Cherry and R.D. Locy, 1997. Purification and characterization of glutamate decarboxylase from Cowpea. Phytochemistry, 46 (1): 39-44.

Leisner, J.J., M. Vancanney, R. Van der Meulen, K. Lefebvre, K. Engelbeen, B. Hoste, B.G. Laursen, L. Bay, Rusul, L. De Vuyst and J. Swings, 2005. *Leuconostoc durionis* sp. nov, a heterofermenter with no detectable gas production from glucose. Int. J. Syst. Evolutionary Microbiol., 55: 1267-1270. DOI: 10.1099/ijs.0.63434-0. PMID: 15879266. http://ijs.sgmjournals.org/cgi/content/full/55/3/1267.

Park., K.B. and S.H. Oh, 2007. Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. Bioresour. Technol., 98: 1675-1679. DOI: 10.1016/j.biortech.2006.06.006. PMID: 17055264. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6V24-4M4TNKX-1-5&_cdi=5692&_user=4704852&_orig=search&_coverDate=05%2F31%2F2007&_sk=999019991&view=c&wchp=dGLbVtz-zSkzk&md5=57c38a95fa8f3c4b95441b8f32ac762f&ie=/sdarticle.pdf.

Kobayashi, T., M. Kajimara, M. Wahyuni, T. Kitakado, N. Hamada-Sado, C. Imada and E. Watanabe, 2003. Isolation and characterization of halophilic lactic acid bacteria isolated from terasi shrimp paste: A traditional fermented seafood product in Indonesia. J. General Applied Microbiol., 49: 279-286. DOI: 10.2323/jgam.49.279. PMID: 14673751. http://www.jstage.jst.go.jp/article/jgam/49/5/279/_pdf.

Komatsuzaki, N., J. Shima, S. Kawamoto, H. Momose and T. Kimura, 2005. Production of γ-Aminobutyric Acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. Food Microbiol., 22: 497-504. DOI: 10.1016/j.fm.2005.01.002. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6WFP-4G241FT-2-X&_cdi=6800&_user=4704852&_orig=search&_coverDate=12%2F31%2F2005&_sk=999779993&view=c&wchp=dGLbVlb-zSkWz&md5=954815e797726f650ea8c2a844b07b73&ie=/sdarticle.pdf.

Komatsuzaki, N., Nakamura, T. Kimura and J. Shima, 2008. Characterization of glutamate decarboxyalse from a high γ-Aminobutyric Acid (GABA) Producer, *Lactobacillus paracasei.* Biosci. Biotechnol. Biochem., 72 (2): 278-285. DOI: 10.1271/bbb.70163. http://www.jstage.jst.go.jp/article/bbb/72/2/278/_pdf.

Mohd Adnan, A.F. and I.K.P. Tan, 2007. Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. Bioresour. Technol., 98 (7): 1380-1385. DOI: 10.1016/j.biortech.2006.05.034. PMID: 16872826. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6V24-4KGX84R-1-1&_cdi=5692&_user=4704852&_orig=search&_coverDate=05%2F31%2F2007&_sk=999019992&view=c&wchp=dGLbVlb-zSkzV&md5=a201fedd49d3eb8700acfc65da838abb&ie=/sdarticle.pdf.

Nomura, M., H. Kimoto, Y. Someya, S. Furukawa and I. Suzuki, 1998. Production of γ-Aminobutyric Acid by cheese starters during cheese ripening. J. Dairy Sci., 81: 1486-1491. PMID: 9684157. http://jds.fass.org/cgi/reprint/81/6/1486.

Saikusa, T., T. Horino and Y. Mori, 1994. Accumulation of γ-Aminobutyric Acid (GABA) in the rice germ during water soaking. Biosci. Biotechnol. Biochem., 58: 2291-2292.

Sandmeier, E., T.I. Hale and P. Christen, 1994. Multiple evolutionary origin of pyridoxal 5'-phosphate-dependent amino decarboxylase. Eur. J. Biochem., 221: 997-1002. DOI: 10.1111/j.1432-1033/1994.tb18816.x. PMID: 8181483. http://www3.interscience.wiley.com/cgi-bin/fulltext/120768525/PDFSTART.

Stiles, M.E., 1994. Potential for control agents of foodborne disease. Food Res. Int., 27: 245-250. DOI: 10.1016/0963-9969(94)90091-4. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T6V-49KK542-3Y-1&_cdi=5040&_user=4704852&_orig=search&_coverDate=12%2F31%2F1994&_sk=999729996&view=c&wchp=dGLbVzW-zSkzk&md5=d641a4c0d5d9c03e1490ce6a77a38003&ie=/sdarticle.pdf.

Tarawneh, K.A., M. Wedyan, M.A. Al-zou'bi, K.M. Khleifat and A. Tarawneh, 2008. Isolation and characterization of halophilic bacteria from the Dead Sea Coast, Jordan. Adv. Environ. Biol., 2 (2): 63-69. http://www.aensi.org/aeb/2008/63-69.pdf.

Tanasupawat, S. and K. Komagata, 1998. Lactic acid bacteria in fermented foods in Thailand. J. General Applied Microbiol., 44 (3): 193-200. DOI: 10.2323/jgam.44.193. http://www.jstage.jst.go.jp/article/jgam/44/3/193/_pdf.

Tsuchiya, K., K. Nishimura and M. Iwahara, 2003. Purification and characterization of glutamate decarboxylase from *Aspergillus oryzae*. Food Sci. Technol. Res., 9 (3): 283-287. DOI: 10.3136/fstr.9.283. http://www.jstage.jst.go.jp/article/fstr/9/3/283/_pdf.

Tsushida, T. and T. Murai, 1987. Conversion of glutamic acid to γ-aminobutyric acid in tea leaves under anaerobic conditions. Agric. Biolog. Chem., 51: 2865-2871. DOI: JOIJST.Journalarchive/bbb1961/51.2865. http://www.journalarchive.jst.go.jp/jnlpdf.php?cdjournal=bbb1961&cdvol=51&noissue=11&startpage=2865&lang=en&from=jnlabstract.

Ueno, H., 2000. Enzymatic and structural aspect on glutamate decarboxylase. J. Mol.Catalysis, 10: 67-79. DOI: 10.1016/S1381-1177(00)00114-4. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TGN-40T9JY0-7-F&_cdi=5259&_user=4704852&_orig=search&_coverDate=10%2F04%2F2000&_sk=999899998&view=c&wchp=dGLzVtz-zSkzV&md5=c65c9ba4973e0e2b21a43bfd7d2c7668&ie=/sdarticle.pdf.

Yang, S.Y., F.X. Lü, Z.X. Lu, X.M. Bie, Y. Jiao, L.J. Sun and B. Yu, 2008. Production of γ-Aminobutyric Acid (GABA) by *Streptococcus salivarious* subsp. thermophilus Y2 under submerged fermentation. Amino Acids, 34: 473-478. DOI: 10.1017/s00726-007-0544-x. PMID: 17514494. http://www.springerlink.com/content/5483u6474x056661/fulltext.pdf?page=1.

Rossetti, V. and A. Lombard, 1996. Determination of glutamate decarboxylase by high-performance liquid chromatography. J. Chromatogr. B., 681: 63-67. DOI: 10.1016/0378-4347(96)88202-8. PMID: 8798913. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TG9-3V9965F-19-1&_cdi=5249&_user=4704852&_orig=search&_coverDate=05%2F31%2F1996&_sk=993189998&view=c&wchp=dGLzVtz-zSkWz&md5=7094e10d1f3210760990a4acfb77fe5e&ie=/sdarticle.pdf.

Yokohama, S., J.I. Hiramatsu and K. Hayakawa, 2002. Production of γ-aminobutyric acid from alcohol distillery lees by *Lactobacillys brevis* IFO-12005. J. Biosci. Bioengin., 93: 95-97. DOI: 10.1016/S1389-1723(02)80061-5. PMID: 16233172. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6VSD-46G3T2V-M-1&_cdi=6260&_user=4704852&_orig=search&_coverDate=01%2F31%2F2002&_sk=999069998&view=c&wchp=dGLbVzz-zSkzS&md5=a320d2308f1f582bb4c1b9c9b358ece0&ie=/sdarticle.pdf.

Watanabe, N., Y. Endo, K. Fujimoto and H. Aoki, 2006. Tempeh-like fermented soybean (GABA-tempeh) has an effective influence on lipid metabolism in rats. J. Oleo Sci., 55 (8): 391-396. DOI: JOI JST.JSTAGE/jos/55.391. PMID: 06A0569772. http://www.jstage.jst.go.jp/article/jos/55/8/391/_pdf.