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Short communication

Antioxidant activity and γ-aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods

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ABSTRACT

GABA-producing lactic acid bacteria were isolated from kimchi and salt-fermented Jot-gal, which are traditional Korean fermented foods. The strain, BJ-20, isolated from salt-fermented Jot-gal (cod gut), possessed the highest GABA-producing ability in MRS broth with 1% monosodium glutamate (MSG), as determined by thin layer chromatography. The BJ-20 strain was identified as *Lactobacillus brevis* and designated as *L. brevis* BJ20. A sea tangle solution was fermented over 5 days to produce GABA using *L. brevis* BJ20. During fermentation, the GABA concentration dramatically increased, while the glutamic acid concentration decreased. This result indicates that the glutamic acid was converted to GABA by *L. brevis* BJ20 in the fermented sea tangle solution. Furthermore, the fermented solution exhibited strong antioxidant activities, such as DPPH scavenging, superoxide scavenging, and xanthine oxidase inhibition, which were higher than those of BHA as a positive control.

1. Introduction

Seaweeds provide essential bioactive compounds, such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins, and minerals, and are a nutritional food choice for growing children and pregnant women (Athukorala, Kim, & Jeon, 2006). In addition, many studies have reported that seaweed extracts possess biological activities, such as anticoagulation, protection against oxidative cell damage, antioxidant and immunomodulatory effects (Athukorala, Lee, Kim, & Jeon, 2007; Kim & Joo, 2007). As a result, seaweed is generally believed to be a good candidate for the production of safe biologically-active substances.

Antioxidants are substances that delay or prevent the oxidation of cellular oxidisable substrates by scavenging free radicals and reactive oxygen species (ROS), preventing the generation of free radicals and ROS, and/or activating a battery of detoxifying proteins. Until recently, several synthetic antioxidants, including BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and TBHQ (*t*-butyl hydroquinone), were commonly used to maintain foodstuffs; however, the use of these products has been restricted because they are now suspected to be carcinogenic (Kumar, Ganesan, & Rao, 2008). Accordingly, naturally-occurring antioxidants from bioresources such as seaweeds have received a great deal of attention during the past decade.

γ-Aminobutyric acid (GABA) is a non-protein amino acid that is widely distributed in nature and acts as a major inhibitory neurotransmitter in the central nervous system (Manyam, Katz, Hare, Kaniefski, & Tremblay, 1981). GABA is produced by glutamate decarboxylase that catalyses the irreversible decarboxylation of L-glutamate to GABA (Bown & Shelp, 1997). GABA is well-known for its physiological functions, such as the induction of hypotension, and diuretic and tranquiliser effects (Siragusa et al., 2007). Owing to these physiological functions, the commercial demand for GABA is increasing and GABA-enriched functional foods have been reported as follows: GABA-enriched green tea by anaerobic or cyclic treatments of tea leaves or shoots (Ohmori et al., 1987), GABA-enriched rice germ by soaking in water (Saikusa, Horino, & Mori, 1994), GABA-enriched brown rice by high-pressure treatment and germination (Kinefuchi, Sekiya, Yamazaiki, & Yamamoto, 1999), tempeh-like fermented soybeans (Aoki, Furuya, Endo, & Fujimoto, 2003), and dairy products (Nomura, Kimoto, Someya, Furukawa, & Suzuki, 1998; Park & Oh, 2007). Recently, lactic acid bacteria (LAB), including *Lactobacillus brevis* isolated from kimuchi

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(Ueno, Hayakawa, Takahashi, & Oda, 1997) and alcohol distillery lees (Yokoyama, Hiramatsu, & Hayakawa, 2002), as well as *Lactococcus lactis* from cheese starters (Nomura et al., 1998), were used for the mass production of GABA.

The aims of this study were to screen 20 traditional Korean fermented foods in order to isolate and identify GABA-producing lactic acid bacteria, to produce GABA by fermenting sea tangle using a selected strain, and to evaluate the antioxidant activity of the fermented sea tangle solution.

2. Materials and methods

2.1. Materials

Twenty-four traditional Korean fermented foods were considered in this study. Ten kinds of kimchi, such as Baechu kimchi (fermented for 10 days), Mul kimchi (fermented for 7 days), Baik kimchi (fermented for 7 days), Chonggak kimchi (fermented for 7 days), Kimchi altari (fermented for 5 days), Yulmoo kimchi (fermented for 7 days), leaf mustard kimchi (fermented for 15 days), Codulbaegi kimchi (fermented for 15 days), garlic jangachi (fermented for 17 days), and Oisobaki (fermented for 5 days) were used. Fourteen kinds of salt-fermented Jot-gal, such as anchovy (fermented for 6 months, 1 and 2 years), squid (fermented for 3 months), octopus (fermented for 3 and 6 months), sand lance (fermented for 1 and 2 years), cod gut (fermented for 15 days), Alaska pollack roe (fermented for 15 days), oyster (fermented for 4 months), yellow croaker (fermented for 3 months), and shrimp (fermented for 6 months and 1 year), were used to screen for GABA production. The sea tangle was purchased from Food & Food Co., Ltd. (Busan, Korea), and GABA, monosodium glutamate (MSG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), xanthine, xanthine oxidase, and nitro blue tetrazolium (NBT) were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals and reagents used in this study were of analytical grade and commercially available.

2.2. Isolation of LAB and identification of GABA by thin layer chromatography (TLC)

One millilitre samples of kimchi and jot-gal juice, serially diluted, were plated on MRS agar (Oxoid Ltd., Basingstoke, Hampshire, England) and placed at 30 °C for 24 h under anaerobiosis to isolate presumptive mesophilic LAB. At least 10 colonies, possibly with different morphology, were isolated on the MRS plates.

In order to select a LAB with high GABA-producing ability, the bacteria were grown in MRS broth containing 1% MSG at 30 °C for 24 h, and then the supernatants were filtered with a 0.2-μm membrane filter. GABA was identified by TLC on activated silica gel plates (Silica gel 60 F_{254}, Merck Co., Darmstadt, Germany) using *n*-butanol: acetic acid: water (5:2:2, v/v/v). The chromatogram was viewed after spraying with a 2% ninhydrin solution and developing at 105 °C for 5 min.

2.3. Identification of isolated LAB

The genomic DNA was extracted using a CoreOne bacterial DNA extraction kit (Coretech. Co. Ltd., Seoul, Korea) from 1.5 ml of the MRS culture broth of the selected LAB. The 16S rDNA gene was amplified from a single isolated colony using 9F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1512R (5′-ACGG(H)TACCTTGTTACGACTT-3′) primers. The PCR reaction was carried out by a T1 Thermocycle (Biometra, Goettingen, Germany). Amplicons were obtained with a PCR cycling program of 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and a final 7 min extension at 72 °C. The amplified products were visualised by electrophoresis separation on 1% agarose gel stained with ethidium bromide.

The PCR fragments were purified by using a QIAquick PCR purification kit (Bioneer, Seoul, Korea). The expected amplicons of 1400 bp were sequenced with the same primers, using an ABI Prism Bio Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an automatic DNA sequencer (ABI Prism Model 3700, Applied Biosystems). The nearly complete 16S rDNA gene sequences were used to search the GenBank database. A phylogenetic tree was constructed by generating a complete alignment of the 16S rDNA gene sequences of the selected members in GenBank by using MEGA software bootstrap values (Tamura, Dudley, Nei, & Kumar, 2007).

2.4. Preparation of fermented sea tangle solution using LAB

The isolated LAB strain was cultured in Lactobacilli MRS broth at 37 °C. For the production of GABA in fermented sea tangle, the sea tangle was added to water at a ratio of 1:15 (w/v) and 2% (w/w) of rice flour based on the amount of sea tangle added to the mixture to aid fermentation. Final volume of the fermentation process was 1 L and the fermentation process was performed with 5 days. After autoclaving at 121 °C for 30 min, a sea tangle solution was obtained and the LAB culture broth was added to the solution at a concentration of 2% (v/v), which was well mixed and then incubated at 37 °C. The intact solution (control) had no LAB.

The GABA content in the culture broth was measured as follows. A 1-ml sample was taken and diluted 20-fold using 0.02 N HCl. The diluted solution (2 ml) was filtered through a 0.2-μm membrane filter (DISMIC-25CS, Toyo Roshi Kaisha, Tokyo, Japan). The filtered sample (20 μl) was subjected to an amino acid analyser (AAA L-8900, Hitachi High-Technologies Co., Tokyo, Japan) with a flow rate of 0.3 ml/min.

2.5. DPPH scavenging assay

The DPPH-scavenging activity of the fermented sea tangle solution was measured according to the method described by Heo, Park, Lee, and Jeon, 2005. Briefly, DPPH solution (0.4 mM, 100 μl) was mixed with the fermented sea tangle solution (100 μl), after which the mixture was incubated at room temperature for 30 min. The absorbance of the mixture was then determined at 517 nm using a microplate reader (ELx 808™, BioTek, Winooski, VT). BHA was used as the positive control (50 and 100 μg/ml).

Scavenging activity (%) = $[(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance of the sample.

2.6. Superoxide scavenging assay

The superoxide scavenging activity of the fermented sea tangle solution was measured using the xanthine/xanthine oxidase method with slight modifications (Mao, Pan, Que, & Fang, 2006). The fermented solution was added to a mixture of 0.5 mM xanthine and 1 mM EDTA in 100 mM phosphate buffer (pH 7.5). Xanthine oxidase (50 mU/ml) was then added and the resulting mixture was incubated in a water bath at 37 °C for 1 h. After incubation, 0.5 mM NBT was added and this resulting mixture was re-incubated at 37 °C for 20 min. The reaction was terminated by adding 2.0 N HCl and the absorbance was measured at 560 nm. BHA was used as the positive control.

Scavenging activity (%) = $[(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance in the presence of sample.

2.7. Xanthine oxidase inhibition assay

The xanthine oxidase inhibition assay was performed according to the protocol of Kong, Zhang, Pan, Tan, and Cheng (2000), with slight modifications. The assay mixture, consisting of 50 μl of the fermented sea tangle solution, 100 μl of 100 mM phosphate buffer (pH 7.5), and 50 μl of 50 mU/ml xanthine oxidase was prepared immediately before use. After preincubation at 25 °C for 10 min, the reaction was initiated by the addition of 50 μl of 0.5 mM xanthine. The reaction was monitored for 1 h at 290 nm.

Scavenging activity (%) = $[(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the blank and A_1 is the absorbance in the presence of sample.

2.8. Statistical analysis

All experiments were performed in triplicate. Data are presented as mean ± SD. Statistical significance was determined by Student's *t*-test. Values with $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Screening and identification of GABA-producing LAB

A total of 22 isolates producing GABA in MRS culture medium were isolated from kimchi and salt-fermented Jot-gal. To select LAB strains that would produce GABA at a high concentration in the culture medium, the 22 isolates were cultivated in MRS medium containing 1% MSG and GABA was measured in the culture supernatants by using TLC. Six of the strains (BJ-2, BJ-6, BJ-7, BJ-12, BJ-20, and BJ-21) were selected, and the TLC chromatogram is shown in Fig. 1. The chromatogram shows that the culture supernatant of the BJ-20 strain exhibited a strong GABA spot compared to the other strains. A lactic acid bacterium, the BJ-20 strain was isolated from salt-fermented Jot-gal (cod gut). It is a gram-positive, rod-type strain with high GABA-generating capacity. To confirm the identification of the BJ-20 strain, 16S rDNA was amplified and 1400 bp of the DNA sequence were determined (data not shown). The GenBank database was used to search for genes similar to the 16S rDNA sequence, revealing that its sequence identity was 99% with *L. brevis* (GenBank accession No. AF429529), 99% with *L. brevis* (GenBank accession No. AF429497), and 99% with *L. brevis* (GenBank accession No. AF429496). Therefore, BJ-20 was considered to be a strain of *L. brevis* and designated as *L. brevis* BJ20. The strain was deposited at the Korea Research Institute of Bioscience and Biotechnology (accession No. KCTC 11377BP).

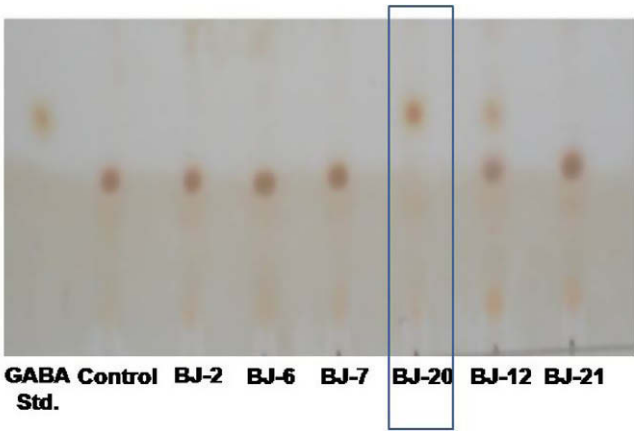

Fig. 1. TLC chromatogram for the selection of GABA-producing LAB. The development solvent consisted of *n*-butanol:acetic acid:water (5:2:2, v/v). The chromatogram was viewed after spraying with a 2% ninhydrin solution and developing at 105 °C for 5 min.

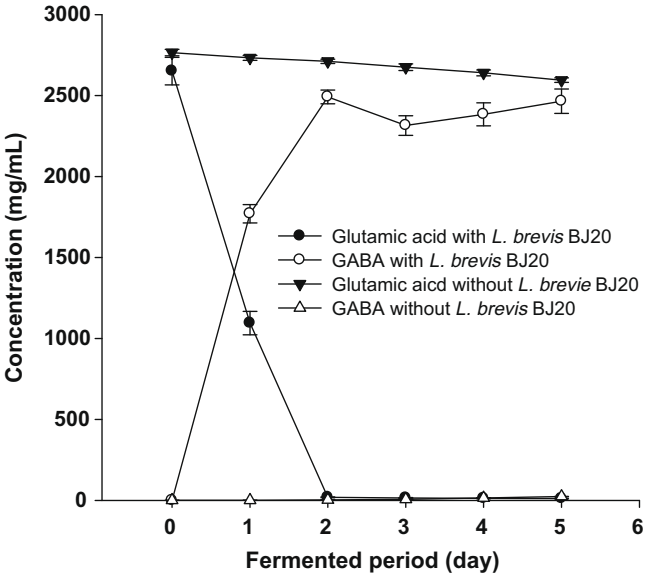

Fig. 2. Changes in GABA and glutamic acid concentrations in fermented sea tangle solution during fermentation with and without *L. brevis* BJ20.

Table 1
Free amino acid contents of fermented sea tangle solutions with and without *L. brevis* BJ20.

Amino acids	Fermented sea tangle solution			
	Without *L. brevis* BJ20 (0 day) mg/l sample	Without *L. brevis* BJ20 (5 days later) mg/l sample	With *L. brevis* BJ20 (0 day) mg/l sample	With *L. brevis* BJ20 (5 days later) mg/l sample
Phosphoserine	14.7	35.9	24.6	33.0
Taurine	21.6	13.9	14.4	26.3
Phosphoethanolamine	5.30	8.65	8.09	7.65
Aspartic acid	1606	1821	1834	1285
Threonine	14.8	9.65	1.43	4.37
Serine	28.0	30.7	32.9	1.67
Glutamic acid	2790	2595	2651	11.4
Glycine	9.62	29.9	7.86	30.2
Alanine	163	227	198	814
DL-2-Aminobutyric acid	5.50	5.91	5.31	33.3
Valine	10.2	33.0	4.79	44.8
Methionine	0.52	3.74	0.00	0.00
Cystathione	6.79	9.98	4.37	10.2
Isoleucine	4.19	18.4	0.97	11.3
Leucine	5.67	26.2	1.05	38.3
Tyrosine	4.44	4.44	0.00	0.00
Phenylalanine	3.39	26.4	0.00	26.7
β-Alanine	1.09	3.22	2.26	10.1
Aminoisobutyric acid	0.00	3.03	1.15	4.55
γ-Aminobutyric acid (GABA)	0.00	23.9	0.00	2465
Aminoethanol	9.02	13.6	14.9	14.2
Ornithine	4.34	10.5	2.84	2.80
Lysine	5.67	18.6	0.00	9.62
Histidine	0.87	6.12	0.00	0.00
Arginine	6.72	0.00	2.40	12.4
Proline	87.0	102	116	85.3
Total	4808	5081	4929	4983

3.2. GABA changes in fermented sea tangle solution during fermentation by L. brevis BJ20

Beneficial nutrients occurring in seaweeds include vitamins, trace minerals, lipids, plant sterols, amino acids, and antioxidants, all of which form part of a healthy diet (Athukorala et al., 2006). Seaweeds are used as a functional food source, and pharmaceuticals and functional foods containing GABA have also been developed. To date, most GABA sources in pharmaceuticals and functional foods are derived from various fruits and vegetables (Kim, Lee, Ji, Lee, & Hwang, 2009). However, there is little information regarding the production of GABA using marine plants such as seaweeds. Therefore, in this study, we produced GABA in a sea tangle solution by fermentation with *L. brevis* BJ20.

In order to produce GABA, a sea tangle solution was obtained after autoclaving at 121 °C for 30 min, and then fermented at 37 °C for 5 days. The GABA-producing capacity of *L. brevis* BJ20 was determined by conducting a time course analysis in the culture supernatant using an amino acid analyser. Fig. 2 shows the GABA and glutamic acid changes in the sea tangle solution during fermentation with *L. brevis* BJ20. The glutamic acid content was 2,789 mg/l before fermentation, however, its concentration dramatically decreased during fermentation, and ultimately, it was no longer detected in the fermented sea tangle solution. GABA was not detected in the intact solution without *L. brevis* BJ20. However, GABA content dramatically increased during fermentation with *L. brevis* BJ20, and it was ultimately detected at the concentration of 2,465 mg/l after 5 days of fermentation. This result indicates that the glutamic acid in the sea tangle solution was completely converted to GABA by *L. brevis* BJ20. The free amino acid contents of the fermented sea tangle solutions with and without *L. brevis* BJ20 are shown in Table 1. After fermentation, most of the glutamic acid was converted to GABA, and some levels of amino acids such as aspartic acid, serine, and threonine decreased. The contents of alanine, valine, glycine, and leucine dramatically increased after fermentation.

Several biological activities of GABA and/or GABA-enriched foods have been reported. Oh and Oh (2003) investigated the

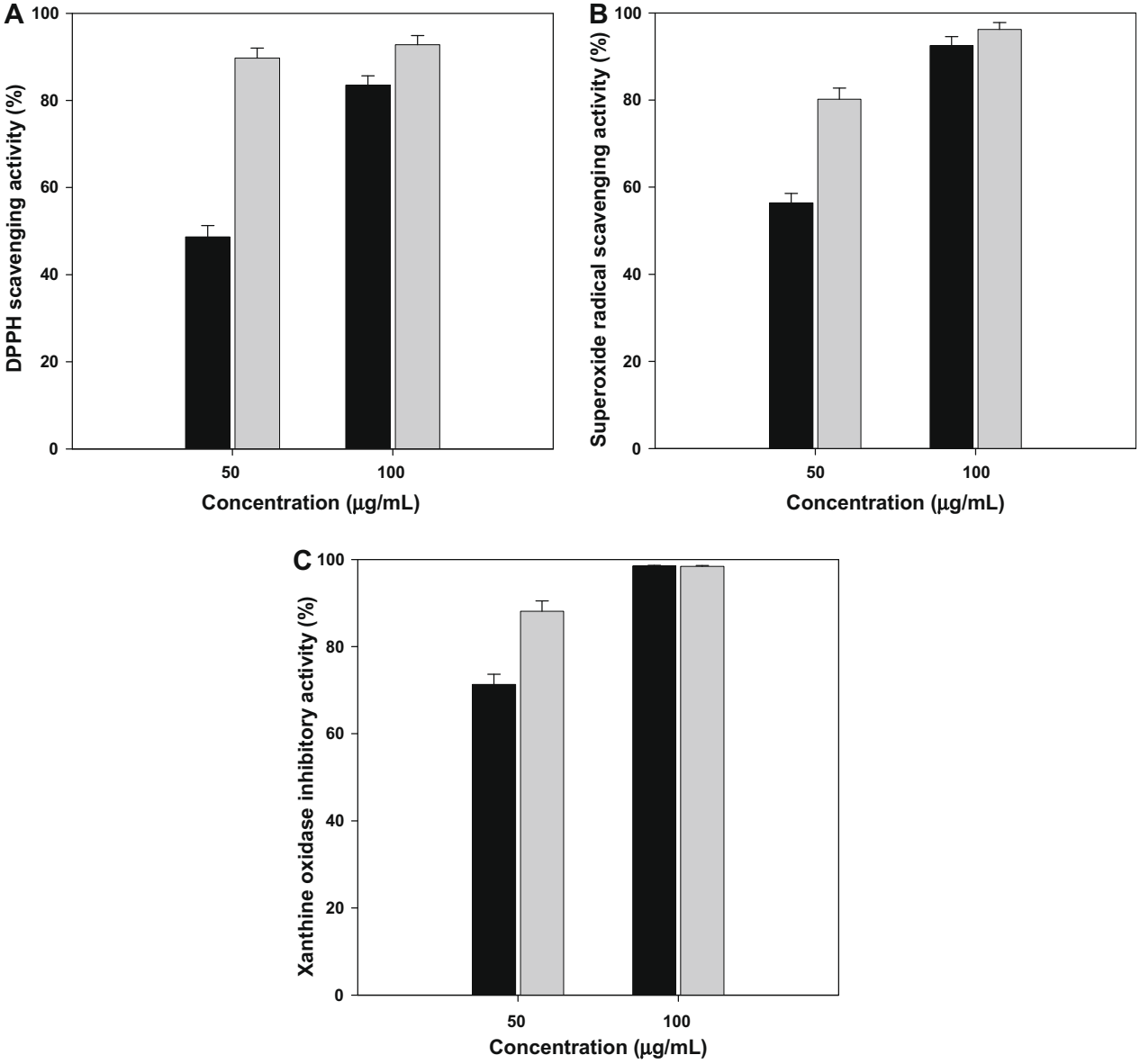

Fig. 3. (A) DPPH-scavenging activity. (B) Superoxide radical-scavenging activity. (C) Xanthine oxidase inhibitory activity of fermented sea tangle solution and BHA as a positive control. Results are means ± S.E. of three independent experiments. (■) BHA, (■) fermented sea tangle solution.

immune cell-stimulating activity of brown rice extracts with enhanced levels of GABA, showing increased cell proliferation of mesenteric lymph node cells. They also reported that brown rice extracts with enhanced GABA levels had inhibitory actions on leukaemia cell proliferation and stimulatory effects on cancer cell apoptosis (Oh & Oh, 2004). GABA has also been reported to lower blood pressure in experimental animals as well as human subjects (Inoue et al., 2003; Kimura, Hayakawa, & Sansawa, 2002). In some cases, MSG is added as an ingredient source for GABA production, but this is not positively received by consumers. However, in this study, we used naturally-occurring glutamic acid in the sea tangle solution as a source for GABA production, and the results revealed that almost all the glutamic acid was converted to GABA during fermentation with *L. brevis* BJ20.

3.3. Antioxidant activities of the fermented sea tangle solution

The DPPH radical model is a widely-used, relatively quick method for evaluating free radical-scavenging activity. The effect of antioxidants on DPPH radical scavenging is believed to be due to their hydrogen-donating ability (Baumann, Wurn, & Bruchlausen, 1979), and this scavenging is visually noticeable as a change in colour from purple to yellow. Therefore, DPPH is often used as a substrate to evaluate antioxidant activity. Fig. 3A shows the DPPH-scavenging activity of the fermented sea tangle solution, in which its scavenging potency ranged from 87.7% to 92.8% at concentrations of 50 and 100 μg/ml, respectively.

Superoxide radicals are known to be very harmful to cellular components as precursors to more reactive oxidative species, such as single oxygen and hydroxyl radicals. As shown in Fig. 3B, the fermented sea tangle solution effectively quenched superoxide radicals in a dose-dependent manner. The scavenging activity reached 96.2% at the concentration of 100 μg/ml.

Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid (Yang, Huang, Lee, & Lu, 2002). It is also related to important biological sources of ROS that play key roles in many diseases, such as cancer, gastric ulcers, Alzheimer's, arthritis, and ischaemic reperfusion (Je, Park, & Kim, 2004). As shown in Fig. 3C, the fermented sea tangle solution significantly inhibited xanthine oxidase in a dose-dependent manner. Several researches regarding antioxidant activity of seaweeds have been reported (Chandini, Ganesan, & Bhaskar, 2008; Heo et al., 2005). Antioxidant compounds were extracted by the solvent method and enzymatic digestion and showed high antioxidant activities at milligram levels against free radical species. In this study, the antioxidant activities of the fermented sea tangle solution were more potent than those of the published literature, indicating that the fermentation process is also a good method for producing antioxidant compounds from seaweeds. We also used BHA as a positive control and it also showed good antioxidant activities against DPPH, superoxide radical and xanthine oxidase inhibition but its activities were lower than that of the fermented sea tangle solution. Currently, natural, effective and safe antioxidants are in high demand as alternatives to commercial synthetic antioxidants such as BHT, BHA and TBHQ, due to their side effects. Therefore, the results indicate that seaweeds are good candidates as natural antioxidants.

4. Conclusion

In summary, a LAB with GABA-producing ability was isolated from salt-fermented Jot-gal (cod gut), and 16S rDNA analysis revealed that this isolate was *L. brevis*. A sea tangle solution was fermented with the *L. brevis* BJ20 strain over 5 days in order to produce a GABA-enriched solution. After fermentation, almost all the glutamic acid in the solution had been converted to GABA. To the best of our knowledge, this is the first report of GABA production using seaweeds. However, further studies on an optimisation of the biotechnological parameters, such as cell density, pH, temperature are needed and these works are in progress. Furthermore, the fermented sea tangle solution exhibited strong antioxidant activities, such as DPPH scavenging, superoxide radical scavenging, and xanthine oxidase inhibition. These results suggest that fermented sea tangle solution is a promising ingredient source for use in functional foods and/or pharmaceuticals.

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References

Aoki, H., Furuya, Y., Endo, Y., & Fujimoto, K. (2003). Effect of γ-aminobutyric acid-enriched tempeh-like fermented soybean (GABA-tempeh) on the blood pressure of spontaneously hypertensive rats. *Bioscience, Biotechnology, and Biochemistry, 67*, 1806–1808.

Athukorala, Y., Kim, K. N., & Jeon, Y. J. (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food and Chemical Toxicology, 44*, 1065–1074.

Athukorala, Y., Lee, K. W., Kim, S. K., & Jeon, Y. J. (2007). Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Bioresource Technology, 98*, 1711–1716.

Baumann, J., Wurn, G., & Bruchlausen, F. V. (1979). Prostaglandin synthetase inhibiting O_2-radical scavenging properties of some flavonoids and related phenolic compounds, Naunyn, Schmiedebergs. *Archives Pharmacology, 308*, 27–32.

Bown, A. W., & Shelp, B. J. (1997). The metabolism and functions of γ-aminobutyric acid. *Plant Physiology, 115*, 1–5.

Chandini, S. K., Ganesan, P., & Bhaskar, N. (2008). *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chemistry, 107*, 707–713.

Heo, S. J., Park, E. J., Lee, K. W., & Jeon, Y. J. (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology, 96*, 1613–1623.

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., et al. (2003). Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *European Journal of Clinical Nutrition, 57*, 490–495.

Je, J. Y., Park, P. J., & Kim, S. K. (2004). Free radical scavenging properties of heterochitooligosaccharides using an ESR spectroscopy. *Food and Chemical Toxicology, 42*, 381–387.

Kim, J. Y., Lee, M. Y., Ji, G. E., Lee, Y. S., & Hwang, K. T. (2009). Production of γ-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA100. *International Journal of Food Microbiology, 130*, 12–16.

Kim, M. H., & Joo, H. J. (2007). Immunostimulatory effects of fucoidan on bone marrow-derived dendritic cells. *Immunology Letters, 115*, 138–143.

Kimura, M., Hayakawa, K., & Sansawa, H. (2002). Involvement of γ-aminobutyric acid (GABA) B receptors in the hypotensive effect of systemically administered GABA in spontaneously hypertensive rats. *Japanese Journal of Pharmacology, 89*, 388–394.

Kinefuchi, M., Sekiya, M., Yamazaiki, A., & Yamamoto, K. (1999). Accumulation of GABA in brown rice by high pressure treatment. *Nippon Shokuhin Kagaku Kaishi, 46*, 323–328.

Kong, L. D., Zhang, Y., Pan, X., Tan, R. X., & Cheng, C. H. K. (2000). Inhibition of xanthine oxidase by liquiritigenin and isoliquiritigenin isolated from *Sinofranchetia chinesis*. *CMLS Cellular and Molecular Life Sciences, 57*, 500–505.

Kumar, K. S., Ganesan, K., & Rao, P. V. S. (2008). Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) Doty – An edible seaweed. *Food Chemistry, 107*, 289–295.

Manyam, B. V., Katz, L., Hare, T. A., Kaniefski, K., & Tremblay, R. D. (1981). Isoniazid-induced elevation of cerebrospinal fluid (CSF) GABA levels and effects on chorea in huntington's disease. *Annals of Neurology, 10*, 35–37.

Mao, L., Pan, X., Que, F., & Fang, X. (2006). Antioxidant properties of water and ethanol extract from hot air–dried and freeze–dried daylily flowers. *European Food Research and Technology, 222*, 236–241.

Nomura, M., Kimoto, H., Someya, Y., Furukawa, S., & Suzuki, I. (1998). Production of γ-aminobutyric acid by cheese starters during cheese ripening. *Journal of Dairy Science, 81*, 1486–1491.

Oh, C. H., & Oh, S. H. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *Journal of Medicinal Food, 7*, 19–23.

Oh, S. H., & Oh, C. H. (2003). Brown rice extracts with enhanced levels of GABA stimulate immune cells. *Food Science and Biotechnology, 12*, 248–252.

Ohmori, M., Yano, T., Okamoto, J., Tsushida, T., Murai, T., & Higuchi, M. (1987). Effect of anaerobically treated tea on blood pressure of spontaneous hypertensive rats. *Nippon Nogeikagaku Kaishi, 61*, 1449–1451.

Park, K. B., & Oh, S. H. (2007). Production of yogurt with enhanced levels of gamma-aminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresource Technology, 98*, 1675–1679.

Saikusa, T., Horino, T., & Mori, Y. (1994). Accumulation of γ-aminobutyric acid (GABA) in the rice germ during water soaking. *Bioscience, Biotechnology and Biochemistry, 58*, 2219–2292.

Siragusa, S., De Angelis, M., Di Cagno, R., Rizzello, C. G., Coda, R., & Gobbetti, M. (2007). Synthesis of γ-aminobutyric acid (GABA) by lactic acid bacteria isolated from italian cheese varieties. *Applied and Environmental Microbiology, 73*, 7283–7290.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology Evolution, 24*, 1596–1599.

Ueno, Y., Hayakawa, K., Takahashi, S., & Oda, K. (1997). Purification and characterization of glutamate decarboxylase from *Lactobacillus brevis* IFO 12005. *Bioscience, Biotechnology and Biochemistry, 61*, 1168–1171.

Yang, M. L., Huang, T. S., Lee, Y., & Lu, F. J. (2002). Free radical scavenging properties of sulfinpyrazone. *Free Radical Research, 36*, 685–693.

Yokoyama, S., Hiramatsu, J. I., & Hayakawa, K. (2002). Production of γ-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO-12005. *Journal of Bioscience and Bioengineering, 93*, 95–97.