Biotechnology and Bioprocess Engineering 28: 568-576 (2023)
DOI 10.1007/s12257-023-0073-9

pISSN 1226-8372
eISSN 1976-3816

RESEARCH PAPER

Evaluation of Pretreatment and GABA Production Using *Levilactobacillus brevis* Fermentation of the Seaweed *Saccharina japonica*

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Received: 24 March 2023 / Revised: 25 May 2023 / Accepted: 28 May 2023

Abstract The seaweed has a high content of easily degradable carbohydrates, making it a potential substrate for the production of γ-aminobutyric acid (GABA). In this study, response surface methodology pretreatment and enzymatic saccharification (*E*s) were conducted on a flask culture of *Saccharina japonica* seaweed. The optimal hydrolytic conditions were: 10.8% (w/v) slurry content, 0.7% H_2SO_4, and 121°C for 30 min. *E*s using enzyme cocktails (Celluclast 1.5 L + Viscozyme L) at 16 U/mL produced 6.26 g/L glucose with an efficiency of 92%. The concentrations of laminarin and fucose (prebiotics) were 10.4 and 0.48 g/L after pretreatment and saccharification, respectively. The suitable monosodium glutamate (MSG) addition was 2% (w/v), and further increase in MSG addition (3–5% (w/v)) had no significant effect on GABA production. The pyridoxal 5′-phosphate (10 μM) addition time of 48–72 h was determined based on the GABA fermentation. Adapted *Levilactobacillus brevis* KCL010 to high concentrations of mannitol improved the synbiotic fermentation efficiency of *S. japonica* hydrolysates, further improving the consumption of mixed monosaccharides.

Keywords: response surface methodology, prebiotics, probiotics, *Saccharina japonica,* γ-aminobutyric acid

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1. Introduction

Seaweed is an underexploited and potentially sustainable crop that is a rich source of dietary factors, including complex carbohydrates, such as polysaccharides. It provides abundant nutrients and substrates for microbial metabolism in the gut, affecting the members and their functionality [1]. Polysaccharides account for the majority of seaweed biomass (up to 76% of dry weight) in some species [2], and together with functional polysaccharides of fucoidan, laminarin, and alginate have been the key focus of many studies of seaweed-derived compounds [3]. Regarding specific benefits on human health, seaweeds have demonstrated antioxidant effects and preventive effects against several non-transmissible diseases, such as cardiovascular diseases, hypertension, obesity, diabetes, and cancer [4-8]. Thus, *Saccharina japonica* was selected as the biomass for the prebiotic (especially laminarin and fucoidan) ingredient in this study.

Response surface methodology (RSM) has been applied for the optimum process conditions by combining the experimental designs with interpolation by first- or second-polynomial equation in the sequential testing procedure [9]. Thus, RSM was used for optimizing the pretreatment conditions and assess their effectiveness in increasing the monosaccharides (mannitol). High-salt stress in lactic acid bacteria (LAB) significantly impedes the cell growth and formation of γ-aminobutyric acid (GABA) from seaweed hydrolysates. The salt tolerance and ion transport mechanisms have been extensively studied [10]. Higher production of monosaccharides and GABA was obtained from *S. japonica* hydrolysates owing to the use of the optimized process.

Probiotics are known to promote health by stimulating

native gut microbiota (GM), host immunity, cholesterol reduction, and several other functions. LAB species (*Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Enterococcus*) and *Bifidobacterium* are among the best- known probiotics [11,12]. In this study, the biosynthetic production of natural GABA by LAB (for food manufacture) exploits the health-promoting properties of GABA and LAB, which are probiotics. Notably, GABA is considered a bioactive compound with multiple physiological functions in nonneural tissues, including neurotransmission, the induction of hypotensive effect, the diuretic effect, and the tranquilizer effect [13]. The GABA-producing ability varies individually among the LAB strains, *Levilactobacillus brevis* have shown great promise potential in large-scale fermentation [14]. Thus, *L. brevis* was selected as the optimal probiotic and GABA-producing LAB. To further increase the yield of GABA, the effects of the pyridoxal 5′-phosphate (PLP) cofactor and initial monosodium glutamate (MSG) concentrations were evaluated by *L. brevis* using *S. japonica* hydrolysates. Synbiotic fermentation is a promising option for preparing functional fermented foods [15].

In this study, RSM was used for optimizing pretreatment conditions and assessing the influence of pretreatment on the saccharification of *S. japonica*. After pretreatment and enzymatic saccharification, *S. japonica* and *L. brevis* were synbiotically fermented to produce prebiotics and probiotics.

2. Materials and Methods

2.1. Raw materials

L. brevis KCL010 was obtained from the Korean Culture Collection of Probiotics (KCCP) and cultured in Difco Lactobacilli de Man, Rogosa, and Sharpe (MRS) broth (Becton, Dickinson and Co.). *S. japonica* (Sea tangle, Dasima) was obtained from Gijang Local Products Co., Ltd. in Busan, Korea. The seaweed was dried in sunlight or hot air and then ground in a hammer mill. The seaweed powder was separated using a 200-mesh sieve prior to thermal acid hydrolysis pretreatment. The compositions of *S. japonica* were analyzed using the AOAC method at the Feed and Foods Nutrition Research Center at Pukyong National University in Korea [16].

2.2. Optimization of the pretreatment conditions using RSM

Response surface experiments were designed for evaluating the relationships between three independent variables (H_2SO_4 [%, v/v, X_1], slurry content [%, w/v, X_2], and thermal acid hydrolysis [min, X_3]) and three dependent variables (mannitol concentration [g/L], GABA content [g/L], and salinity [%, g/g]). Notably, *S. japonica* hydrolysates were then adjusted to pH 5.0 with 5 N NaOH. The hydrolysates, which were fermented at 30°C and 150 rpm with *L. brevis* KCL010 serving as fermentation media (with 4% [w/v] MSG) for GABA production.

Here, a face-centered central composite design (CCD) was used for optimizing the levels of these parameters. Each factor in the design was studied at five different levels (–α, –1, 0, +1, +α) as shown in Table 1. A set of 17 experiments was conducted and all variables were taken at a central coded value considered as zero. The minimum and maximum ranges of variables were investigated; the full experimental plan with respect to their values in the actual and coded form is listed in Table 1. The experimental data were analyzed according to the response surface regression procedure to fit the following second-order polynomial Eq. (1) for each response variable:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

where Y is the predicted response; β_0 is the intercept; β_1, β_2, β_3 are linear coefficients; β_{11}, β_{22}, β_{33} are squared coefficients; β_{12}, β_{23}, $\beta 1_3$ are interaction coefficients. The fitted quality for the polynomial model equation was expressed by the coefficient of determination (R^2). All statistical calculations were performed with the RSM using SAS version 9.4 (SAS Institute) [17].

2.3. Enzymatic saccharification

The thermal acid hydrolysates were enzymatically saccharified using Viscozyme L (β-glucanase, 121 U/mL; Novozymes) and Celluclast 1.5 L (cellulase, 854 U/mL; Novozymes). These two enzymes were then diluted, and an enzyme concentration of 16 U/mL was used. Saccharification proceeded at 45°C and 150 rpm for 48 h after

Table 1. Concentration ranges of the three components used in a central composite design

Variable	Medium component	Range studied (%)	Concentration of the component (%)				
			– α	– 1	0	+ 1	+ α
X_1	H_2SO_4	0.3-1.7	0.3	0.5	1	1.5	1.7
X_2	Slurry contents	5.2-10.8	5.2	6	8	10	10.8
X_3	Thermal acid hydrolysis	23.8-66.2	23.8	30	45	60	66.2

thermal acid hydrolysis. To achieve a synergistic effect compared with single and cocktail enzymes were prepared at a 1:1 ratio with 16 U/mL of each enzyme. The efficiency of enzymatic saccharification (E_s, %) was determined using Eq. (2) as the glucose concentration (ΔS_{glu}, g/L) increased because of the fiber content (F, g/L) during this process after pretreatment, as follows:

$$E_s(\%) = \frac{\Delta S_{glu}}{F} \times 100 \quad (2)$$

2.4. Analysis of fucoidan and laminarin

The analysis methods were based on the determination of common chemical features (*e.g.,* fucose, sulfated polysaccharides, and fuco-oligosaccharides) of fucoidan. The fucose was quantified using an enzymatic L-fucose assay kit (K-FUCOSE; Megazyme) according to the manufacturer's instructions. The fucoidan content was determined by measuring the amount of fucose released from *S. japonica* hydrolysates according to the procedures described by Gajdos *et al*. [18]. Briefly, 0.1 mL of the reaction mixture was added to 2.5 mL of mixed reagents (2.0 mL dH_2O, 0.4 mL reaction buffer [pH 9.5], 0.1 mL $NADP^+$, and 0.05 mL L-fucose dehydrogenase suspension). The mixture was held at room temperature for 20 min, after which the optical density (OD) was measured using a UV–visible spectrophotometer (EMC-18PC-UV; EMCLAB) at 340 nm. The blank was prepared as above, but 0.1 mL of distilled water was added instead of the sample supernatant (0.1 mL). The fucose concentration was calculated using the following equation: fucose (g/L) = 0.6905 × $\Delta A_{L\text{-fucose.}}$

The laminarin was purified using the procedures reported by Rajauria *et al*. [19]. Briefly, the sample (1 mL) was mixed with 100% ethanol in 1:4 (v/v) ratio and centrifuged at 994 × *g* for 5 min. The supernatant was discarded, and the pellet was dissolved in some ultrapure water and left overnight at 4°C. The suspension was then mixed with 3 mL calcium chloride (2%, w/v) and centrifuged at 994 × *g* for 5 min. The supernatant was analyzed using an HPLC system equipped with a refractive index detector (RID). Degassed 5 mM sulfuric acid at a flow rate of 0.6 mL/min and a temperature of 65°C was used with a Bio-Rad Aminex HPX-87H column (300.0 × 7.8 mm).

2.5. Synbiotic fermentation of *S. japonica* hydrolysate

A seed culture of LAB was grown at 30°C and 120 rpm for 18 h in a 30 mL MRS medium, and an inoculate of 5% (w/v, 2.8 g dcw/L) was transferred to a 250 mL Erlenmeyer flask containing 100 mL of *S. japonica* hydrolysate. Mannitol adaptation was performed with *L. brevis* KCL010 cultured in MRS medium containing 80 g/L mannitol (MRSM) for 48 h. Then, the adapted cells were washed twice with fresh MRS medium by centrifugation (1,390 × *g*, 10 min) to remove the MRSM medium and transferred to *S. japonica* hydrolysate for synbiotic fermentation.

The *S. japonica* hydrolysate obtained from thermal acid hydrolysis and enzymatic saccharification using the optimized conditions was centrifuged (1,390 × *g*, 10 min). The liquids were filtered through 0.2-μm filters for removing contamination from the liquid hydrolysate. The seaweed hydrolysates were fermented at 30°C and 150 rpm with non-adapted and adapted *L. brevis* KCL010 to a high concentration of mannitol. Nutrient supplements of 2.0 g/L yeast extract, 5 g/L K_2HPO_4, 0.25 g/L $MgSO_4$, 10 μM PLP, and 40 g/L MSG were added to the *S. japonica* hydrolysate medium and mixed together before inoculation. Samples were collected regularly for the measurement of GABA, mannitol, glucose, fucose, laminarin, and OD_{600} and stored at -20°C before analysis.

2.6. Analytical methods

The cell growth was monitored by measuring OD_{600} using a UV–visible spectrophotometer (EMC-18PC-UV; EMCLAB®). The OD_{600} value was converted into the cell concentration using a standard curve between OD_{600} and dry cell weight with OD_{600} = 1.0 corresponding to 0.45 g dcw/L. Salinity was measured by a digital salinity refractometer (ES-421; ATAGO). MSG and GABA contents were determined through pre-column derivatization with 2-hydroxynaphthaldehyde followed by HPLC analysis. The detailed derivatization and HPLC procedure have been well established [20,21]. Glucose and mannitol concentrations were determined using an HPLC system (Agilent 1200 Series; Agilent Inc.) equipped with an RID. The Bio-Red Aminex HPX-87H column (300.0 × 7.8 mm) was maintained at 65°C, and the samples were eluted with 5 mM H_2SO_4 at 0.6 mL/min. Each sample was analyzed in triplicate, and the mean values were calculated.

3. Results and Discussion

3.1. CCD and RSM

The response of the optimal pretreatment conditions of 0.3–1.7% of H_2SO_4 (%, v/v, X_1), 5.2–10.8% of slurry content (%, w/v, X_2), and 23–66 min of thermal acid hydrolysis (min, X_3) was evaluated in CCD. All the experiments were performed under the aforementioned conditions, and interactions with mannitol concentration (g/L), GABA content (g/L), and salinity (%, g/g) were determined. The regression coefficients were calculated, and the data were fitted to a second-order polynomial equation.

Mannitol concentration (Y_1, Eq. (3)), GABA content (Y_2, Eq. (4)), and salinity (Y_3, Eq. (5)) were expressed in terms of the following regression equations:

$$Y_1 = -10.255 + 8.542\,X_1 + 3.496\,X_2 - 0.010\,X_3 - 2.077\,X_1^2 + 0.0002\,X_2^2 + 0.001\,X_3^2 - 0.472\,X_1X_2 - 0.015\,X_1X_3 - 0.015\,X_2X_3 \quad (3)$$

$$Y_2 = -0.035 + 1.069\,X_1 + 0.131\,X_2 + 0.029\,X_3 - 0.284\,X_1^2 + 0.004\,X_2^2 - 0.0002\,X_3^2 - 0.071\,X_1X_2 + 0.002\,X_1X_3 - 0.001\,X_2X_3 \quad (4)$$

$$Y_3 = 2.964 + 0.092\,X_1 - 1.132\,X_2 + 0.080\,X_3 + 1.805\,X_1^2 + 0.106\,X_2^2 - 0.0007\,X_3^2 - 0.175\,X_1X_2 - 0.003\,X_1X_3 - 0\,X_2X_3 \quad (5)$$

Analysis of variance (ANOVA) showed that the R^2 values for Mannitol concentration (Y_1), GABA content (Y_2), and salinity (Y_3) were 0.9858, 0.9078, and 0.8753, respectively (Table 2). The closer the R^2 to 1.00, the stronger the model and the better the response prediction [22]. The interactions and three-dimensional response surface plots according to the second-order polynomial equation are shown in Fig. 1. Fig. 1A clearly shows the increase in mannitol concentration with an increase in the slurry level. However, the mannitol concentration decreased slightly when the H_2SO_4 level increased beyond 1.0% (v/v). Thus, the conditions that resulted in the maximum mannitol concentration of 25.47 g/L were an H_2SO_4 concentration of 0.7%, slurry content of 10.8%, and thermal acid hydrolysis time of 30 min.

GABA production was an important factor for probiotic fermentation and related to salinity content, as shown in Fig. 1B and 1C. After 30 min of thermal acid hydrolysis (Fig. 1B), GABA production increased from 1.60 to 2.26 g/L as the slurry content increased from 5.2 to 10.8% (w/v). However, GABA production decreased from 2.26 to 1.71 g/L as the H_2SO_4 concentration increased from 0.7 to 1.7% (w/v). Fig. 1C shows an increase in the salinity contents with an increase in the slurry and H_2SO_4 levels. These results suggest that high-salt stress of *L. brevis* KCL010 significantly impeded GABA production during the fermentation of thermal acid-hydrolyzed seaweed medium. A similar phenomenon was observed for environmental conditions affecting GABA production in *Lactococcus lactis* NCDO 2118 [23]. In summary, considering the GABA production, the optimal thermal acid hydrolysis conditions were 10.8% (w/v) slurry, 0.7% H_2SO_4, and 121°C for 30 min.

Table 2. Response surface level combinations of independent variables in the experimental design and responses of dependent variables

Design point	Independent variable[a]			Dependent variable		
	H_2SO_4 (%, v/v)	Slurry contents (%, w/v)	Thermal acid hydrolysis (min)	Mannitol concentration (g/L)	GABA[b] contents (g/L)	Salinity (%, g/g)
	X_1	X_2	X_3			
1	–1	–1	–1	11.03	1.64	1.9
2	–1	–1	1	12.31	1.66	1.7
3	–1	1	–1	22.32	2.11	3.6
4	–1	1	1	20.71	1.89	4.8
5	1	–1	–1	12.78	1.81	3.9
6	1	–1	1	12.55	1.84	5.0
7	1	1	–1	21.12	1.93	6.3
8	1	1	1	20.12	1.85	6.0
9	–α	0	0	16.53	1.78	2.5
10	α	0	0	16.10	1.88	5.1
11	0	–α	0	10.11	1.74	3.3
12	0	α	0	24.56	2.27	4.2
13	0	0	–α	17.85	1.82	2.4
14	0	0	α	18.22	1.90	2.8
15[c]	0	0	0	16.96	1.97	3.4
16[c]	0	0	0	16.94	1.94	3.5
17[c]	0	0	0	16.23	1.93	3.6

[a]Independent variable

X_1: H_2SO_4	– α = 0.3%	– 1 = 0.5%	0 = 1%	+ 1 = 1.5%	+ α = 1.7%
X_2: Slurry contents	– α = 5.2%	– 1 = 6%	0 = 8%	+ 1 = 10%	+ α = 10.8%
X_3: Thermal acid hydrolysis	– α = 23.8 min	– 1 = 30 min	0 = 45 min	+ 1 = 60 min	+ α = 66.2 min

[b]γ-aminobutyric acid.
[c]Central points.

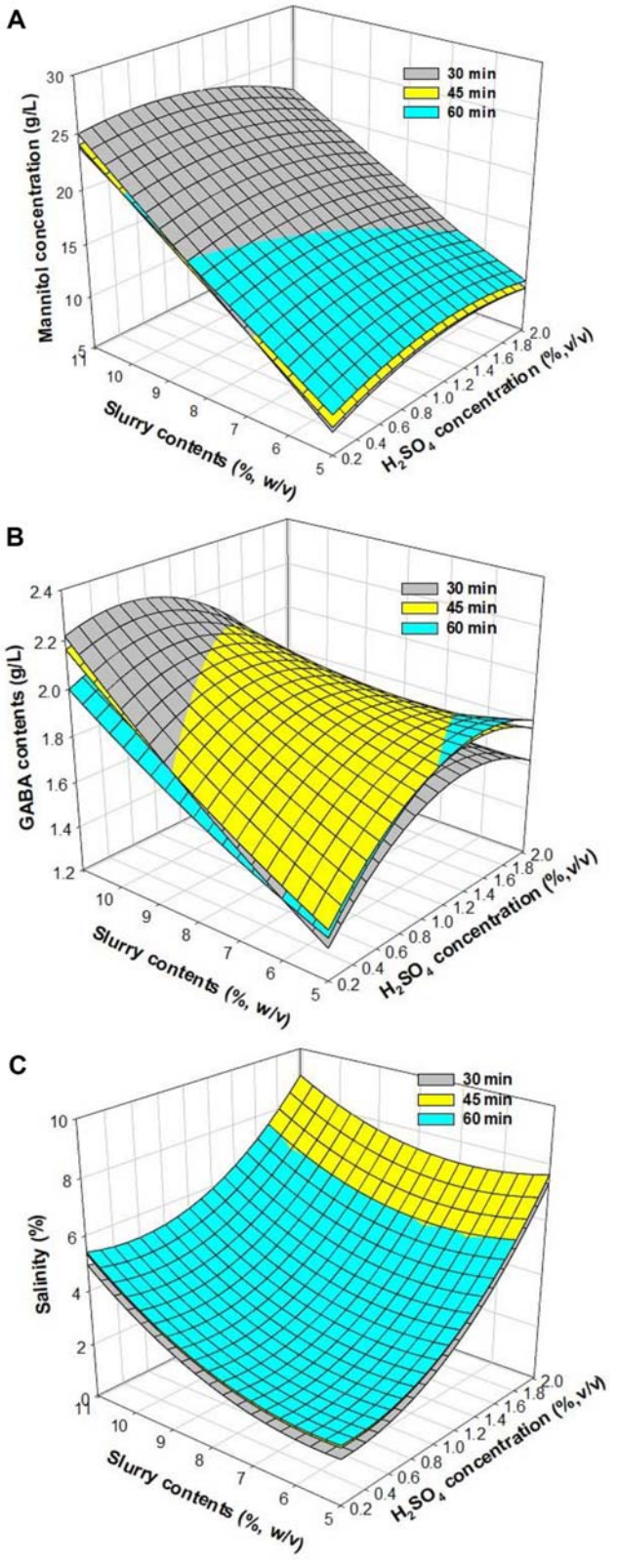

Fig. 1. Response surface showing the effects of H_2SO_4 concentration, *Saccharina japonica* slurry contents, and thermal acid hydrolysis time on (A) Mannitol concentration, (B) GABA contents, and (C) Salinity. GABA: γ-aminobutyric acid.

3.2. *S. japonica* composition and enzymatic saccharification

The composition of *S. japonica* was analyzed by the AOAC method and was as follows: 59.7% carbohydrate, 6.3% crude fiber, 10.6% crude protein, 1.6% crude lipid, and 21.8% crude ash.

The thermal acid hydrolysis conditions were optimized with a 10.8% (w/v) seaweed slurry, 0.7% (v/v) H_2SO_4, and 30 min thermal hydrolysis in a 250 mL Erlenmeyer flask containing 100 mL of *S. japonica* hydrolysate. Seaweed slurry hydrolysates were then neutralized to pH 5.0 with 5 N NaOH. The effects of single enzyme and enzyme cocktails treatments on glucose release of *S. japonica* hydrolysate were evaluated as shown in Fig. 2A. Among these enzyme treatments, a single treatment of Celluclast 1.5 L (C) was preferred over that of Viscozyme L (V). The enzyme cocktail (C + V) treatment to *S. japonica* hydrolysate showed a synergistic effect; the maximum E_s was 92% with 6.26 g/L of glucose from the crude fiber of 6.80 g/L from 108 g dcw/L *S. japonica* slurry. Fig. 2B shows that the enzyme cocktail (C + V) dosage was assessed with the

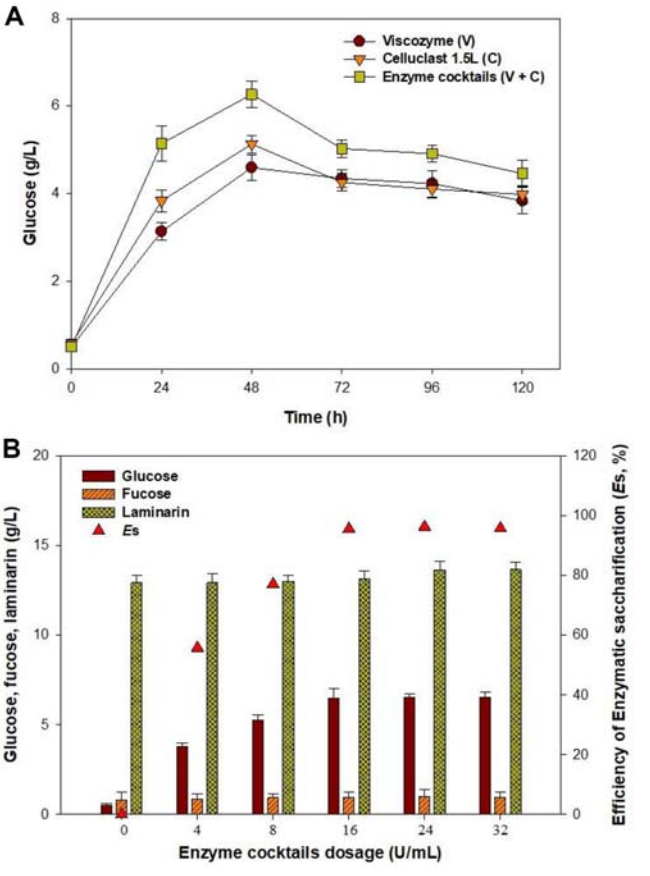

Fig. 2. Effect of (A) enzyme type and (B) enzyme dosage on glucose release of *Saccharina japonica* hydrolysate with 10.8% slurry at pH 5.5, 45°C, and 150 rpm for 48 h after thermal acid hydrolysis.

activities of 0–32 U/mL at 45°C and 150 rpm for 48 h. The best enzyme cocktail (C + V) activity was observed at 16 U/mL and a further increase in enzyme activities at up to 32 U/mL had no significant effect on E_s. Thus, the enzyme cocktail (C + V) dosage of 16 U/mL was selected for enzymatic saccharification.

The GM contributes to the human energy balance and nutrition by extending the host metabolic capacity to indigestible polysaccharides [1]. As shown in Fig. 2B, in terms of prebiotic production, the laminarin and fucose concentrations were respectively 12.9–13.6 and 0.82–0.95 g/L after the pretreatments, and a further increase in the enzyme levels did not significantly increase it. In other research, laminarin has been shown to promote an immune response [24] and could be useful for inhibiting the production of putrefactive substances from undigested proteins [25]. Usov *et al*. [26] determined the polysaccharide compositions of 17 species, particularly the fucoidan contents calculated as twice the fucose contents, assuming the average fucose content to be 50% that of fucoidan. Several studies have reported that the administration of whole brown seaweed or brown seaweed-extracted polysaccharides increased short chain fatty acids (SCFA), stimulating growth of beneficial bacteria, such as *Lactobacillus*, *Bifidobacterium* or *Faecalibacterium* [27-29]. Therefore, *S. japonica* hydrolysates may serve as a valuable prebiotic ingredient.

3.3. MSG and PLP enhance GABA production

Effects of different MSG and PLP concentrations on GABA production were investigated as shown in Fig. 3. MSG and PLP are required when GAD converts L-glutamate to GABA [30]. GAD catalyzes the irreversible decarboxylation of glutamate to GABA, which is activated by the coenzyme PLP. Fig. 3A shows the GABA production by *L. brevis* KCL010 with MSG range of 1–5% (w/v). The suitable MSG addition was 2% (w/v) and a further increase in MSG addition of 3–5% (w/v) had no significant effect on GABA production. The GABA production amount was 2.18 g/L used in subsequent experiments. Villegas *et al*. [31] reported a gradual increase in GABA yield as the MSG level increased from 0 to 270 mM (4.6%). Higher MSG concentrations decreased the GABA yield, possibly because an increase in osmotic pressure compromised the bacterial metabolism.

As shown in Fig. 3B, the effects of the PLP concentration of 10–50 μM were determined. The results suggest that GABA production slightly increased with an increase in the PLP concentration. However, further increase in PLP concentration (up to 50 μM) had no significant additional effect on GABA production. Yunes *et al*. [32] reported a similar result: the addition of PLP to the fermentation medium of *L. plantarum* 90sk did not affect GABA production. A possible explanation for these results is the degradation of PLP in the early phase of growth.

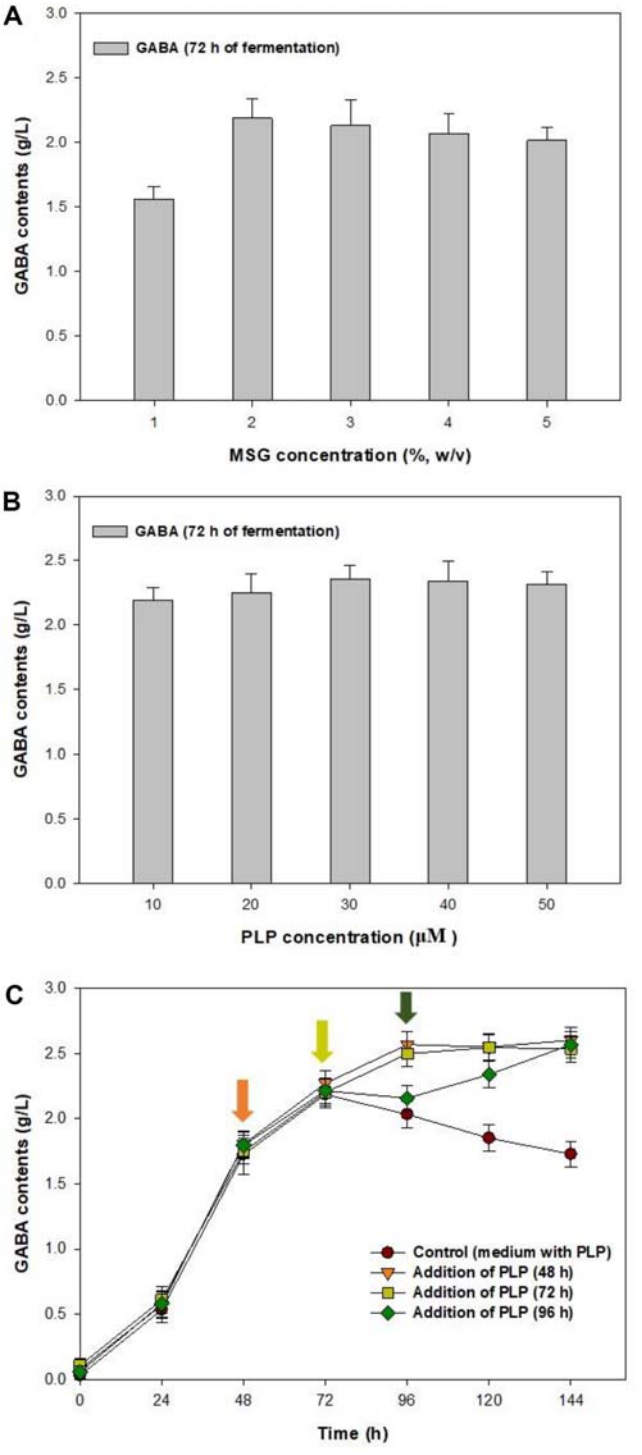

Fig. 3. Effects of (A) MSG, (B) PLP concentrations, and (C) PLP were added at 48, 72, and 96 h on GABA production by *Levilactobacillus brevis* KCL010. GABA: γ-aminobutyric acid, MSG: monosodium glutamate, PLP: pyridoxal 5′-phosphate.

Dependence on the timing of PLP addition was previously reported by Yang *et al*. [33]. Hence, we investigated the effect of PLP addition of 10 μM at 48, 72, and 96 h during bacterial growth as shown in Fig. 3C. When PLP of 10 μM was added at 48, 72, and 96 h of fermentation, GABA production (2.53–2.60 g/L) was higher than that with the control group (medium with PLP; 1.72 g/L). However, the addition of PLP after 96 h delayed the overall fermentation of GABA production. Therefore, PLP (10 μM) addition at 48–72 h was determined based on the GABA fermentation.

3.4. Fermentation of *S. japonica* hydrolysates using *L. brevis* KCL010

Fig. 4 show the synbiotic fermentation results of 10.8% (w/v) *S. japonica* hydrolysate containing mixed monosaccharides with non-adapted or adapted *L. brevis* KCL010, respectively. After the exhaustion of glucose at 24 h, mannitol was consumed due to the preference for glucose to mannitol (Fig. 4A). Mannitol was not completely consumed until 120 h and 10.2 g/L of mannitol remained in the fermentation broth with non-adapted *L. brevis* KCL010. This probably reflects the preference of LAB for glucose compared to other monosaccharides; mannitol consumption is inefficient. This appears as a strain-specific phenomenon [34]. The growth of *L. brevis* KCL010 with MSG had no lag time and entered a stationary phase after 72 h. With 10.8% (w/v) *S. japonica* hydrolysate, the fermentation using non-adapted *L. brevis* KCL010 produced a GABA concentration of 2.45 g/L at 72 h of fermentation. The initial fucose concentration was 0.93 g/L, which decreased to 0.76 g/L at the end of the fermentation. The initial laminarin concentration of 13.4 g/L showed almost no decrease during fermentation.

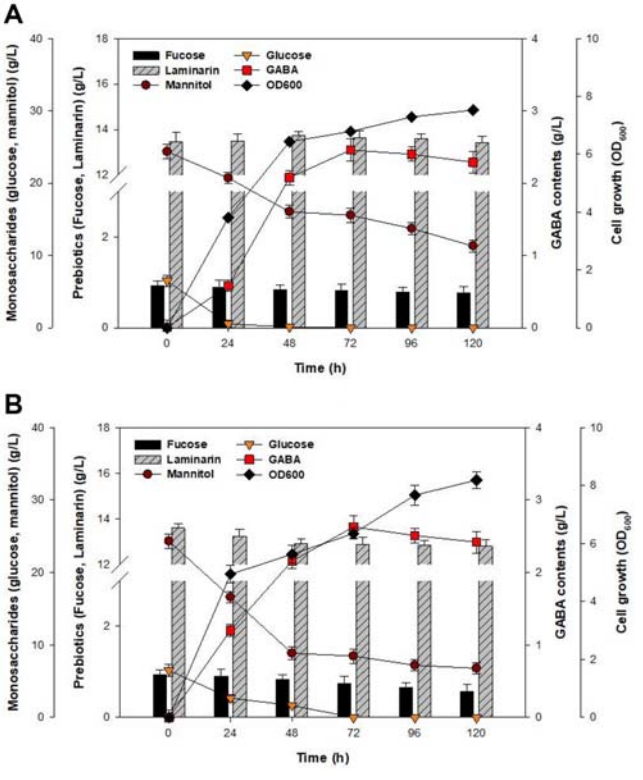

Fig. 4. Comparison of synbiotic fermentation using (A) non-adapted *Levilactobacillus brevis* KCL010 and (B) *L. brevis* KCL010 adapted to high mannitol concentrations in 250 mL Erlenmeyer flasks containing 100 mL of *Saccharina japonica* hydrolysate. GABA: γ-aminobutyric acid.

To overcome these problems caused by glucose repression, *L. brevis* KCL010 was adapted to high concentrations of mannitol, as shown in Fig. 4B. Glucose and mannitol were simultaneously consumed within 72 h. Mannitol was consumed after 120 h; only 6.7 g/L of mannitol remained in the fermentation medium. In contrast, the adapted *L. brevis* KCL010 showed a high mannitol uptake rate and produced 2.63 g/L of GABA. Choe *et al*. [35,36] reported that the preferential utilization of glucose over mannitol by bacteria requires precise regulation of the mannitol operon, in which the mannitol operon repressor (MtlR) plays an important role as a transcriptional repressor. In the presence of glucose, histidine phosphocarrier protein (HPr) is mostly dephosphorylated, and the MtlR recognizes dephosphorylated HPr to form the MtlR-HPr complex, which serves as a strong repressor of the mannitol operon. In the presence of mannitol alone, however, more than 50% of HPr exists in phosphorylated forms, which cannot interact with HtlR. Therefore, in the presence of both glucose and mannitol, repression by glucose seems to predominate over induction by mannitol, since the expression of the mannitol operon is not induced until glucose is exhausted. Similar results were obtained previously for glucose repression of xylose utilization by *Lactococcus lactis* IO-1, which showed a mixture of glucose and xylose causing diauxic cell growth because of carbon catabolite repression [37]. Also, *L. brevis* parallelly utilizes glucose and xylose as co-metabolites [38,39]. Consequently, the adapted *L. brevis* KCL010 could facilitate the efficient utilization of mannitol, and glucose, for the synbiotic fermentation from *S. japonica* hydrolysate.

4. Conclusion

The seaweed *S. japonica* was used for optimizing RSM pretreatment using various levels of slurry content, thermal hydrolysis time, and H_2SO_4 concentration. The results show that *S. japonica* hydrolysate might be a potential prebiotic ingredient. MSG and PLP concentrations levels for further improving the GABA fermentation were determined. The comparison between the non-adapted and

adapted *L. brevis* KCL010 to a high concentration of mannitol showed that glucose repression can be overcome using the adaptation process. Therefore, the optimization of fermentation parameters could provide a new opportunity for improving the production efficiency of synbiotic fermentation from *S. japonica* seaweed.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No.2022R1F1A1074594).

Ethical Statements

The authors declare no conflict of interest.
Neither ethical approval nor informed consent was required for this study.

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