Enhanced Production of Gamma-Aminobutyric Acid by Optimizing Culture Conditions of Lactobacillus brevis HYE1 Isolated from Kimchi, a Korean Fermented Food

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Introduction

Gamma-aminobutyric acid (GABA), a non-protein amino acid, plays a major role as an inhibitory neurotransmitter in mammalian nervous systems and is commonly found in nature [1]. GABA is principally biosynthesized by glutamate decarboxylase (GAD; E.C. 4.1.1.15), which is responsible for the irreversible α-decarboxylation of glutamate to GABA [2]. Since GABA exerts an antihypertensive and diuretic effect and mitigates depression and sleeplessness, it has been widely used as a bioactive natural compound in foods and pharmaceuticals [3, 4]. As a result, interests in the commercial production of GABA itself and GABA-fortified functional foods have increased [5].

GABA production is mainly conducted by fermentation of microorganisms such as yeast, fungi, and bacteria [6]. Lactic acid bacteria (LAB) in particular have been reported to be GABA producers, since most LAB as generally

This study evaluated the effects of culture conditions, including carbon and nitrogen sources, l-monosodium glutamate (MSG), and initial pH, on gamma-aminobutyric acid (GABA) production by Lactobacillus brevis HYE1 isolated from kimchi, a Korean traditional fermented food. L. brevis HYE1 was screened by the production analysis of GABA and genetic analysis of the glutamate decarboxylase gene, resulting in 14.64 mM GABA after 48 h of cultivation in MRS medium containing 1% (w/v) MSG. In order to increase GABA production by L. brevis HYE1, the effects of carbon and nitrogen sources on GABA production were preliminarily investigated via one-factor-at-a-time optimization strategy. As the results, 2% maltose and 3% tryptone were determined to produce 17.93 mM GABA in modified MRS medium with 1% (w/v) MSG. In addition, the optimal MSG concentration and initial pH were determined to be 1% and 5.0, respectively, resulting in production of 18.97 mM GABA. Thereafter, response surface methodology (RSM) was applied to determine the optimal conditions of the above four factors. The results indicate that pH was the most significant factor for GABA production. The optimal culture conditions for maximum GABA production were also determined to be 2.14% (w/v) maltose, 4.01% (w/v) tryptone, 2.38% (w/v) MSG, and an initial pH of 4.74. In these conditions, GABA production by L. brevis HYE1 was predicted to be 21.44 mM using the RSM model. The experiment was performed under these optimized conditions, resulting in GABA production of 18.76 mM. These results show that the predicted and experimental values of GABA production are in good agreement.

Keywords: Gamma-aminobutyric acid, Lactobacillus brevis, optimization, response surface methodology, fermented food
regarded as safe bacteria produce a large amount of GABA compared with other microorganisms and are widely used as probiotics with specific characteristics, including resistance to bile acid and gastric acidity and intestinal homeostasis [7]. In addition, LAB are capable of inhibiting some pathogenic bacteria, resulting in stability improvement during storage of fermented foods [8]. So far, several GABA-producing LAB isolated from several fermented foods have been reported, such as Lactobacillus plantarum [9], L. paracasei [10], and Lc. lactis [11] from cheese, L. buchneri [12] and L. brevis [13] from the Korean traditional fermented food kimchi, and L. senmaiuzukei [14] from pickles. Although there have been many studies on LAB-derived GABA production, the isolation of GABA-producing LAB and the development of their GABA production need to be further explored since fermented foods as isolate sources for GABA producers have various kinds and diverse LAB depending on the raw materials and preparation methods [15].

Microbial GABA production is dependent on several fermentation factors, including pH, temperature, glutamate concentration, medium composition, and fermentation time [6]. These environmental and nutritional factors have been optimized by conventional or statistical methods, providing basic information to develop the bioprocess for commercial GABA production [16]. One-factor-at-a-time (OFAT) strategy, as a conventional method, varies only one independent factor at a time while keeping all others fixed. However, the conventional OFAT method is limited since interactions between each factor cannot be considered in a complex system, resulting in just local optimum points being obtained. On the other hand, response surface methodology (RSM), as a statistical method, is more rapid and reliable for studying interactions among factors. The statistical RSM method uses a sequence of designed experiments to obtain an optimal response, and the experiments can be easily arranged and interpreted using an efficient design [17, 18]. In addition, the RSM method reduces the total number of experiments for optimization of each factor, which saves experimental resources and time [19]. Most studies on optimization of GABA production by LAB have focused on the conventional OFAT method to determine optimal carbon and nitrogen sources and glutamate concentration [17, 20–22]. Although the statistical RSM method is more efficient compared with the conventional OFAT method, there have been few studies on culture optimization for GABA production via the RSM method. Recently, culture conditions of Lc. lactis strain B isolated from kimchi and L. plantarum Taj-Apis362 from honeybees were optimized for GABA production by the RSM method based on the Box-Behnken design (BBD) and central composite design (CCD), respectively [23, 24].

In this study, L. brevis HYE1 producing a high level of GABA was isolated from kimchi. The culture conditions of L. brevis HYE1 were also investigated via the conventional OFAT method to determine carbon and nitrogen sources and identify fermentation factors with significant effects on GABA production. Thereafter, the optimal concentration of each fermentation factor, including the selected carbon and nitrogen sources, exogenous l-monosodium glutamate (MSG), and initial pH, were further determined to enhance GABA production by L. brevis HYE1 using statistical RSM while employing a three-level, four-variable BBD.

Materials and Methods

Isolation and Cultivation of GABA-Producing Microorganisms

Various Korean traditional fermented food samples, including kimchi, cucumber pickles, soybean pastes, salted shrimp, and red pepper pastes, were diluted in 10 ml of 0.85% NaCl solution and plated on de Man, Rogosa, Sharpe (MRS) agar (MB Cell, Republic of Korea), followed by incubation at 30°C for 48 h. After re-streaking the randomly selected colonies three times, a pure single colony was cultivated in MRS broth supplemented with 1% (w/v) MSG for 48 h at 30°C to isolate the GABA-producing microorganisms. After cultivation, the culture broth was centrifuged at 9,000 x g for 10 min, and the resultant supernatants were used for analysis of GABA production.

Genetic Analysis of GABA-Producing Microorganisms

In order to genetically analyze the 16S rRNA gene and GAD gene involved in GABA biosynthesis, genomic DNA of GABA-producing microorganisms isolated in this study was extracted with the HiYield Genomic DNA Mini Kit (RBC, Taiwan). The universal primer set 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-CGTATTACGACTT-3’) was used to identify the isolated GABA producers by 16S rRNA gene sequence analysis. The full-length gad gene from genomic DNA was also amplified by using the designed primer sets based on previously reported gad sequences of several L. brevis strains: MME-63 (5’-ATGGCTATGTTGTATGGA-3’) and MME-64 (5’-TTAGTGCGT GAACCCGTA-3’). The polymerase chain reaction (PCR) conditions for gad amplification were initial denaturation at 94°C for 2 min, followed by 30 cycles at 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min, and final extension at 72°C for 5 min.

Analysis of GABA Production

GABA production was preliminarily evaluated by thin-layer chromatography (TLC) analysis [25]. In brief, the culture supernatant-spotted TLC silica gel plate (Merck, Germany) was
developed in n-butanol-acetic acid-water (4:1:1 v/v/v) solvent, followed by addition of 0.2% ninhydrin solution for visualization of the spots on the TLC plate. GABA contents produced by _L. brevis_ HYE1 were also quantified by spectrophotometry assay using GBase enzyme (Sigma, USA) [26]. Briefly, culture supernatants was 100°C for 4 min, followed by a temperature gradient of 100°C, 200°C, and 280°C, respectively. The temperature program of the injector, ion source, and interface were 320°C at 10°C/min and a hold at 320°C for 11 min. Spectra of standards were determined in scan mode and selected ion monitoring (m/z 363 for MSG) within a range of 45-600 m/z.

Gas chromatography-mass spectrometry (GC-MS) was used to verify the GABA production [27]. The freeze-dried samples (10 mg) from culture supernatants were extracted by a 1 ml solvent mixture of methanol, water, and chloroform (2.5:1:1 v/v/v). After two-stage derivatization of oximation followed by trimethylsilyl etherification, the derivatized (1 ml) was separated by using the GCMS-QP2010 Ultra system with an AOC-20i autosampler (Shimadzu, Japan) and a DB-5 capillary column (30 m x 0.25 mm, 1.0 μm, J&W Scientific, USA). The split ratio was set at 1:10, and helium gas was used as the carrier gas at a flow rate of 1 ml/min. The temperature of the injector, ion source, and interface were 280°C, 200°C, and 280°C, respectively. The temperature program was 100°C for 4 min, followed by a temperature gradient of 100–320°C at 10°C/min and a hold at 320°C for 11 min. Spectra of standards were determined in scan mode and selected ion monitoring (m/z 304 for GABA and m/z 363 for MSG) within a detected mass range of 45-600 m/z.

**OFAT-Based Culture Optimization for GABA Production**

The OFAT strategy was first employed to determine the optimal carbon and nitrogen sources and to identify the range of culture parameters, including MSG concentration and initial pH, which have significant effects on GABA production. Several carbon sources (glucose, lactose, sucrose, xylose, fructose, maltose, galactose, arabinose, raffinose, and mannitol) were individually added into a 250 ml Erlenmeyer flask containing 100 ml of modified MRS medium of which 2% glucose was removed. In the same manner, all nitrogen sources (1% proteose peptone No. 3, 1% beef extract, and 0.5% yeast extract) in the defined MRS medium were replaced with 2.5% various nitrogen sources (beef extract, tryptone, soytone, yeast extract, peptone, casitone, caseamino acid, proteose peptone No. 3, malt extract, and control mixture) to determine the optimal nitrogen source in GABA production. Other single parameters were also optimized by altering their ranges, such as MSG concentration (0–15% (w/v)) and initial pH (4.0–8.0).

**RSM-Based Optimization for GABA Production**

RSM with the BBD model using three coded levels was employed for determining the optimal culture conditions of the four variables monitored in the OFAT method. The behavior of the system was explained by the following quadratic equation.

\[
Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \sum_{j=1}^{4} \beta_{ij} X_i X_j + \sum_{i=1}^{4} \beta_{ii} X_i^2
\]

where Y is the predicted response, \(\beta_0\) is constant, and \(\beta_i, \beta_{ij}\), and \(\beta_{ii}\) are coefficients assumed from the model. The symbols \(X_i\) and \(X_j\) represent the levels of independent variables. Various assemblies used in this design are shown in Table 1. Analysis of variance (ANOVA) and regression analysis were performed with the SAS software package (SAS Institute Inc., USA).

**Table 1. Box-Behnken design matrix and corresponding experimental values of GABA production.**

<table>
<thead>
<tr>
<th>Trials</th>
<th>Coded variable levels and actual values</th>
<th>GABA production (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(X_1) Maltose conc. (%)</td>
<td>(X_2) Tryptone conc. (%)</td>
</tr>
<tr>
<td>1</td>
<td>0 (2)</td>
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<tr>
<td>2</td>
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<tr>
<td>5</td>
<td>0 (2)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>6</td>
<td>-1 (0.2)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>7</td>
<td>1 (4)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>8</td>
<td>-1 (0.2)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>9</td>
<td>1 (4)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>10</td>
<td>-1 (0.2)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>11</td>
<td>-1 (0.2)</td>
<td>-1 (1)</td>
</tr>
<tr>
<td>12</td>
<td>-1 (0.2)</td>
<td>0 (3)</td>
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<tr>
<td>13</td>
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<td>0 (3)</td>
</tr>
<tr>
<td>14</td>
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<td>0 (3)</td>
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<td>0 (2)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>27</td>
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</table>
Results and Discussion

Isolation of GABA-Producing *L. brevis* HYE1

In order to isolate the GABA-producing microorganism, over 100 bacterial isolates obtained from traditional fermented foods were screened for GABA production by incubating the bacterial culture with 1% MSG. After TLC and spectrophotometry analyses using GABase enzyme, over 10 isolates showing high GABA production were identified by 16S rRNA gene sequence analysis, resulting in *Lactobacillus* genus strains with taxonomical similarities from 99.6% to 100.0% (data not shown). Sequence analysis of the GAD gene was subsequently conducted to select the novel GAD gene-harboring strain. Each genomic DNA extracted from the isolated GABA-producing LAB was PCR-amplified with the designed PCR primer sets (MME-63 and MME-64). The amplified DNA fragment generated was about 1.4 kbp per the expected size (data not shown). The isolate HYE1 was finally selected based on the GABA production level and sequence analysis of the GAD gene, and exhibited the highest level of GABA production (14.64 mM) compared with the other GABA-producing LAB strains isolated in this study (data not shown). The amino acid sequence of GAD from the isolate HYE1 showed 99% identity to those of *L. brevis* ATCC 367, *L. brevis* KB290, and *L. brevis* BSO 464 (Fig. 1). Additionally, the sequence showed 83%, 82%, and 71% amino acid sequence identities with those from *L. herbarum*, *L. plantarum* 16, and *Enterococcus avium*, respectively. Based on the result of 16S rRNA gene sequence analysis, the isolate HYE1 was identified as *L. brevis* based on 99% sequence similarity to those of *L. brevis* ATCC 367 (GenBank Accession No. CP000416) and *L. brevis* KB290 (AP012167). The 16S rRNA gene and GAD gene sequences from *L. brevis* HYE1 were deposited in GenBank under Accession No. KX458105 and KM649685, respectively.

Cell Growth and GABA Production by *L. brevis* HYE1

The time-course profile for cell growth and GABA production of *L. brevis* HYE1 was monitored upon cultivation in 200 ml of MRS medium containing 1% MSG at 30°C for up to 60 h. Cell growth reached the stationary phase after 24 h of cultivation, whereas GABA production dramatically increased from 9 to 18 h of culture up to a maximum of 16.94 mM after 36 h of cultivation (Fig. 2). GABA production by *L. brevis* HYE1 was also measured by GC-MS analysis of derivatized organic extracts of its culture supernatants. GABA produced by *L. brevis* HYE1 was identical in terms of both the retention time and mass spectrum of GC-MS analysis based on direct comparison with an authentic GABA control (Fig. 3).
Effects of Carbon and Nitrogen Sources on GABA Production by *L. brevis* HYE1

Culture conditions such as medium composition and additives significantly affect microbial GABA production. In particular, medium components such as carbon and nitrogen sources exert a strong influence on GABA production [28]. In order to determine the optimal carbon and nitrogen sources that could be used for further optimization based on RSM, the effect of carbon source on GABA production by *L. brevis* HYE1 was evaluated using modified MRS medium containing 2% of each carbon source and 1% MSG at 30°C. Maltose was determined to be the best carbon source for GABA production (3.04 mM), whereas fructose (2.02 mM) and galactose (2.06 mM) showed similar effects compared with standard MRS medium containing 2% glucose (2.13 mM) (Fig. 4A). On the other hand, sucrose, raffinose, and mannitol had no effect on GABA production, showing similar levels of GABA production to that with no addition of carbon source. The maltose concentration for optimal GABA production was also determined to be 2% (Fig. 4B). Previous studies reported that diverse carbon sources have been optimized according to GABA-producing LAB; 1% glucose for *L. buchneri* MS [12], 4% sucrose for *L. sakei* B2-16 [21], and 3% sucrose for *L. brevis* 340G [15]. Maltose, as an optimal carbon source in our study, was also reported to be the best carbon source for GABA production by *L. brevis* K203 [20].

The effect of nitrogen sources on GABA production by *L. brevis* HYE1 was also investigated in modified MRS medium containing 2% maltose as a carbon source, 1% MSG, and 2.5% various nitrogen sources. Individual addition of beef extract, tryptone, or protease peptone No. 3 had similar effects on GABA production as that of the control mixture of original nitrogen sources in MRS medium, whereas low production of GABA was observed using peptone, casitone, casamino acid, and malt extract (Fig. 4C). Tryptone resulted in maximum GABA production (16.47 mM), followed by beef extract (16.03 mM) and proteose peptone No. 3 (15.71 mM). Most previous studies on optimization of GABA production by LAB reported yeast extract as an optimal nitrogen source, whereas addition of 2.5% yeast extract resulted in just 13.92 mM GABA in our study [15, 20, 21]. However, it should be noted that the mixed nitrogen sources of tryptone and yeast extract have occasionally been reported to be optimal nitrogen sources for GABA production by LAB [29, 30]. Addition of greater than 1.5% tryptone had a significant effect on GABA production, and 3% tryptone resulted in maximum GABA production (17.93 mM) (Fig. 4D). Finally, the optimum carbon and nitrogen sources were determined to be 2% maltose and 3% tryptone for GABA production by *L. brevis* HYE1, respectively.

Effects of MSG Concentration and Initial pH on GABA Production by *L. brevis* HYE1

The effect of initial MSG concentration on GABA production was determined using the already-optimized culture medium (modified MRS medium containing 2%
maltose and 3% tryptone) under fixed culture conditions (initial pH 7.4, 30°C, 48 h of culture time). Maximum GABA production (18.60 mM) was obtained upon addition of 1% MSG, followed by gradual reduction of GABA production up to 6% MSG (Fig. 4E). GABA production was also drastically reduced when the initial MSG concentration exceeded 6% up to 15%. Although MSG is generally regarded as a requisite substrate for GABA generation and a high level of MSG concentration is needed to enhance GABA production [31, 32], our findings suggest that a high level of MSG suppressed GABA production [24]. Our result that MSG concentration was optimal within a relatively low range is consistent with a previous study reporting 0.5–1% MSG for *E. faecium* JK29 [22]. GABA production

![Fig. 4. Effects of carbon sources (A), maltose concentration (B), nitrogen sources (C), tryptone concentration (D), initial MSG concentration (E), and initial pH (F) on the cell growth (closed bars) and GABA production (open bars) of *L. brevis* HYE1.](image-url)
with a low level of MSG as a substrate could address the current bottleneck for industrial production of GABA, such as high cost of the culture medium [6].

Microbial biosynthesis of GABA is mainly regulated by pH, and the pH value for optimized GABA production is species-dependent owing to the diverse biochemical characteristics of their GADs [3, 33]. Investigation of the initial pH effect on GABA production by L. brevis HYE1 revealed that GABA was produced within a pH range between 4.0 and 7.0, suggesting that the optimal initial pH for GABA production (18.97 mM) was 5.0 (Fig. 4F). GABA production and cell growth were strongly inhibited at pH 3.0 and 8.0. In agreement with our results, the pH conditions for most Lactobacillus-genus strains producing GABA have been optimized within the range from 4.5 to 5.5, including the following studies reporting pH 5.0 for L. buchneri MS [12], pH 5.25 for L. brevis K203 [20], and pH 5.31 for L. plantarum Taj-Apis362 [24]. The enhancement of GABA production under acidic culture conditions is closely associated with the characteristics of GAD, which shows increased activity and stability in the presence of hydrogen ions [34, 35]. However, the optimal pH for GABA production by Enterococcus-genus strains has been reported to be 6.5 for E. avium 9184 [36] and 7.5 for E. faecium JK29 [22] and the results were distinctly different from those for Lactobacillus-genus strains in our study. Finally, the optimal MSG concentration and initial pH were determined to be 1% and 5.0, respectively, for GABA production by L. brevis HYE1.

**Further Optimization for GABA Production by RSM**

OFAT-based optimization revealed that maltose and tryptone were the best carbon and nitrogen sources, respectively. Additionally, the suitable pH and concentration ranges of each factor, including maltose, tryptone, and MSG, were preliminarily determined. In the following experiments, the above four factors were further optimized using RSM with the BBD model. Table 1 shows various combinations used with the four variables and three levels, and the corresponding experimental values of GABA production were means of duplicates. Data were analyzed by the quadratic regression model and the following equation was obtained.

\[
Y = - 20.28 + 4.88 X_1 + 4.16 X_2 + 9.99 X_3 + 6.87 X_4 - 1.31 X_1^2 + 0.55 X_1 X_2 - 0.88 X_2^2 - 0.56 X_1 X_3 + 0.48 X_2 X_3 - 2.03 X_3^2 - 0.03 X_1 X_4 + 0.12 X_2 X_4 - 0.23 X_3 X_4 - 0.71 X_4^2
\]

where \(X_1\), \(X_2\), \(X_3\), and \(X_4\) are the concentrations of maltose, tryptone, and initial MSG and the initial pH, respectively.

The significance of the regression coefficient was demonstrated by ANOVA analysis (Table 2). A value of “Model Prob > F” less than 0.0001 indicates that the quadratic regression model is highly significant. Therefore, \(X_3\) is the most significant model term in this case. The lack of fit determines the model failure to represent data in the experimental domain at points not included in the regression [37]. Although the “Lack of Fit F-value” of 1,090.83 implies a strong association with pure error, the probability to generate pure error by noise was just 0.09% [38]. The \(R^2\) value of this model as coefficient of determination was 0.9246, suggesting that this regression model is highly significant and thus reasonable for analyzing trends in the response.

The optimal level of each variable and the effect of their interactions on GABA production as a function of two variables were analyzed by plotting three-dimensional response surface curves (Fig. 5). The response surface representing GABA production is a function of the values of two variables with other variables at an optimal level. The reciprocal interaction between maltose and tryptone concentrations on GABA production was analyzed when the initial MSG concentration and initial pH were fixed at 2.05% and 6.5, respectively. The effects of maltose and initial MSG concentrations on GABA production were also analyzed when the tryptone concentration and initial pH were fixed at 3.0% and 6.5, respectively. When the maltose and tryptone concentrations were fixed at 2.1% and 4.0%, respectively, GABA production was elevated at a reduced initial pH and higher initial MSG concentration up to 2.38%. Finally, the optimum conditions for maximum GABA production using the quadratic regression model

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**Table 2. ANOVA for GABA production.**

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<th>Factors</th>
<th>Sum of squares</th>
<th>DF</th>
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<th>F value</th>
<th>Prob &gt; F</th>
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<td>(X_2)</td>
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<td>Lack of fit</td>
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<td>Pure error</td>
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<td>Correlation total</td>
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equation and response surface curves were 2.14% maltose, 4.01% tryptone, 2.38% initial MSG, and an initial pH of 4.74. The maximum value of GABA production predicted from this model was determined to be 21.44 mM. GABA production by \textit{L. brevis} HYE1 under the optimum conditions was analyzed by triplication to verify the predicted value from this model, resulting in an experimental value of 18.76 mM (87.5% of predicted value). This result suggests that the predicted and experimental values of GABA production are in good agreement.

\textbf{Fig. 5.} Three-dimensional response surface plots showing the effect of different variables on GABA production. Each panel indicates the effect of maltose concentration and tryptone concentration (A), maltose concentration and initial MSG concentration (B), maltose concentration and initial pH (C), tryptone concentration and initial MSG concentration (D), tryptone concentration and initial pH (E), and initial MSG concentration and initial pH (F).
So far, many studies have performed culture optimization based on the OFAT method for enhancement of GABA production by LAB [15, 20–22]. On the other hand, there have been few studies on RSM-based optimization for GABA production. In previous studies, culture conditions of L. brevis NCL912 were optimized by RSM with the BBD model, where four variables, including concentrations of glucose, soy peptone, Tween-80, and MnSO₄·4H₂O, were used with a focus on medium composition [39]. Recently, RSM with the CCD model was used to optimize L. plantarum Taj-Apis362 GABA production, where four variables were analyzed with a focus on environmental culture conditions such as temperature, pH, and time, as well as the concentration of glutamic acid [24]. Optimization of GABA production by the RSM model could be dependent on GABA-producing microorganisms, in particular species, and variables used in the model. Therefore, it was meaningful to optimize GABA production by L. brevis HYE1 via the RSM model with four variables, including the concentrations of maltose, tryptone, and initial MSG and the initial pH in this study. Further studies are needed on culture optimization using medium additives such as pyridoxal-5’-phosphate as a coenzyme of GAD, Tween-80 as a growth-stimulating factor for LAB, and sulfate ions for enhancing GAD activity [20, 40].

Generally, GABA-producing LAB are cultivated in MRS medium at the first research stage, and thereafter, the optimization studies controlling the composition of MRS medium are actively conducted in laboratory-scale [15, 22, 40]. However, optimization of the medium composition for the increase of GABA production is necessary to configure a low-cost medium for the industrial production of GABA in commercial-scale. The culture conditions of L. sakei B2-16 and Pediococcus pentosaceus HN8 were optimized using rice bran extracts and mature coconut water, which are by-products from industrial agricultural and food productions, respectively [21, 41]. Therefore, further study will be needed regarding the commercial production of GABA by L. brevis HYE1 and its culture optimization using a cost-effective medium instead of modified MRS medium. In particular, the fact that studies on culture optimization to directly control the cost-effective medium are limited will encourage the follow-up study regarding the RSM-based culture optimization for the industrial production of GABA. In addition, development of a fermentation strategy such as a two-stage fermentation process, including the first stage for cell growth and second stage for GABA production, will be necessary for the industrial production of LAB-derived GABA [36].

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References


