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Development of Starter Cultures for Precision Fermentation of Kombucha with Enriched Gamma-Aminobutyric Acid (GABA) Content

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Abstract: Kombucha, a fermented tea beverage, is produced through the symbiotic interaction of several microbial strains, including acetic acid bacteria, lactic acid bacteria, and yeast, collectively known as symbiotic culture of bacteria and yeast (SCOBY). As its health benefits and distinctive flavor gain wider recognition, consumer demand and research on kombucha fermentation have increased. This study focused on developing starter cultures to produce functional kombucha through precision fermentation technology using selected microbial strains newly isolated from food sources. The isolated bacterial and yeast strains were evaluated and selected based on their fermentation characteristics. Notably, a lactic acid bacterial strain was chosen for its ability to overproduce the γ-amino butyric acid (GABA), a functional food component known to enhance cognitive function and reduce mental stress. To produce the GABA-fortified kombucha, selected single strains of *Acetobacter pasteurianus*, *Lactiplantibacillus plantarum*, and *Saccharomyces cerevisiae* were mixed and used as starter cultures. By optimizing the inoculation ratios and initial sugar concentration, a functional kombucha enriched with acetic acid, lactic acid, and GABA was successfully produced. The resulting kombucha demonstrated 2.2 mg/L of GABA production and 1.15 times higher antioxidant activity after the fermentation, highlighting its enhanced health-promoting properties.

Keywords: kombucha; starter culture; precision fermentation; γ-amino butyric acid (GABA); fortification

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1. Introduction

Kombucha is a fermented beverage produced by adding sugar to green or black tea leaves, followed by fermentation with a consortium of microorganisms [1]. In recent years, global demand for kombucha has increased significantly [2,3], as its health benefits including antioxidant and anti-inflammatory properties have gained widespread recognition [4–6]. The health-promoting effects of kombucha are attributed not only to the functional components inherent in the tea leaves but also to the metabolites generated during fermentation [6]. These metabolites include organic acids such as acetic acid and lactic acid, along with various vitamins, minerals, and polyphenolic compounds [7].

The fermentation of kombucha is a symbiotic relationship between microorganisms called SCOBY (symbiotic culture of bacteria and yeast), which includes acetic acid bacteria, lactic acid bacteria, and yeast (Figure 1) [3].

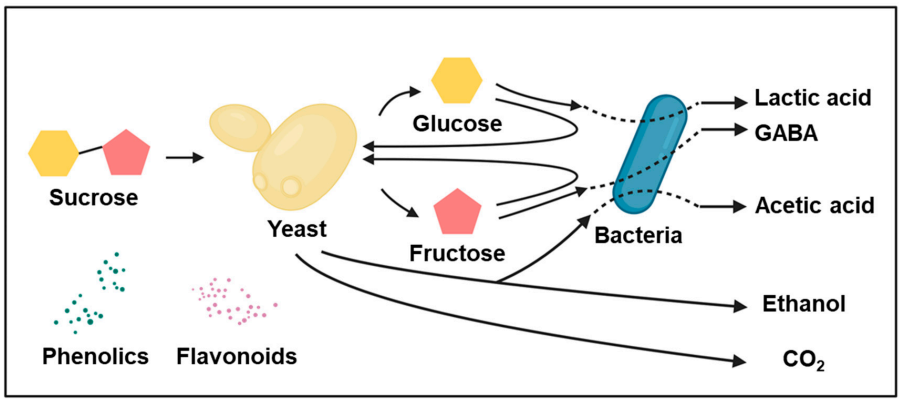

Figure 1. Symbiotic relationship of bacteria and yeast in kombucha fermentation.

During kombucha fermentation, yeast and lactic acid bacteria secrete an enzyme called invertase, which can break down sucrose into glucose and fructose [8]. The yeast utilizes these sugars as carbon sources to produce ethanol, which, in turn, serves as a carbon source for the acetic acid bacteria to produce organic acids such as acetic acid [9]. Lactic acid bacteria also utilize glucose and fructose as a carbon source to produce organic acids such as lactic acid [2,10]. Lactic acid and acetic acid can impact the composition of the human microbiota and improve gut integrity as well as immune function [11]. In addition to these well-known metabolites, various metabolites produced by microorganisms can contribute to the functionality of kombucha [12]. Among them, γ-aminobutyric acid (GABA) has gained attention as a bioactive compound due to its health benefits including cognitive function enhancement, stress reduction, blood pressure regulation, and diuretic effects [13,14]. GABA is known to be produced during fermentation by specific strains of lactic acid bacteria that have been isolated from foods such as kimchi and cheese [14,15]. The use of GABA-overproducing lactic acid bacteria as a starter culture for kombucha fermentation may facilitate the development of functional and value-added kombucha with additional health benefits.

Since the fermentation process relies on the interaction of these microorganisms, variations in the microbial composition can alter the fermentation environment and result in kombucha with distinct characteristics [1,16]. Traditional kombucha production utilizes SCOBY, whose microbial composition is not fully understood, and follows an unstructured fermentation method [16]. As a result, the fermentation process is prone to fluctuations in temperature, pH, and microbial communities, which can lead to inconsistency in flavor and quality between fermentation batches [17,18]. Furthermore, this production process is inefficient for large-scale production, making it challenging to apply to industrial kombucha production. Recently, precision fermentation technology has been reported as a next-generation technology for efficiently producing flavors and specific food components by precisely designing starter cultures and controlling the fermentation process of microorganisms [19,20]. This precision fermentation technology can be used to improve the quality of kombucha products and can also be applied to produce kombucha with specific functions, containing bioactive compounds. Therefore, kombucha fermentation can be precisely designed and developed by employing specialized starter cultures composed of single strains, eliminating the need for traditional SCOBY and alleviating contamination issues. The starter cultures developed in this study can be subsequently applied to effectively enhance the levels of functional components such as GABA, offering a novel approach to kombucha fermentation. This study aimed to design the starter cultures by isolating beneficial strains of acetic acid bacteria, lactic acid bacteria, and yeast from food sources, evaluating the fermentation characteristics of each strain, and utilizing fermentation technology to produce kombucha with enhanced functionality through the enrichment of acetic acid, lactic acid, and GABA.

2. Materials and Methods

2.1. Isolation of Candidate Strains

Yeasts were isolated from various fruits (mangosteen, blueberry, litchi, peach, and cherry) and homemade Makgeolli (Korean rice wine). Lactic acid bacteria were isolated from traditional Kimchi varieties (baechu, dongchimi, yeolmu, and pa kimchi). Acetic acid bacteria were isolated from homemade fruit fermentations, fruit wines, and Makgeolli vinegar. To isolate microorganisms, 1 g of each sample was diluted in 9 mL sterile distilled water and plated on selective media, followed by incubation at 30 °C for 24–48 h. The selective medium for yeast isolation was YPD2 (Yeast Extract 10 g/L, Peptone 20 g/L, Glucose 20 g/L, Agar 18 g/L). For lactic acid bacteria, MRS with BCP (Proteose Peptone 10 g/L, Yeast Extract 5 g/L, Dextrose 20 g/L, Tween80 1 g/L, L-Cysteine 0.1 g/L, Bromocresol purple 0.05 g/L, Agar 18 g/L) was used as the selective medium. The selective medium for acetic acid bacteria was GYCEA (Glucose 50 g/L, Yeast Extract 10 g/L, Ethanol 20 g/L, $CaCO_3$ 30 g/L, Agar 18 g/L). Yeast colonies were identified through 18S rRNA sequencing using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers. Lactic acid bacteria colonies that showed yellow coloration on BCP media were examined, with bacilli isolates identified by 16S rRNA sequencing using 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTAC CTTGTTACGACTT-3′) primers. Acetic acid bacteria colonies that formed a clear zone in the presence of $CaCO_3$ were also identified using 16S rRNA sequencing. All sequencing results were analyzed using NCBI Blast for strain identification.

2.2. Screening and Selection of Beneficial Isolates

Microorganisms suitable for kombucha fermentation were selected by evaluating the characteristics of yeast, lactic acid bacteria, and acetic acid bacteria. For yeast screening, acid tolerance was assessed by performing a spot assay on YPD2 medium adjusted to pH 2.5 with HCl. Overnight-cultured yeasts were prepared by serial decimal dilutions using sterile distilled water, and 5 μL of each dilution was spotted onto the medium. Plates were incubated at 30 °C for 36 h, and growth was observed to evaluate acid tolerance. Lactic acid bacteria were screened for their ability to produce GABA by culturing them in MRS medium supplemented with 10 g/L of monosodium glutamate (MSG), a substrate for GABA synthesis, for 24 h. The lactic acid production in the culture medium was measured, and the GABA production was assessed in both the culture supernatant and cell extracts. Acetic acid bacteria were screened by culturing them in GYE (Glucose 20 g/L, Yeast Extract 10 g/L, Ethanol 20 g/L) medium for 24 h, followed by the measurement of acetic acid production.

2.3. Media and Culture Conditions

To analyze the characteristics of the identified strains, yeast was cultured in YPD2 (Yeast Extract 10 g/L, Peptone 20 g/L, Glucose 20 g/L) medium at 200 rpm and 30 °C. Lactic acid bacteria were cultured in MRS (Proteose Peptone 10 g/L, Beef Extract 10 g/L, Yeast Extract 5 g/L, Dextrose 20 g/L, Polysorbate 80 g/L, Ammonium Citrate 2 g/L, Magnesium Sulfate 0.1 g/L, Manganese Sulfate 0.05 g/L, Dipotassium Phosphate 2 g/L) medium supplemented with MSG (10 g/L) at 200 rpm and 30 °C. Acetic acid bacteria were cultured in GYE (Glucose 20 g/L, Yeast Extract 10 g/L, Ethanol 20 g/L) medium at 200 rpm and 30 °C.

2.4. Characterization of Fermentation

The optical density of microorganisms was measured at 600 nm using a UV spectrophotometer (SHIMADZU, Kyoto, Japan). For the analysis of fermented products, culture supernatants were appropriately diluted after centrifugation of the fermentation medium and kombucha. The concentrations of glucose, glycerol, acetic acid, lactic acid, and ethanol

were measured using the 1260 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with an Aminex® HPX-87H column (300 × 7.8 mm) (Bio-Rad, Hercules, CA, USA). The mobile phase consisted of a 5 mM H_2SO_4 solution, and the flow rate was set to 0.6 mL/min. The column was maintained at 60 °C, and 10 µL of the diluted supernatant sample was injected for analysis.

2.5. Kombucha Fermentation

White tea leaves (6.6 g) were added to 1 L of water at 80 °C and steeped for 3 min. After filtering out the tea leaves, 100 g (first fermentation experiment) or 50 g (second fermentation experiment) of commercially available table sugar (sucrose) was dissolved in the tea solution. Following this, the isolated yeast, lactic acid bacteria, and acetic acid bacteria were inoculated under various conditions. The concentration of microorganisms used in kombucha production was determined based on the OD_{600} value. Initially, the yeast, lactic acid bacteria, and acetic acid bacteria were set at a 1:1:1 ratio with an OD_{600} value of 30, and then the ratios were adjusted for inoculation during the kombucha fermentation process. The fermentation was carried out for 7 days at 25 °C and 40–50% humidity under static conditions, avoiding direct sunlight (Figure 2).

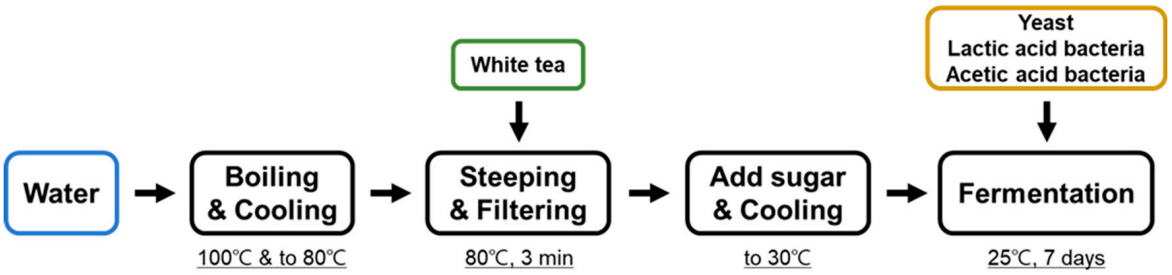

Figure 2. Diagram for kombucha fermentation process.

2.6. Analysis of γ-Aminobutyric Acid (GABA)

The γ-aminobutyric acid (GABA) content in the fermentation supernatant was analyzed using an amino acid analyzer (Sykam S433; Sykam, Eresing, Germany) [21,22]. The fermentation supernatant was mixed with 8% sulfosalicylic acid, then centrifuged at 10,000 rpm for 10 min and filtered through a 0.45 µm membrane. Analysis was performed using a cation exchange column (LCA K07/Li; Sykam, Germany). The physiological reagents containing lithium citrate buffers (pH 2.9, pH 4.2, and pH 8.0) was used for amino acid separation. All reagents were stored in an inert nitrogen gas atmosphere. The sample injection volume was 50 µL. Amino acid separation was carried out in a gradient at 80 °C, followed by post-column derivatization in the reaction chamber using a ninhydrin reagent at 130 °C. Nitrogen was used as the carrier gas, and the flow rate was maintained at 0.45 mL/min. Detection was performed at wavelengths of 570 nm and 440 nm, and the detection reagent was a ninhydrin solution.

2.7. DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined according to the method of Villarreal-Soto et al. (2019) [23]. A 1 mL kombucha supernatant was centrifuged (15,000 rpm, 10 min), and 20 µL of the supernatant was mixed with 180 µL of 0.2 mM DPPH solution. The mixture was then reacted in the dark at 25 °C for 30 min. Afterward, the absorbance was measured at 517 nm using a spectrophotometer (BioTek, Winooski, VT, USA). The DPPH scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (A(\text{blank}) - A(\text{sample}))/A(\text{blank})$$

3. Results and Discussion

3.1. Isolation of Starter Culture Candidates for Kombucha Fermentation

Starter strains for kombucha fermentation were mainly isolated from traditional Korean fermented foods. Lactic acid bacteria were isolated from Kimchi, while the yeast was from Makgeolli and acetic acid bacteria was from Makgeolli vinegar. A total of 11 yeast strains, 19 lactic acid bacteria strains, and 2 acetic acid bacteria strains were isolated (Table S1). To select suitable strains for kombucha fermentation, the identified strains were screened. Since a significant amount of acid is produced during kombucha fermentation, acid-tolerant yeast strains were chosen for selecting the starter. As a control, *Saccharomyces boulardii*, known for its high acid tolerance, and a laboratory strain of *S. cerevisiae* (D452-2) were used for comparative evaluation. Among the isolated yeasts, *S. cerevisiae* FBEL0143 exhibited higher acid tolerance than *S. boulardii*, making it the chosen starter yeast strain for kombucha fermentation. After 18 h of incubation, *S. cerevisiae* FBEL0143 began forming colonies, and by 36 h, it demonstrated superior colony size and density compared to other strains under acidic conditions (Figure 3A). Lactic acid and GABA are metabolites known for their potential health benefits. Therefore, in selecting a lactic acid bacteria starter strain, we focused on strains that produce high levels of both lactic acid and GABA. The production levels of the metabolites varied among the tested lactic acid bacteria strains. Previous studies have similarly reported significant variation in lactic acid and GABA production by lactic acid bacteria isolated from traditional fermented foods, even under identical culture conditions [24–26]. This suggests that the differences in lactic acid and GABA production observed in this study can be attributed to the inherent genetic characteristics of the strains. Among the isolated lactic acid bacteria strains, *Lactiplantibacillus plantarum* FBEL0112 was confirmed to produce both lactic acid (23.75 g/L) and GABA (642.1 mg/L), and selected as the starter strain for kombucha fermentation (Figure 3B,C). Finally, fermentations were performed to select acetic acid bacteria capable of high acetic acid production, which contributes to the sour taste and flavor of kombucha. Among the two isolated strains, *Acetobacter pasteurianus* FBEL0144, which exhibited high acetic acid production, was selected for starter cultures (Figure 3D).

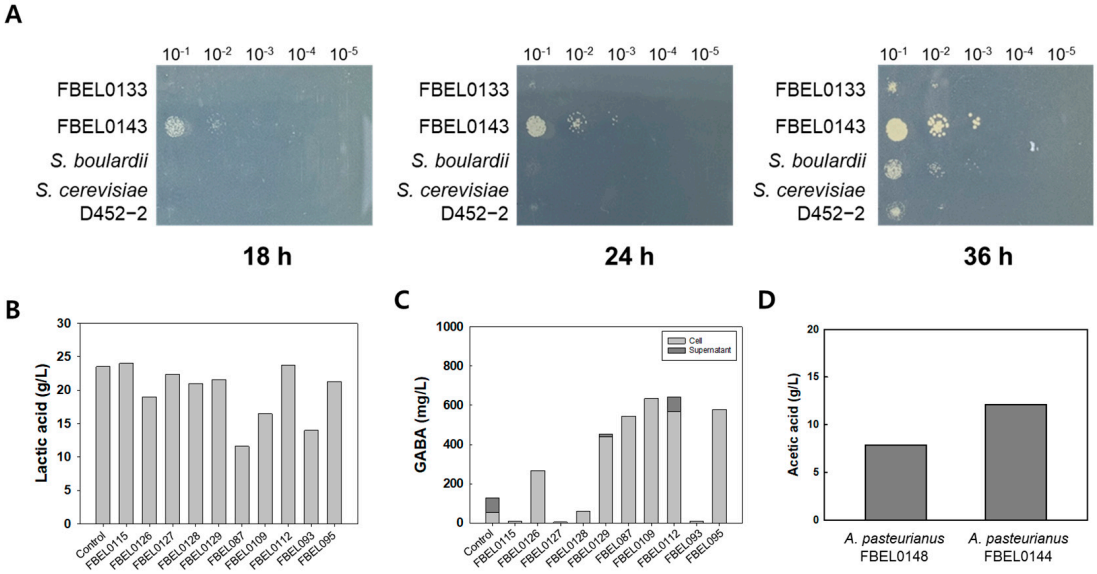

Figure 3. Screening results for the selection of starter culture strains for kombucha fermentation. (**A**) Acid tolerance screening for yeast isolates; (**B**) Lactic acid production by lactic acid bacteria isolates; (**C**) GABA production by lactic acid bacteria isolates; (**D**) Acetic acid production by acetic acid bacteria isolates.

3.2. Characterization of Kombucha Starter Strains

To investigate the fermentation characteristics of each starter strain for the fermentation of kombucha, the growth and metabolic profiles were analyzed. The cultivation of *S. cerevisiae* FBEL0143 confirmed that the OD_{600} reached 23.23 at 24 h. Glucose was completely consumed within 12 h of fermentation. At 24 h, the final production concentrations of glycerol and acetic acid were 0.33 g/L and 0.17 g/L, respectively. Ethanol production peaked at 12 h with a concentration of 7.54 g/L but subsequently decreased, resulting in 2.74 g/L at 24 h (Figure 4). *S. cerevisiae* FBEL0143 primarily focuses on ethanol production while producing small amounts of glycerol and acetic acid. It exhibits the characteristic of consuming ethanol for biomass production once all glucose is depleted. With enough sugar sources, it is expected to produce significant amounts of ethanol. When applied to the precision fermentation of kombucha, *S. cerevisiae* FBEL0143 can serve as an appropriate strain for production of ethanol, a substrate for *Acetobacter* fermentation [27].

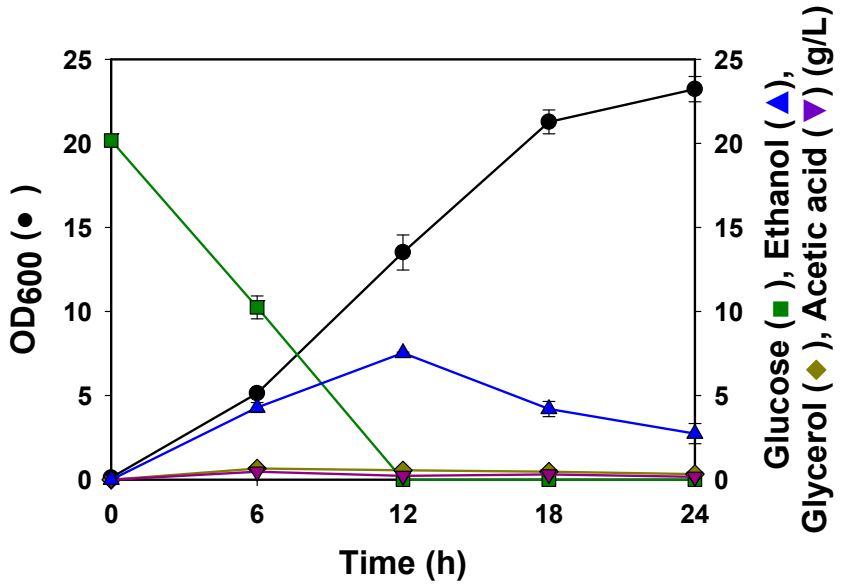

Figure 4. Growth and metabolic profiles of *S. cerevisiae* FBEL0143. The *S. cerevisiae* FBEL0143 strain was cultivated in YPD2 medium at 30 °C, 200 rpm, for 24 h in flask conditions.

For *L. plantarum* FBEL0112, the OD_{600} reached 11.46 at 24 h but showed a decrease after 24 h, decreasing to 9.31 at 48 h (Figure 5). Glucose was completely consumed within 24 h of fermentation. At 48 h, the production of acetic acid and lactic acid was 3.71 g/L and 22.74 g/L, respectively. The final analysis of GABA production at 48 h revealed 349.1 mg/L within the cells and 418.5 mg/L in the supernatant, resulting in a total GABA production of 767.6 mg/L (Figure 5). These results differ from the screening results obtained after 24 h of fermentation, as presented in Figure 3C, where the intracellular GABA content was higher than the extracellular content. This difference can be attributed to the extended fermentation time. Previous studies have shown that intracellularly produced GABA can be transported to the extracellular environment via the GABA antiporter [28,29]. *L. plantarum* FBEL0112 produces acetic acid and lactic acid, which is consistent with the role of lactic acid bacteria in organic acid production during the fermentation of kombucha [30]. Additionally, GABA production was observed under cultivation conditions with the addition of MSG, and other studies have reported that the use of *L. plantarum* strains producing GABA to ferment soy milk and yogurt resulted in an increase in GABA content in the fermented dairy products [31,32]. Therefore, GABA production can also be expected during the fermentation of kombucha.

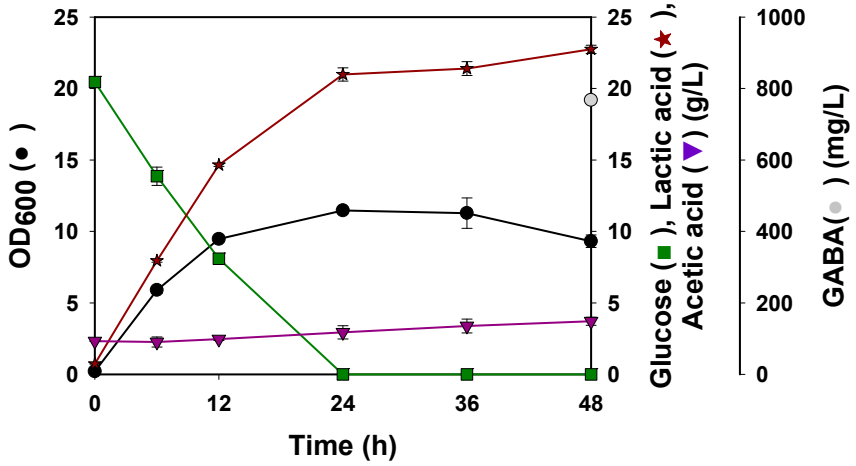

Figure 5. Growth and metabolic profiles of *L. plantarum* FBEL0112. *L. plantarum* FBEL0112 was cultivated in MRS medium supplemented with MSG (10 g/L) at 30 °C, 200 rpm, for 48 h in flask conditions.

The cultivation of *A. pasteurianus* FBEL0144 showed an OD_{600} of 1.7 at 48 h, with minimal glucose consumption observed during the fermentation period. Ethanol consumption was confirmed at 0.8 g/L by 36 h and was completely depleted by 48 h. Acetic acid production reached a final concentration of 19.45 g/L at 48 h (Figure 6). *A. pasteurianus* FBEL0144 is characterized by its preference for ethanol over glucose as a primary substrate and its ability to convert ethanol into acetic acid. In traditional kombucha, the SCOBY, also known as the "black tea fungus", is formed by acetic acid bacteria that produce cellulose, resulting in the SCOBY being intertwined with cellulose during the fermentation process. However, in industrial-scale production, the formation of biofilms, such as cellulose produced by microorganisms, can lead to contamination issues [33]. Therefore, in this study, *A. pasteurianus* FBEL0144, which produces acetic acid without generating cellulose, was utilized for the precision fermentation of kombucha.

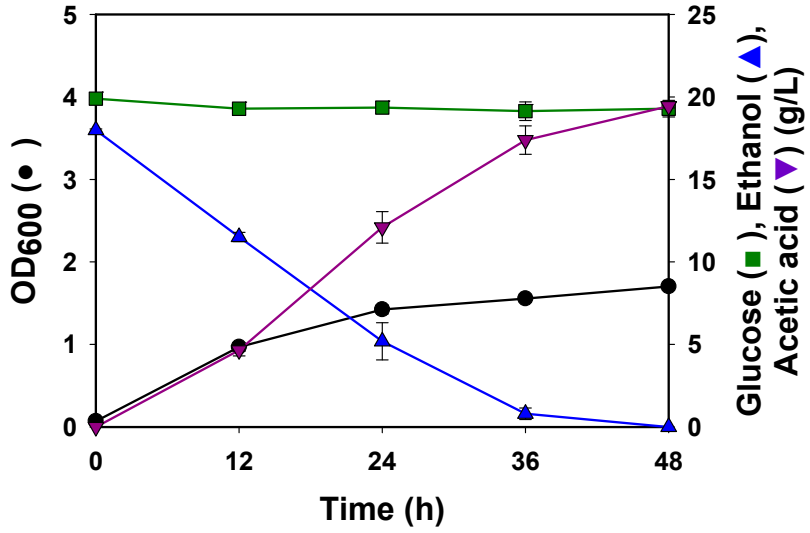

Figure 6. Growth and metabolic profiles of *A. pasteurianus* FBEL0144. *A. pasteurianus* FBEL0144 was cultivated in GYE medium at 30 °C, 200 rpm, for 48 h in flask conditions.

Each starter strain is anticipated to perform essential roles in kombucha fermentation. The fermentation strategy leveraging these strains includes the following: *L. plantarum* FBEL0112 for the production of lactic acid and GABA, *S. cerevisiae* FBEL0143 for initial ethanol production, and *A. pasteurianus* FBEL0144 for converting the produced ethanol into acetic acid.

3.3. Precision Fermentation of Kombucha

Kombucha was produced under various conditions by varying the inoculation ratios and the amount of added sugar for the three starter strains. White tea leaves were used for preparing the kombucha base due to their diverse range of bioactive compounds, including alkaloids, polyphenols, and flavonoids, which are beneficial for health [34]. Furthermore, previous studies have demonstrated that white tea enhances antioxidant capacity and bioaccessibility during the kombucha fermentation process [35]. For the first kombucha fermentation experiment, 100 g of table sugar (sucrose) was added before fermentation. Various inoculation ratios among the selected starter culture strains—*S. cerevisiae* FBEL0143, *L. plantarum* FBEL0112, and *A. pasteurianus* FBEL0144—were utilized to optimize kombucha fermentation. The results of the first fermentation showed that ethanol concentrations exceeded 7 g/L under all conditions (Table 1). Acetic acid concentrations were 4.04 g/L and 3.19 g/L in conditions B and F, respectively, where the inoculation ratio of acetic acid bacteria was 2. Notably, lactic acid was not detected under any of the conditions. These results suggested that the high sugar concentration during kombucha fermentation may hinder the growth of lactic acid bacteria (LAB). Lactic acid bacteria are more sensitive to high sugar concentrations, resulting in their slow growth and no lactic acid production.

Table 1. Metabolic profile of kombucha fermentation.

	Inoculation Ratio (*S. cerevisiae* FBEL0143: *L. plantarum* FBEL0112: *A. pasteurianus* FBEL0144)	**Acetic Acid (g/L)**	**Ethanol (g/L)**	**Lactic Acid (g/L)**
First Fermentation Experiment	A (1:1:1)	0.238	8.392	N.D.
	B (1:1:2)	4.039	8.450	N.D.
	C (1:2:1)	0.111	8.513	N.D.
	D (2:1:1)	0.184	10.791	N.D.
	E (2:2:1)	0.688	8.712	N.D.
	F (2:1:2)	3.187	7.318	N.D.
Second Fermentation Experiment	G (2:5:2)	1.382	7.762	1.737
	H (2:5:5)	2.133	9.5	4.46
	I (2:10:5)	1.811	10.84	1.829

N.D.: Not detected.

To overcome this problem, sugar concentration was decreased to 50 g in the second kombucha fermentation. The inoculation ratio was also optimized based on the results from the first kombucha fermentation. The results of the second kombucha fermentation showed that lactic acid was produced under all conditions (Table 1). This result suggested that the initial sugar concentration is an important factor to facilitate the growth of lactic acid bacteria during kombucha fermentation. Interestingly, ethanol concentrations varied from 7.762 g/L to 10.84 g/L, even though the yeast strain has a fixed inoculation ratio under the three conditions. In condition H, lactic acid was produced up to 4.46 g/L, and acetic acid was produced up to 2.133 g/L, which is the highest acid production condition (Table 1). These results indicated that increasing the inoculation ratio of lactic acid bacteria and reducing the amount of added sugar had a significant impact on their growth. However, based on the result of condition I, further increase of *L. plantarum* inoculum did not help to enhance lactic acid production in kombucha. These results suggest that due to the complex symbiotic relationship between the microorganisms involved

in kombucha fermentation, the optimal ratio of each microorganism is crucial for the successful fermentation of kombucha.

3.4. Analysis of Antioxidant Activity and GABA Production of Kombucha

Among the various fermentation conditions with different inoculation ratios of starter culture, the G, H, and I conditions were selected for the further analysis of kombucha functionality. These conditions showed a considerable amount of lactic acid production, indicating that the lactic acid bacteria were actively involved in the symbiotic interaction of kombucha fermentation. The analysis of antioxidant activity through DPPH radical scavenging found that the kombucha base (white tea leaf infusion without fermentation) harbors an antioxidant activity of 78%. In conditions G, H, and I, the antioxidant activities of kombucha increased to 89.4, 89.76, and 89.92%, respectively, indicating a significant improvement up to 1.15 times after the fermentation (Figure 7A). Similarly, a study demonstrated that kombucha fermentation significantly increases antioxidant capacity, total phenolic content, and bioaccessibility across different tea varieties, with enhanced antioxidant activity measured by the ABTS and CUPRAC methods [23]. These results suggest that the antioxidant effect can be enhanced by fermenting kombucha with the starter cultures developed in this study.

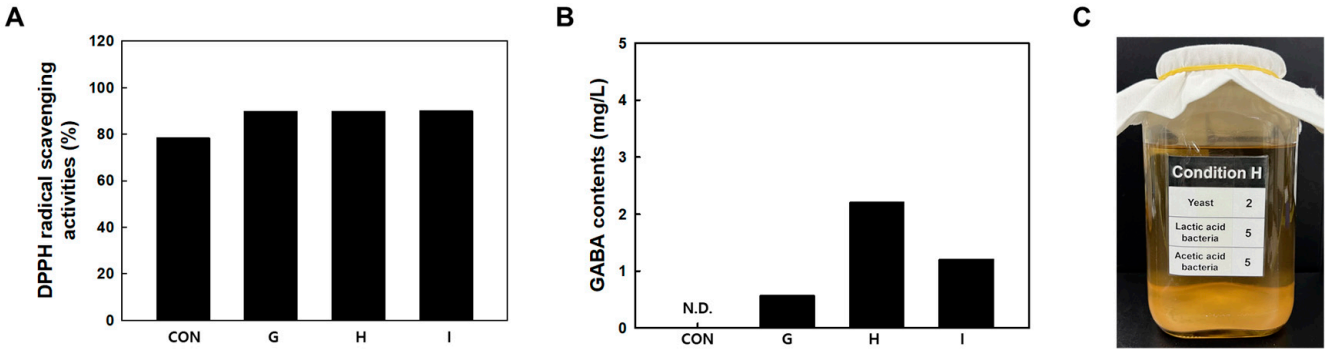

Figure 7. Antioxidant activity and GABA content in kombucha. (**A**) DPPH radical scavenging activity (%) in kombucha under different conditions (CON, G, H, I). (**B**) GABA content (mg/L) in kombucha. CON: White tea leaf base without fermentation; N.D.: Not detected. (**C**) Kombucha produced under condition H.

Regarding GABA production, no GABA was detected in the kombucha base, while GABA was produced in the kombucha fermented under the G, H, and I conditions. GABA concentrations were 0.5 mg/L in condition G, 2.2 mg/L in condition H, and 1.02 mg/L in condition I (Figure 7B). These results suggested that the starter cultures including GABA-overproducing *L. plantarum* used in this study successfully enhanced the GABA contents in kombucha, providing a positive impact on health functionalities. According to other studies, GABA in beverages has been shown to provide various health benefits, including antihypertensive effects [36], improved sleep quality [37], and obesity reduction through the modulation of gut microbiota [38]. These findings indicate the potential of GABA-enriched beverages to support diverse aspects of health, and our study aims to further explore this potential. Also, optimization of the inoculation ratio of starter strains is an important factor in increasing the GABA content in kombucha. The interactions between microorganisms and the production of metabolic byproducts during kombucha fermentation may play a key role in increasing antioxidant activity and GABA content.

4. Conclusions

This study demonstrates that functional kombucha can be produced using characterized starter cultures consisting of each representative single strain of bacteria and yeast without relying on the cellulose-based traditional SCOBY. Yeast, lactic acid bacteria, and acetic acid bacteria isolated from traditional Korean fermented foods were selected according to specific criteria beneficial for the properties of kombucha. The selected strains were analyzed for their fermentation characteristics to be used as starter cultures for kombucha production. The strategy of utilizing these strains as starter cultures is suitable for producing kombucha with the desired characteristics and the precise control of the fermentation process. By adjusting the inoculation ratio of the characterized starter strains and the amount of added sugar, kombucha was successfully developed with lactic acid and acetic acid contents, providing a unique taste and flavors of kombucha. Additionally, functional analysis of the kombucha confirmed the presence of antioxidant activity and the bioactive compound GABA. Utilizing well-characterized starter cultures could aid in the mass production and industrialization of kombucha by addressing issues related to quality control, reproducibility, consistency, and contamination. This study successfully developed the functional kombucha fortified by GABA through precision fermentation using newly developed starter cultures, expecting its enhanced health-promoting properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation11010017/s1, Table S1: List of identified yeast, lactic acid bacteria, and acetic acid bacteria in this study.

Author Contributions: G.-H.K. and K.-R.B.: Experiment, methodology, data curation, writing—original draft; G.-E.L., J.-H.L. and J.-H.M.: Experiment, methodology, investigation, data curation; S.-O.S.: Writing—review and editing, supervision, conceptualization; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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