Article

Development of Blood Sugar Regulatory Products from *Momordica cochininensis* via Probiotic Fermentation

Po-Hua Wu [1,2], Huei-Rong Guo [3], Yi-An Liu [4], Chien-Hui Wu [4], Chun-Chen Huang [1], Jer-An Lin [5] and Chang-Wei Hsieh [1,6,*]

1 Department of Food Science and Biotechnology, National Chung Hsing University, 145 Xingda Road, Taichung City 40227, Taiwan; danielwu7997@gmail.com (P.-H.W.); jchuang8259@gmail.com (C.-C.H.)
2 Best News Biomedical Co., Ltd., Pingtung County, Pingtung City 90846, Taiwan
3 Ph.D. Program of Aquatic Science and Technology, National Kaohsiung University of Science and Technology, Kaohsiung City 81157, Taiwan; I110180101@nkust.edu.tw
4 Department of Seafood Science, National Kaohsiung University of Science and Technology, Kaohsiung City 81157, Taiwan; f110176109@nkust.edu.tw (Y.-A.L.); chwu@nkust.edu.tw (C.-H.W.)
5 Graduate Institute of Food Safety, National Chung Hsing University, 145 Xingda Road, Taichung City 40227, Taiwan; lja@nchu.edu.tw
6 Department of Medical Research, China Medical University Hospital, Taichung City 404327, Taiwan
* Correspondence: welson@nchu.edu.tw; Tel.: +886-4-22840385 (ext. 5010)

Abstract: Type II diabetes is the most important health issue in the whole world. Besides the use of prescribed drugs to control blood glucose level, recently, the development of health supplements is being actively explored. Owing to its high nutritional value, *Momordica cochinchinensis* Spreng. (Gac) is potentially a good source for developing this supplement. In recent years, the aril of Gac has been utilized as a substrate for developing various forms of supplements, but the pulp has been neglected as a byproduct. However, the pulp contains lots of phytochemicals that could provide health benefits, and the investigation using lactobacilli to ferment the pulp juices to lower blood glucose is not yet to be explored. Therefore, we set out to investigate the potential to develop the pulp-based juices for controlling blood glucose level by selecting an optimal strain of lactobacillus to ferment the pulp juice and measuring the inhibitory action of the fermented juice on α-glucosidase. This enzyme is crucial for controlling postprandial glucose absorbed into the bloodstream because it is the enzyme that hydrolyzes the carbohydrates to release glucose. First, we have successfully isolated a strain of lactobacillus which was capable of fermenting the pulp to produce α-glucosidase-inhibitory activity. Through a 16S rRNA sequence, this lactobacillus was named *Lactiplantibacillus plantarum* GBI 001. The optimal conditions for its growth in commercial culture medium were found to be 35 °C for 16 h to produce the highest α-glucosidase activity (72.03%). The optimal conditions for the strain to grow in Gac pulp juice were: 20% pulp juices as substrate with an initial pH adjusted to 4.0, growing at 35 °C for 16 h. Under these conditions, the fermented juice exhibited α-glucosidase activity of 24.36%, which is a 2.17-fold increase over the control group (11.23%). From its increase in α-glucosidase potency, using *L. plantarum* GBI 001 to ferment the pulp juices of Gac as soft drinks has great potential to develop a helpful drink as a food supplement to control postprandial blood glucose in patients with diabetes.

Keywords: *Momordica cochinchinensis*; lactic acid bacteria; fermentation; α-glucosidase inhibitory activity; diabetes

1. Introduction

In the past few decades, the prevalence of diabetes mellitus (DM) increased [1]. DM is mainly classified into three types: type 1 diabetes (T1DM), type 2 diabetes (T2DM), and gestational diabetes [2]. Type 2 diabetes mellitus (T2DM) is among the most prevalent metabolic diseases in the whole world, resulting from insufficient insulin secretion and

insulin resistance. It is characterized by high blood sugar and is controlled through diet control, life style adjustment, and various drugs [3]. T2DM accounts for the majority of diabetes cases and has become a worldwide problem. The International Diabetes Federation (IDF) reported that the number of diabetes cases reached a staggering number of 463 million in 2019 with a projection to increase to approximately 578 million in 2030 and 700 million in 2045 [4,5]. T2DM is defined as a metabolic disorder characterized by hyperglycemia [6]. Without effectively controlling blood sugar, severe diabetes leads to many complications such as cardiovascular diseases, nerve damage, abnormal lipid metabolism, and kidney dysfunction. Thus, it is of paramount importance to maintain a stable blood sugar [7,8]. α-glucosidase inhibitor is able to slow the hydrolysis of carbohydrates, resulting in the slowing absorption of glucose into the bloodstream, thus reducing postprandial surge in the blood glucose concentration. As a result, the glycemic index of food intake can be moderated. Acarbose, miglitol, and voglibose belong to these types of drugs [3]. However, they exhibit gastrointestinal side effects such as bloating, abdominal pains, and diarrhea [9,10], reducing patients' preference for choosing these drugs. Therefore, recently, many investigators have attempted to develop potential α-glucosidase inhibitors from natural resources such as plants, animals, and microbes with few side effects [11–13].

Many vegetables, fruits, microbiomes, fermented products, and proteolytic products exhibit biological activities which have been shown to be beneficial to human health. Thus, these could be potential sources for developing the prevention and treatment of diseases related to diabetes [14–17]. Curcumin supplementation can significantly reduce blood glucose and triglyceride levels [18]. *Gegen Qinlian* Decoction (GQD) has been used for the long-term management of T2DM. GQD plays a protective role in T2DOP by upregulating IGFBP3 expression and downregulating the IGFBP3/MAPK/NFATc1 signaling pathway [19]. Laurolitsine was found to have potent antidiabetic effects with hypoglycemic activity in vivo. It improved insulin resistance, glucose tolerance, and lipid metabolism and protected the liver and renal and pancreatic functions [20]. Probiotics reduced the impact of phthalates and bisphenol A mixture on type 2 diabetes mellitus development [21]. The enzyme-inhibitory activities of *Stachys riederi var. japonica* mediated the enzyme-inhibitory effect against α-amylase and α-glucosidase [22]. Some foods, due to their chemical composition, are implemented as prebiotic sources because they are considered essential for people's health. Therefore, prebiotic foods play an important role in the microbiota and are beneficial for the human gastrointestinal tract [23]. *Momordica cochinchinensis* Spreng. (Gac) belongs to the calabash family of perennial plants. Gac has been coined the fruit of heaven owing to its high nutritional value. It has been identified to contain high concentrations of phytochemicals such as β-carotenoids, lycopene, and lutein. In addition to their antioxidant, anti-obesity, and anti-inflammatory activities, they have anticancer properties and are able to modulate immunological responses [24–26]. Additionally, Gac is active in improving diabetes and its associated complications; for example, Gac is able to lower postprandial blood glucose level owing to its contents of flavonoids and phenolic acids such as myricetin, quercetin, and ferulic acid, which exhibit inhibitory action via α-glucosidase [27–29]. Via the inhibition of α-glucosidase, the digestion of carbohydrates can be slowed to moderate the postprandial surge in blood glucose [30]. Therefore, one way to control postprandial blood glucose surges is to search for inhibitors of α-glucosidase. Using ARPE-19 (human retinal pigment epithelial cell) cell lines as the model system to study proliferate diabetic retinopathy, Abdulqader et al. [31] studied the effects of the extracts of various parts of Gac (Figure 1) including the peel, pulp, aril, and seed on ARPE-19 cells and found that the extracts could reverse high glucose (30 mmol/L)-induced reactive oxygen species (ROS) increase. In addition, the extracts reduced the vascular endothelial growth factor (VEGF), which is a stimulator of new blood vessel formation (angiogenesis), and increased the pigment-epithelium-derived factor (PEPF), which is an inhibitory factor in angiogenesis. If these results could be reproduced in vivo in retinal pigment epithelial cells, the extracts would be expected to slow new blood vessel formation and these could potentially become a remedy for proliferate diabetic retinopathy.
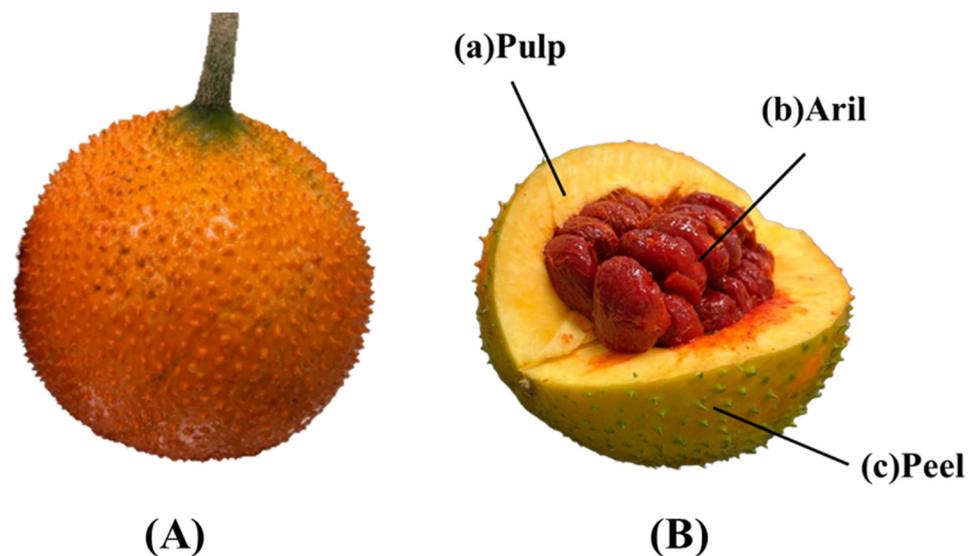
Figure 1. Anatomical components of Gac (**A**) fruit (**B**) a. Pulp b. Aril c. Peel with spines.

Lactic-acid-producing bacteria are commonly called Lactobacilli (LAB). LAB form a large class of non-spore-forming, catalase-negative, Gram-positive, and facultatively anaerobic bacteria that can utilize carbohydrates to produce lactic acid [32]. LAB is beneficial to our health because they ferment many foodstuff-producing products that lower blood sugar and exert anti-inflammatory actions. Thus, they are useful in many applications such as food processing, medicinal development, and cosmetic and other chemical industries [33–37].

Recently, the role of lactobacilli in the management of type II diabetes has received much attention. In an animal model of type II diabetes which was induced by streptozotocin in high-fat-fed mice, Wang et al. [38] found that lactobacilli could help produce mid-chain fatty acids to reduce inflammation and lower insulin resistance, leading to lower blood glucose levels. In in vitro studies, Chen et al. [39] found that the order of inhibition of α-glucosidase is as follows: *Lacticaseibacillus rhamnosus* GG (29.57%), *Lacticaseibacillus rhamnosus* Z7 (29.21%), *Lactobaciseibacillus bulgaricus* L24 (24.71%), *Lactobaciseibacillus casei* 2W (21.27%), and *Bifidobacterium bifidum* F-35 (20.50%).

Despite the observations that both Gac and LAB are beneficial to our health, there are very limited studies on their potential application in diabetes following the fermentation of Gac by LAB. Therefore, the present study attempts to find the optimal condition to ferment the pulp of Gac by LAB to increase the viability of LAB and the fermented products to inhibit α-glucosidase with the hope to develop an oral drink to control the blood sugar level of the patients with type II diabetes.

2. Materials and Methods
2.1. Isolation and Identification of Lactic Acid Bacteria (LAB)
2.1.1. Isolation of LAB

Following Masi et al.'s [40] method to isolate and purify LAB isolates, 25 g Gac flesh from Jia Cheng Agricultural Biotechnology Company (Pingtung, Taiwan) was incubated in 225 mL MRS medium for 24 h; later, 1 mL of broth after being serially diluted was streaked on MRS agar and incubated at 37 °C for 24 h in an aerostat. The single colony was incubated in MRS agar in 4 isolated sections at 37 °C for 24 h, and the follow-up biochemical test was run on the incubated single colony.

2.1.2. Gram Staining, Catalase, and Oxidase Tests

Following Masi et al.'s [41] method to progress Gram staining, the bacteria were identified as Gram positive if they were purple in color, and they were identified as Gram

negative if they were pink in color. The bubble produced were 1 loop of bacteria was mixed with 3% H_2O_2 was regarded as catalase positive. One drop of 1% N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride was dropped on the bacteria colony; if it turned blue, it was regarded as oxidase positive [34]. LAB are Gram positive, catalase negative, and oxidase negative.

2.1.3. α-Glucosidase Inhibitory Activity Assay

Following Zheng et al.'s method [42] with slight modification, the supernatant of the Gac fermentation broth was used for α-glucosidase inhibitory activity. The sample and reagents were all prepared with phosphate buffer (0.1 M, pH 6.8). A total 50 μL of sample was mixed with 50 μL of α-glucosidase (0.25 U/mL), oscillated homogeneously, and set down for 10 min in 37 °C. Then, 50 μL 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added and reacted for 15 min in 37 °C; then, 50 μL of 0.1 M sodium carbonate was added to terminate the reaction, the absorbance measurement was 405 nm, and acarbose (10 mg/mL) was used as a positive control.

α-glucosidase inhibitory rate (%) was calculated as follows: α-glucosidase inhibitory rate (%) = [(A$_{sample}$ − A$_{blank}$)/A$_{control}$] × 100

A$_{sample}$: Absorbance of sample
A$_{blank}$: Absorbance of mixture with sample and α-glucosidase
A$_{control}$: Absorbance of control

Acarbose is a bacterial-derived α-glucosidase inhibitor clinically used to treat patients with type 2 diabetes [43].

2.2. 16S rRNA Gene Sequencing Analysis

The DNA extraction was carried out using a bacteria DNA extraction kit (Solarbio-Science and Technology Co. Ltd., Shanghai, China), and amplification of the 16S rDNA coding region of each LAB was performed via polymerase chain reaction (PCR) with primer. The LAB 16S rRNA sequences obtained were pre-analyzed; we compared them with the sequences gathered in the National Center for Biotechnology Information (NCBI) GenBank database with the Standard nucleotide Basic Local Alignment Search Tool program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (accessed on 31 March 2022.) to determine whether both sequences matched with the same microorganism sequence. The PCR reaction procedure was as described; 94 °C pre-denaturation for 5 min, 94 °C denaturation for 30 min, 42 °C renaturation for 30 s, 72 °C elongation for 2 min, a total of 30 cycles, and finally, 72 °C elongation for 8 min [44].

2.3. Scanning Electron Microscopy of L. plantarum GBI 001

The colonies were grown on MRS medium for 16 h of incubation at 35 °C under anaerobic conditions. Scanning electron microscopy (SEM) was used to analyze the morphology of *L. plantarum* GBI 001 cells. The cells were fixed in McDowell–Trump fixative reagent, pH 7.2 (Agar Scientific Limited, Stansted, UK), for at least 2 h. The cells were washed with 0.1 M phosphate-buffered saline and centrifuged at 5000 rpm for 10 min. The resulting pellet was fixed for 1 h in 1% osmium tetroxide (Sigma-Aldrich Co., LLC, St. Louis, MO, USA) prepared in phosphate-buffered saline. The sample was washed twice with distilled water for 10 min and then dehydrated for 10 min in ethanol (Merck, Darmstadt, Germany) at concentrations of 50%, 75%, 95%, and 99.5%. Afterwards, 1 mL hexamethyldisilazane (Agar Scientific Limited, Stansted, UK) was added to the sample tube for 10 min. Hexamethyldisilazane was decanted from the tube, and the cells were air dried at room temperature. The sample specimen was coated with gold and viewed with a Hitachi Scanning Electron Microscope SU3500 (Hitachi, Tokyo, Japan).

2.4. Optimal Culture Conditions of L. plantarum GBI 001 for Producing α-Glucosidase Fermentation Activity

L. plantarum GBI 001 was incubated in MRS medium to determine the optimum culture condition, and the culture temperatures were 30, 35, and 40 °C, respectively, for 16 h. Then, they were incubated at an optimal 35 °C from 0 to 32 h to evaluate the optimum time, and the samples were tested for LAB cell count, pH value, and α-glucosidase inhibitory activity to determine the optimal fermentation conditions of Gac juice with *L. plantarum* GBI 001.

L. plantarum GBI 001 was incubated in MRS medium for 16 h at 35 °C. The sample was centrifuged and washed twice with sterile PBS. The pellet was incubated in Gac juice to survey the optimum fermentation condition, such as the amount of substrate 30% (*w/v*), fermentation temperature (30~40 °C), fermentation time (0~32 h) and initiate pH value (pH 3~6), and the samples were tested for LAB cell count, pH value, and α-glucosidase-inhibitory activity.

2.5. LAB Cell Count

LAB cell count was measured with the standard plate count method. OA total of 1 mL of Gac fermentation broth was added to 9 mL of sterile dilution and stirred homogeneously; after serial dilution to an appropriate concentration, 0.1 mL of dilution solution was streaked on MRS agar plate. LAB cell count was conducted after being incubated at 37 °C for 48 h in an aerostat [45].

2.6. pH

The pH value of the fermentation broth was measured with a pH meter (Metrohm, Switzerland) at room temperature.

2.7. Statistical Analysis

The statistical analysis was conducted using SPSS statistical software (20.0 version; SPSS, Inc., Chicago, IL, USA). For Duncan's test, $p < 0.05$ was regarded as statistical significance.

3. Results and Discussion

3.1. Isolation and Identification of LAB with α-Glucosidase-Inhibitory Activity

The Gac used in this study is shown in Figure 1. Figure 1A shows the whole fruit and B shows peel, pulp, and arils, which cover the seeds. Twenty (MC1 to MC20) lactic-acid-producing LAB strains were isolated from the surroundings and are listed in Table 1. They were all Gram positive, lacking oxidase and catalase activities, and their inhibitory actions on α-glucosidase are listed in the last column of Table 1. Using acarbose as a reference with 79.85% inhibitory action, 20 strains all exhibited an inhibitory action on α-glucosidase, ranging from 3.24% to 74.23%, with descending order of MC11 (74.23%), MC9 (64.86%), and MC18 (63.87%).

Table 1. Isolation and identification of LAB.

Stain No.	Gram Test	Oxidase Test	Catalase Test	α-Glucosidase-Inhibitory Activity (%)
Acarbose (10 mg/mL)	N.D	N.D	N.D	79.58%
MC1	+	-	-	11.52
MC2	+	-	-	3.238
MC3	+	-	-	20.38
MC4	+	-	-	22.59
MC5	+	-	-	14.75
MC6	+	-	-	11.14
MC7	+	-	-	49.59
MC8	+	-	-	10.22
MC9	+	-	-	64.86

Table 1. *Cont.*

Stain No.	Gram Test	Oxidase Test	Catalase Test	α-Glucosidase-Inhibitory Activity (%)
MC10	+	-	-	55.35
MC11	+	-	-	74.23
MC12	+	-	-	57.55
MC13	+	-	-	15.01
MC14	+	-	-	35.33
MC15	+	-	-	58.21.
MC16	+	-	-	56.37
MC17	+	-	-	14.85
MC18	+	-	-	63.87
MC19	+	-	-	5.42
MC20	+	-	-	5.12

"+" means positive reaction; "-" means negative reaction. N.D means none detected.

The strain belonged to *Lactiplantibacillus plantarum*, as per the identification by 16S rRNA shown in Figure 2 and its phylogenetic tree (Figure 2), and it was named *L. plantarum* GBI 001. The scanning electron microscopic graphs showed that MC11 was typical rod shape (Figure 3).
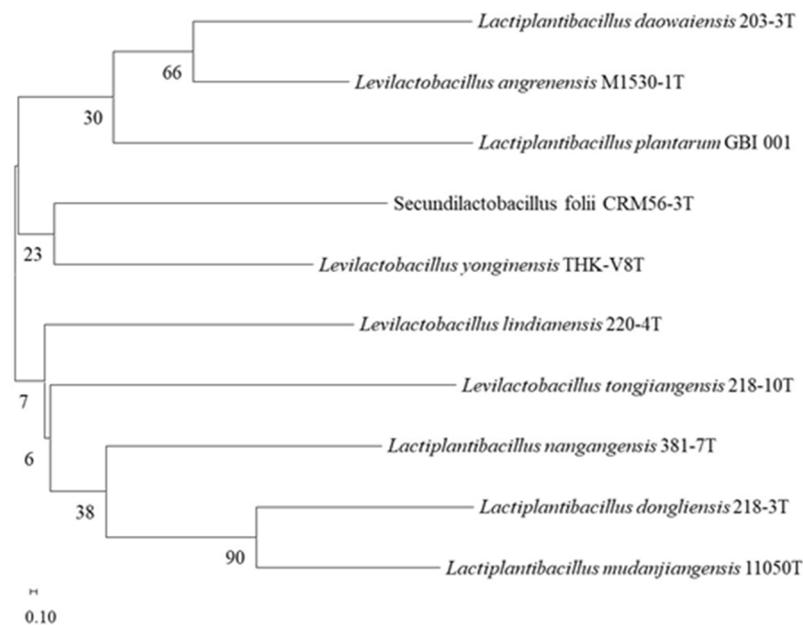

Figure 2. Phylogenetic tree showing the relationships between *Lactiplantibacillus plantarum* GBI 001 strain and related species based on 16S rRNA gene.

Staple foods such as rice, flour, and corn mainly contain carbohydrates. Carbohydrates with a high glycemic index lead to a surge in blood glucose because they are good substrates for α-glucosidase, which hydrolyzes carbohydrates to release glucose. Therefore, we can reduce the decomposition of sugar after meals and delay the absorption of glucose by inhibiting the activity of carbohydrase. Lactobacilli are good natural donors that produce α-glucosidase inhibitors. Cai et al. [46] have utilized principal component analysis (PCA) and analytic hierarchy process (AHP) methods to select bacterial strains for controlling blood glucose level. From fermented foods, they isolated 148 strains with α-glucosidase inhibitory action: *Lactiplantibacillus plantarum* 152 (14.57%) > *Lactiplantibacillus rhamnosus* GG (LGG) (14.18%) > *Pediococcus acidilactici* 004 (10.75%). In the present study, we chose *L. plantarum* GBI 001 with 74.23% inhibitory action, which is 5 to 6.9 times higher than the three above-mentioned strains. Thus, *L. plantarum* GBI 001 is expected to be the most potent strain for developing remedies for lowering blood glucose. Depending on the

sources, lactobacilli vary in their α-glucosidase inhibitory potency: from pickled vegetables, Kwun et al. [47] found that *Lactobacillus sakei* MBEL1397 exerted 3.91 ± 0.25% inhibition on α-glucosidase; from milk and infant feces, respectively, Zeng et al. [48] isolated *Lactobacillus rhamnosus* and *Lactobacillus plantarum* with inhibitory activities ranging from 2.5 to 13.7%; from yogurts, Wang and Li [49] isolated *Lactobacillus lactis*, *Lactobaciseibacillus casei*, and *Lactobacillus paracasei* with α-glucosidase inhibitory action: 29.17%, 28.78%, and 26.17%, respectively; and from infant feces, the same authors isolated *Lacticaseibacillus rhamnosus* MG5411 with α-glucosidase-inhibitory action: 19.8% [50].
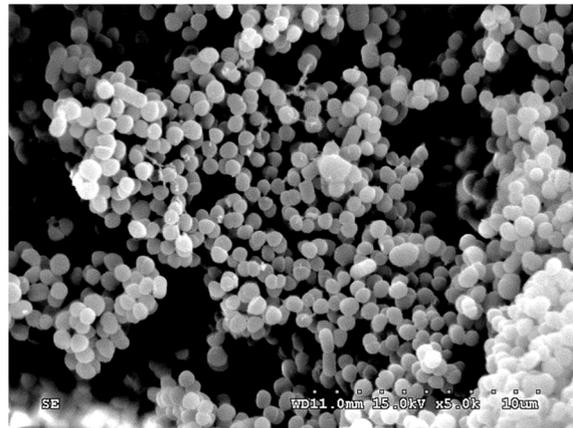

Figure 3. Observation of *Lactiplantibacillus plantarum* GBI 001 using scanning electron micrographs.

3.2. Optimal Culture Conditions for Producing α-Glucosidase-Inhibitory Activity

Our attempt to find an optimal condition to culture the *L. plantarum* GBI 001 strain is illustrated in Figure 4a. First, we compared *L. plantarum* GBI 001 strains plated in MRS medium for 16 h at different temperatures: 30, 35, and 40 °C. Then, the number of cells, the pH of the medium, and the inhibitory activity on α-glucosidase were measured. As illustrated, the LAB counts were between 8.85 and 9.08 log CFU/mL, the pH values were between 4.08 and 4.13, and the inhibitory activities on α-glucosidase ranged from 68% to 73%. Thus, the best temperature for the culture was 35 °C out of the different temperatures: 30, 35, and 40 °C. Because it had provided higher cell counts (9.08 Log CFU/mL) and yielded the most active inhibitory action on α-glucosidase (73.12%), 35 °C was chosen for further experiments to further characterize the culture of the *L. plantarum* GBI 001 strain.
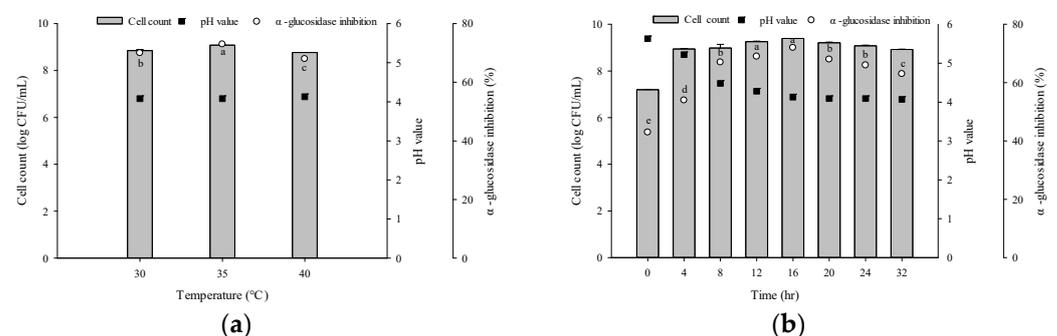

Figure 4. Effects of culture temperature and time on the α-glucosidase-inhibitory activity of *Lactiplantibacillus plantarum* GBI 001 in MRS: (**a**) temperature; (**b**) time. Results are presented as means ± SD of triplicate independent experiments (n = 3). Different lowercase alphabetical letters represent significant differences in the same sample at different concentrations at $p < 0.05$.

At 35 °C, the time course of changes in the *L. plantarum* GBI 001 strain in MRS medium was monitored in terms of cell population, pH values of the medium, and the inhibitory action on α-glucosidase (Figure 4b). The cell count was steadily increased, starting at

7.20 log CFU/mL for up to 16 h, reaching a maximum value of 9.39 log CFU/mL. Then, it started to slowly decrease for the next 16 to 32 h. pH values declined more drastically from 5.63 to around 4.13 from 0 to 12 h, reaching a steady value around 4.0. Part of these changes might reflect the fact that the *L. plantarum* GBI 001 strain grows in population producing acidic products. The inhibitory action on α-glucosidase reached a maximum value of 72.03% at 16 h in culture. Therefore, 16 h culture time was chosen for the rest of the experiments.

Earlier investigations revealed that the fermentation of carbohydrates by microorganisms produces organic acids such as acidic acid, gluconic acid, and other exopolysaccharides (EPS), which are capable of decreasing the pH value of the culture medium. Thus, the rate at which pH value decreases and the time when the culture medium reaches the final pH value depend on the activities of the microorganisms, the density of the organisms, the fermentation time, the substrates, and the fermented products [51]. The decrease in the pH of the fermented quinoa was probably due to the production of organic acids from the fermentation of carbohydrates by the Lactobacill strain [52,53]. Lactobacill species are less efficient producers of EPS compared to other LAB. EPS produced by these LAB are mainly synthesized during the exponential growth phase, and there is a decrease in their concentration at the end of the fermentation, which suggests that EPS could be used as alternative carbon sources [54]. As for the study of the glycemic index, by measuring the inhibitory action on α-amylase and α-glucosidase activities, Yang et al. [55] found that *L. acidophilus*, *L. casei*, *L. mesenteroides*, and *L. lactis* inhibited α-amylase by 48.23%, 55.47%, 44.79%, and 50.65%, respectively, and that their inhibitions on α-glucosidase activity were 56.65%, 60.47%, 48.79%, and 51.23%, respectively. These inhibitory actions could slow glucose absorption into the bloodstream, reducing the blood glucose surge after the ingestion of carbohydrates. Thus, the glycemic index is reduced. These authors postulated that the reduction in the glycemic index by these lactobacilli could be due to exopolysaccharides (EPS) because EPS carry net negative charges that can inactivate α-glucosidase activity by forming a complex with the α-glucosidase enzyme. The direct action of EPS on a-glucosidase was confirmed by the experiments by Bajpai et al. [56] and Sasikumar et al. [57]. They purified the EPS following fermentation with *Lactobacillus sakei* pro Bio 65 found that EPS (10–200 mg/mL) inhibited α-glucosidase activity by 7.05–60.18% [56], and with Lb. plantarum BR2 EPS (100 µg/mL), it inhibited α-glucosidase by 67% [57]. The compositions of EPS depend on the strains of the microorganisms and substrates [58]. In this respect, the lactobacilli are the best choice because they produce EPS, exhibiting a great reduction in the glycemic index and because they are safe on normal cells while exerting anticancer activity. Thus, the application of lactobacilli to produce fermented oral products could potentially be an option to control type II diabetes [59,60].

3.3. Optimal Fermentation Conditions of Gac Pulp

3.3.1. Matrix Content

First, we determined the amount of substrates in the fermentation reaction by varying the amount of pulp juices because they served as substrates for fermentation by lactobacilli. As illustrated in Figure 5a for Gac pulp juices at 10, 20, and 30%, following 16 h of incubating with *L. plantarum* GBI 001, the cell counts were more or less similar (around 9.0 log CFU/mL), and so were the pH values (4.09 to 4.15) of the media. However, the inhibitory action on α-glucosidase activity reached the maximum at 20% juice content. Thus 20% juice content was chosen for further experiments.

Nagarani et al. [61] investigated the composition of Gac fruits and found that they contain 88.6% water, 7.6% carbohydrates, 1.5% proteins, 1.1% crude fibers, and 0.3% crude fats. They also contain many phytochemicals such as carotene (66 µg/g), lycopene (424.6 µg/g), lutein (1.1 µg/g), total phenols (28.9 mg GA/100 g), and flavonoids (8.8 mg QE/100 g). Owing to its antioxidant, lowering blood glucose, anti-inflammatory, and anticancer biological activities, Gac is an excellent candidate for developing organic drinks.
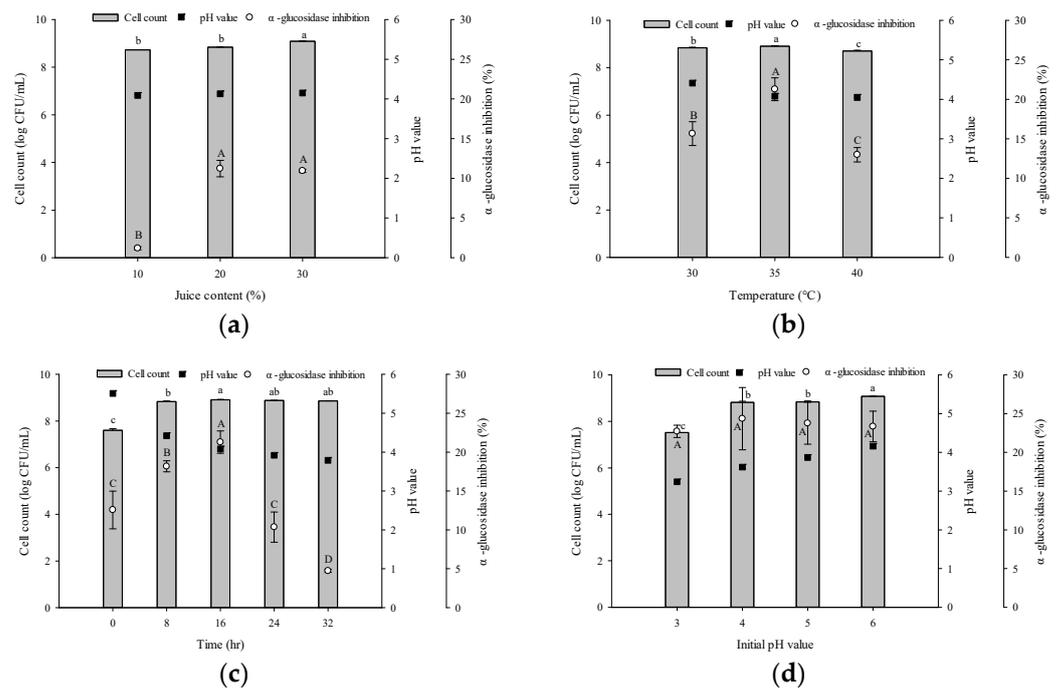
Figure 5. Optimal Gac fermentation condition of *Lactiplantibacillus plantarum* GBI 001: (**a**) Substrate content; (**b**) temperature; (**c**) time; (**d**) initial pH. Results are presented as means $\pm$ SD of triplicate independent experiments (n = 3). Different uppercase alphabetical letters represent significant differences within the same concentration at $p < 0.05$. Different lowercase alphabetical letters represent significant differences in the same sample at different concentrations at $p < 0.05$.

Lactobacilli have been used in the fermentation process in the food industry for many years. Metabolic products such as organic acids, amino acids, and carbohydrates help add flavor and taste to fermented foods. For example, L-proline adds a sweet taste, and glutamic acid adds umami, and lactic acid adds an acidic taste. In addition to flavor- and taste-enhancing action, some exopolysaccharides are capable of inhibiting the growth of pathological bacteria, prolonging the shelf life of the food products [62,63]. In recent years, lactobacilli culture and their wide application in food processing are other ways they have enhanced the nutritional values of foods. Whether it is due to the bacterial strain itself as a probiotic or due to the fermented liquids as nutritional drinks, they have many health benefits. For example, *Lactiplantibacillus plantarum* MG4229 can be used for lowering blood glucose [64], *Lactobacillus paracasei* PS23 for inhibiting colon inflammation [65], *Lactobacillus acidophilus* for anti-obesity [66], *Lactococcus lactis* KX881782 for reducing blood pressure [67], and *Bifidobacterium longum* MC-42 for reducing blood pressure [68]. Latic acid and fermented juice have almost no side effects different from those of hypoglycemic drugs used in clinical settings such as acarbose, miglitol, and voglibose (Dirir, Daou, Yousef, and Yousef, 2021). Numerous animal studies have demonstrated that vegetables and fruits fermented by *Lactobacillus plantarum* exhibit an excellent function to improve type II diabetes. Utilizing *Lactobacillus plantarum* FNCC 0027 to ferment Jamaica cherries (*Muntinga calabura Linn.*), Frediansyah et al. [69] found that there was an increase in total phenolic products, antioxidant activity, and diabetic-improving related enzymatic function. Li et al. [70] found that *Lactobacillus plantarum* NCU116-fermented carrot juices in type II diabetic rats stimulated the low-density lipoprotein (LDL) receptor, cholesterol 7-α-hydroxylase (CYP7A1), and glucose transporter-4 (GLUT-4), and inhibited peroxisome-proliferator-activated receptor-γ (PPAR-γ) inflammatory responses. Pathological slide examinations showed there were morphological improvements in the pancreas and kidneys. Moreover, Gao et al. [71] found that *Lactobacillus plantarum* NCU116-mediated fermentation altered polysaccharide structures in bitter melons to improve intestinal floras that favored the production of short-chain

fatty acids. As a result, the antidiabetic action of bitter melons was enhanced. Taking the above-discussed results together, it is clear that the application of lactobacilli to facilitate the fermentation process using various vegetables or fruits as substrates can provide many benefits: besides producing many biological active products, the fermented liquids exert stronger activity against diabetes.

3.3.2. Fermentation Temperature, Time, and Initial pH

The optimal temperature for carrying out the fermentation of Gac pulp juices was compared at 30, 35, and 40 °C, as shown in Figure 5b. After 16 h of fermentation reaction, the cell counts were similar around 9 log CFU/mL (8.71~8.91 Log CFU). The pH values of the media decreased from 4.4 to around 4.0. The inhibitory action on α-glucosidase activity increased from 13% at 30 °C to 21.25% at 35 °C and started to decline as the temperature further increased to 40 °C. It is clear that 35 °C is the optimal temperature for carrying out fermentation reaction and it was, therefore, chosen for the rest of the experiments for evaluating other factors influencing the fermentation reaction.

The time course of the fermentation reaction was monitored as illustrated in Figure 5c. Cells increased in numbers in the first 8 h from 7.6 to 8.9 log CFU/mL and then remained nearly constant for the 24 h. The pH of the medium decreased from 5.51 to around 4, and the greatest decline occurred in the first 8 h. At 16 h, the inhibitory action on α-glucosidase activity reached the maximum level of 21.28% compared to 12.56% at 0 h, representing a 1.7-fold increase.

To improve the production of anti-α-glucosidase activity by the fermentation of Gac pulp juice, the initial pH of the culture medium varied from 3 to 6. As illustrated in Figure 5d, with the exception of pH 3, the initial pH varying from 4 to 6 did not affect the cell proliferation, as the cell counts remained constant around 9 log CFU/mL. The final pH values of the culture media increased from 3.24 for an initial pH of 3 to 3.62 for an initial pH of 4, to 3.87 for an initial of 5 and finally to 4.16 for an initial pH 6. As for the inhibitory action on α-glucosidase activity, the initial pH exerted moderated effects: changing the initial pH from 3 to 6 produced inhibitory action varying from 22.72% to 24.36%, peaking at pH 4 (24.36%). Compared to the previous study, without adjusting the initial pH that caused an inhibitory action of 21.28%, the adjusted initial pH of 4 (24.36%) enhanced it 1.15-fold. Thus, the initial pH of the culture medium was set to 4.0 for further study.

Many substrates (mainly vegetables and fruits) and various lactobacilli have been utilized to process fermented fluids for antidiabetic purposes. Using apple juices fermented by *Lactobacillus fermentum* 21828, Wang et al. [72] found that, after fermentation, the inhibitory action on α-glucosidase was 93.7% compared to 40% of unfermented juice. Thus, the fermentation of apple juice increased anti-α-glucosidase activity by 144.5%. Frediansyah, Romadhoni, Nurhayati, and Wibowo [69] using Jamaica cherries (*Muntinga calabura Linn*) fermented by *Lactobacillus plantarum* FNCC 0027 and found that the ability of DPPH to reduce free radicals was 77.81%, the ability of ABTS to remove free radicals was 69.25%, and its inhibitory actions on digestive enzymes including α-glucosidase and α-amylase, and its amyloglucosidase activity, were greatly enhanced. Part of these effects might be due to an increase in the contents of gallic acid, dihydrokaempferol, and 5,7-dihydroxyflavon. Using soybeans as the substrates for *Lactobacillus plantarum* TWK10 and following 48 h of fermentation at 37 °C, Liu et al. [73] discovered that this lactobacillus possessed α-glucosidase, which was able to convert glucoside isoflavones to aglycone isoflavones. The fermented soybean products might help improve the recognition of diabetic mice. Other fermented products such as blueberry juices [74], mango juices [45], and chickpea [75] have been shown to have excellent effects to lower the glycemic index.

In addition to different kinds of substrates for fermentation, the activity of lactobacillus and its number are expected to exert an influence on the rate of progression of fermentation and its metabolic products. Other important factors include but are not limited to: the temperature, time, and pH. For example, the culture temperature and pH could alter the contents of five main fatty acids of cell membrane of lactobacill: C14:0, C16:0, C18:0, C18:1,

and cycC19:0, leading to different activities. Depending on the fatty acid chain length and its degree of saturation, the cell membrane behaves differently when the temperature of the medium changes. For example, the shorter fatty acids (C14:0 and C15:0) and unsaturated fatty acids such as C18:2 have higher anti-freezing characteristics, and their membrane compositions are less likely to be altered when the temperature is changed. The composition of C18:0 in the cell membrane at 30 °C is 7% and increases to 19% when the temperature is raised [76]. Previous studies have indicated that the optimal conditions vary depending on the strain of lactobacilli: for *Lactobacillus acidophilus* RD7585, the best conditions are 37 °C and pH 6 [77]; for *L. plantarum* PMO 08: 15~35 °C and pH 3.5~8.0 [78]. Consistent with these previous results, our selected *L. plantarum* GBI 001 showed the optimal conditions of 35 °C and pH 3~6.

4. Conclusions

We successfully isolated the α-glucosidase-inhibitory activity of probiotic *L. plantarum* GBI 001 from Gac pulp and established the formation condition of Gac juice. In conclusion, Gac-based fermented juice is a potential natural carrier to cultivate *L. plantarum* GBI 001 for the development of a functional hypoglycemia beverage.

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