Deep Ocean Water Research, 25(1), 9–18, 2024

Original

The α-glucosidase inhibitory activity of soy milk fermented by coastal seaweed-derived lactic acid bacteria with various plants and seaweed powder

Chiaki Imada[1, 2], Jun Wakasugi[1], Enjuro Harunari[3], and Yasuhiro Igarashi[3]

Abstract

Using 12 lactic acid bacterial strains isolated from deep-sea water (DSW) from Izu Akazawa, coastal marine sediment and seaweed (SSW), and fermented foods (FF), fermented soy milk products were prepared with the addition of powders from plants, mushrooms, and seaweed, each reported to have α-glucosidase inhibitory activity (α-GI) or postprandial hyperglycemia improvement and preventive effects on diabetes and obesity. The α-GI of these products was measured. Results showed that guava leaf powder addition increased the α-GI activity of 2 strains from SSW (H-6, 116) and 4 strains (R8, R4, R24, R43) from FF compared to the control without additives. Similarly, persimmon leaf matcha powder addition increased the α-GI activity of strain 1–14 from DSW, strain 116 from SSW, and 3 strains (R4, R24, R43) from FF. *Cordyceps sinensis* powder addition increased the α-GI activity of strain 109 from SSW and 3 strains (R24, R8, R43) from FF. Furthermore, adding Suginori *(Chondracanthus tenellus*) powder increased the α-GI activity of strain KM-2 and strain 116 from SSW. Notably, strain KM-2 exhibited no α-GI activity without Suginori addition, but significant activity was observed with supplementation. High-performance liquid chromatography (HPLC) analysis revealed that daidzin and genistin (glycosidic isoflavones) were present in the control without Suginori, while daidzein and genistein (aglycone isoflavones) were absent. Conversely, Suginori-added fermented product lacked daidzin and genistin but contained daidzein and genistein. HPLC analysis of Suginori-added product showed peaks of daidzein and genistein, both exhibiting α-GI activity.

Key Words: α-glucosidase inhibitor, deep-sea water, lactic acid bacteria, *Lactobacillus*

Introduction

It is well-known that certain plants (Matsuura *et al.*, 2004), mushrooms (Zhang *et al.*, 2023), and microbial cultures (Kim *et al.*, 2002) contain α-glucosidase inhibitors (α-GI), which suppress the activity of α-glucosidase. These inhibitors are known to mitigate the absorption of ingested sugars, thereby inhibiting the postprandial rise in blood. Improving postprandial hyperglycemia through α-GI has gained attention as a strategy for preventing diabetes and obesity (Kuboyama *et al.,* 2006).

Lactic acid bacteria have long been utilized in the fermentation of food, and some strains have been reported to exhibit α-GI activity (Panwar *et al.*, 2014). However, all these lactic acid bacteria are terrestrial origin, and there are no reports on α-GI activity in marine-derived lactic acid bacteria. Given the unique environmental conditions of the ocean, it is anticipated that marine-derived lactic acid bacteria may possess unique characteristics. Marine environments differ from terrestrial ones in terms of low temperature, low nutrients, high pressure, and high salt concentration, resulting in microorganisms with distinct

[1] Tokyo University of Marine Science and Technology (4–5–7 Konan, Minato-ku, Tokyo 108–8477)
[2] Institute of Ocean Energy Saga University (1–Honjomachi, Sagashi, Saga 840–8502)
[3] Toyama Prefectural University (5180 Kurokawa, Imizu, Toyama 939–0398)

characteristics, including salt tolerance (Jansen *et al.*, 2005). Frantzmann *et al.* (1991) first isolated lactic acid bacteria from the marine environment. Subsequently, Ishikawa *et al.* (2005) proposed a new species of lactic acid bacteria, "*Marinilactibacillus psychrotolerans*," with properties such as salt and alkali resistance distinct from terrestrial lactic acid bacteria. Since then, research institutions have been exploring marine lactic acid bacteria, gradually revealing their ecological and taxonomic characteristics.

This study aims to isolate lactic acid bacteria having α-GI activity from deep-sea water obtained from the Izu-Akazawa deep-sea water collection facility (referred to as DSW), coastal marine sediments and seaweed (referred to as SSW).

Additionally, for comparison, lactic acid bacterial isolates from fermented foods (referred to as FF) were used. The α-GI activity of these isolates was measured by preparing fermented soy milk with added powders from plants, mushrooms, and seaweeds. These powders have been selected for their reported α-GI activity or confirmed ability to inhibit the elevation of blood glucose, contributing to the prevention of obesity and diabetes.

In this study, various strains of lactic acid bacteria were incorporated into commercially available soy milk along with various plant, mushroom and seaweed powders to prepare fermented soy milk. The reason for using soy milk as a fermentation medium is that soy milk is readily available, cost-effective, and ensures safety as a food product. This choice is made with the consideration that soy milk provides a convenient and affordable option if the resulting fermented product is easily applicable to health foods. The α-glucosidase inhibitory (α-GI) activity of the fermented product was investigated.

2. Materials and methods

2.1. Isolation of lactic acid bacteria

Twelve strains of lactic acid bacteria were provided for this study, comprising three strains isolated from deep-sea water (DSW) using a bag-type filter (Nagoya Filter Sales Co., Ltd. TR1045 model) employed for removing suspended solids from DSW obtained from DHC, a company located in Izu-Akazawa, Ito City, Shizuoka Prefecture. Additionally, 5 strains were isolated from estuarine marine sediment and seaweed in various regions, including Kumejima Town, Okinawa Prefecture. Another 4 strains were isolated from commercially available pickled foods such as kimchi and cucumbers, collectively referred to as fermented food (FF) strains.

The isolation procedures for these lactic acid bacteria followed those outlined in a previous report (Imada *et al.*, 2024).

2.2. Identification of isolates by 16S rDNA sequencing

After culturing the 12 isolates on Lactobacilli MRS Agar (manufactured by Difco Laboratories) for three days, the cultures were centrifuged (20,000 × g, 4℃, 20 min), and bacterial DNA was extracted and purified from the collected bacterial cells using the Soil DNA Isolation Kit (Funakoshi) following standard procedures (Fujimoto and Fukui, 2005). The obtained DNA was subjected to sequence PCR, and 16S rRNA gene analysis was performed. The genetic analysis was outsourced to Technosuruga Labo Co., Ltd.

2.3. Plant, mushroom, and seaweed powders used for the fermentation of soy milk

The plant powders included Guava leaves (Yamauchi *et al.,* 2008, Kawamura Farm Co., Ltd.), *Gymnema sylvestre* (Matsuura *et al.*, 2004, Okinawa Chosei Yakuso Honsha Co., Ltd.), Persimmon leaf matcha (Masuda *et al.,* 2003, product name: Kakinko, Xenoa Cosmetics Honpo Co., Ltd.), Noni leaves (Koyama, 2003, Olive Garden Co., Ltd.), and Long life grass (Maeda *et al.,* 2016, Okinawa Chosei Yakuso Honsha Co., Ltd.). Additionally, mushroom powders included *Cordyceps sinensis* (Zhang *et al.,* 2020, Okisu Co., Ltd.), Maitake (Matsuura *et al.,* 2002, Keyfoods Namatame Co., Ltd.), and Shiitake (Zhang *et*

al., 2023, Matsuoka Shiitake Production and Sales Co., Ltd.). Furthermore, seaweed powders included Mekabu, Suginori, Hijiki, and Kelp (Nishizawa, 1993, all from Kaneryo Seaweed Co., Ltd.). A total of 12 powders were obtained. The α-GI activity of these powders was measured using the method described in 2.4. and four powders aligning with the objectives of this study were selected.

2.4. Preparation of soy milk fermentation broth enriched with plant, mushroom, and seaweed powder

In this study, four selected plant, mushroom, and seaweed powders were individually added at a concentration of 1.0% (0.20 g) to 20 mL of soy milk. The mixture was then filled into 50 mL volume Falcon tubes and subjected to autoclaving (121℃, 15 min) for sterilization. After cooling, each selected strain was inoculated, and the tubes were statically cultured at 37℃ for 3 days. The α-GI activity of the resulting soy milk fermentation product was measured according to the procedures outlined in a previous publication (Imada *et al.*, 2024). After adding various plant, mushroom, and seaweed powders to soy milk, a "synthetic soy milk fermentation broth" was prepared by adding lactic acid (manufactured by Fuji Film Wako Pure Chemical Corporation, 4987481283909) to soy milk (non-inoculated with microbes) following sterilization. The pH was adjusted to 4.1 ± 0.1. Subsequently, the α-GI activity of this synthetic broth was measured, and the α-GI activity inherent to each powder was calculated. Measurement was performed at $n = 3$, and the average value was used as the data.

2.5. Preparation of samples for α-GI activity measurement

A total of 500 μL of the obtained soy milk fermentation product (including synthetic soy milk fermentation broth) was aliquoted and transferred to a 1.5 mL microtube. Using a heat block (Taiyo Kagaku Kogyo Co., Ltd.), the sample was thoroughly dried (50℃, 12–16 h) to remove all moisture. Subsequently, 500 μL of 99.5% methanol (Fujifilm 131–01826, high purity) was added to the resulting dry matter. The mixture was vigorously stirred using a test tube mixer, followed by a 10-min settling period. The supernatant was then collected as a sample for α-glucosidase inhibitory activity measurement after centrifugation (20,630 × *g*, 10 min). The α-GI activity of each sample was determined following the procedures outlined in a previous report (Imada *et al.*, 2024).

2.6. High performance liquid chromatography (HPLC) analysis of soy milk fermentation product

HPLC analysis was conducted on various soy milk fermentation products, specifically on strain KM-2 fermentation products derived from SSW with and without the addition of Suginori powder (10 μL). The column used for analysis was COSMOSIL 3C18AR-II (4.6 × 100 mm). The solvent system consisted of 0.1% formic acid/acetonitrile, with acetonitrile concentrations progressing as follows: 0–3 min (15%), 25 min (85%), 29 min (85%), and 32 min (15%). The flow rate was set at 1.2 mL/min, and analysis of soy milk fermentation products (100 μL) was carried out under these elution conditions with UV detection at a wavelength of 254 nm.

Glycoside-type isoflavones, namely daidzin (Fujifilm 040–27741), genistin (Fujifilm 302–05151), and aglycone-type isoflavones daidzein (Fujifilm 045–31081) and genistein (Wako 073–05531), were obtained and utilized as standard substances for HPLC analysis.

2.7. Fractionation of soy milk fermentation products, HPLC analysis, and α-GI activity

After concentrating and drying 10 mL of soy milk fermentation broth using an evaporator, the resulting material was dissolved in 100 μL of water. Subsequently, preparative HPLC (the column used for analysis was COSMOSIL 3C18AR-II (10 × 250 mm). The stepwise elution was performed. The solvent system consisted of 0.1% formic acid/acetonitrile, with acetonitrile concen-

trations progressing as follows: 0–3 min (15%), 25 min (85%), 29 min (85%), and 32 min (15%). The flow rate was set at 3.0 mL/min, and analysis of soy milk fermentation products (100 μL) was carried out under these elution conditions with UV detection at a wavelength of 254 nm, leading to the isolation of 11 fractions. The solvent used for HPLC elution (0.1% formic acid/acetonitrile) was removed from each fraction through freeze-drying.

Following the freeze-drying process, these samples were reconstituted in a phosphate buffer, and the α-GI activity of each fraction was measured.

2.8. Comparison of carbohydrate fermentation and growth temperature between strain KM-2 and its reference strain

Carbohydrate fermentation tests were conducted for strain KM-2 and its reference strain (*Lactobacillus delbrueckii* subsp. *delbrueckii* NBRC3202^{T}) using the bacterial identification test kit (API 50 CHL, BioMerieux, Marcy I'Etoile). Each strain was pre-cultured in MRS broth (37℃, 24 h), then streaked onto MRS agar for the main culture (37℃, 24 h). After cultivation, the obtained bacterial cells were suspended in sterile physiological saline, and the suspension was inoculated into the bacterial identification test kit. Subsequently, static cultivation was performed (37℃, 48 h), and the presence or absence of carbohydrate fermentation was determined based on visual observations.

Additionally, each strain was inoculated into MRS broth, followed by 15 days of static cultivation at temperatures of 4, 15, 20, 27, 37, 40, 50, and 55℃. The presence or absence of growth was visually assessed after the incubation period.

3. Results

The identification results of the 12 strains of lactic acid bacteria used in this study is presented in Table 1. As evident from the table, all strains derived from DSW were identified as *Lactiplantibacillus plantarum*. On the other hand, strains from SSW were identified as various species, including *Pediococcus pentosaceus*, *Lactobacillus delbrueckii* subsp. *delbrueckii*, and *Leuconostoc mesenteroides*, in addition to *Lactiplantibacillus plantarum*. Furthermore, strains from FF were identified as three different species: *Lactobacillus sakei*, *Lactobacillus brevis*, and *Lactiplantibacillus plantarum*.

Prior to creating fermented soy milk products, the presence of α-GI in various plant, mushroom, and seaweed powders used in this study was examined. These powders have been selected for their reported α-GI activity or confirmed ability to inhibit the elevation of blood glucose, contributing to the prevention of obesity and diabetes.

Figure 1 illustrates the α-GI activity of artificially fer-

Table 1. Identification of lactic acid bacteria isolated from DSW, SSW and FF.

Isolation source	Strain code	Similar strain	Similarity (%)	Isolation	place	Sample
DSW	1–11	*Lactiplantibacillus plantarum* JCM1149	100	Shizuoka	Izu-Akazawa	Bag filter
DSW	1–14	*Lactiplantibacillus plantarum* JCM1149	99.8	Shizuoka	Izu-Akazawa	Bag filter
DSW	2–12	*Lactiplantibacillus plantarum* JCM1149	100	Shizuoka	Izu-Akazawa	Bag filter
SSW	SSW116	*Pedincoccus pentosaceus* DSM20336		Chiba	Minamiboso	Sediment
SSW	SSW109	*Lactiplantibacillus plantarum* JCM1149	99.8	Okinawa	Kumejima	Seaweed
SSW	KM-2	*Lactobacillus delbrueckii* subsp. *delbrueckii* NBRC 3202	100	Okinawa	Kumejima	Seaweed
SSW	H-6	*Lactiplantibacillus plantarum* JCM1149	99.9	Iwate	Otsuchi town	Seaweed
SSW	OKI-9–2	*Leuconostoc mesenteroides*	92.7	Chiba	Tateyama city	Seaweed
FF	R4	*Lactobacillus sakei* DS4	99.8	Chiba	Commercial product	Kimuchi
FF	R8	*Lactobacillus sakei* DS4	99.8	Chiba	Commercial product	Kimuchi
FF	R24	*Lactobacillus brevis*	100	Chiba	Commercial product	Kimuchi
FF	R43	*Lactiplantibacillus plantarum* JCM1149	99.4	Chiba	Commercial product	Pickles

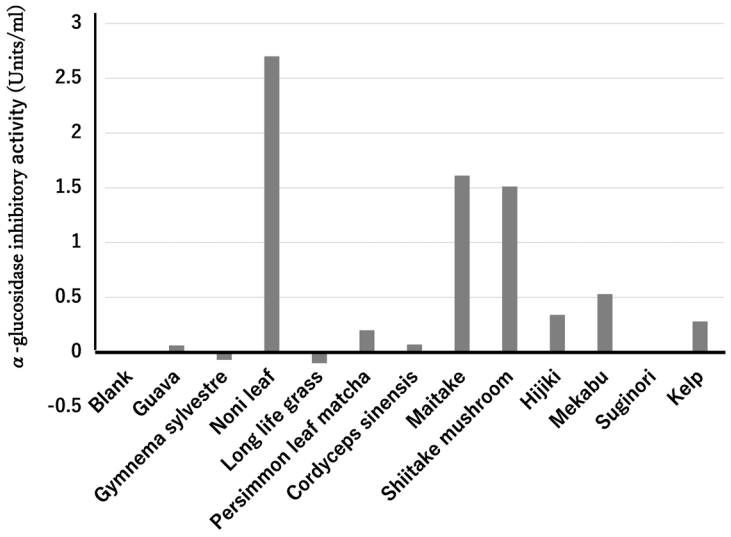

Fig. 1. α-glucosidase inhibitory activity of various plant, mushroom and seaweed powders.

mented soy milk with 0.1% addition of various powders. It is evident from this figure that Shiitake, Maitake, and Noni leaf powders exhibit strong α-GI activity on their own. Additionally, powders such as Hijiki, Mekabu, and Kelp also showed α-GI activity, making it challenging to measure the α-GI activity of lactic acid bacteria-fermented soy milk (products) with these powders. Therefore, in this study, we chose powders (Guava leaf, Persimmon leaf tea, *Cordyceps sinensis*, and Suginori) that either showed no α-GI activity or minimal α-GI activity on their own for preparing lactic acid bacteria-fermented soy milk products.

Figures 2a and 2b depict the α-GI activity of 1 strain of soy milk fermented with 0.1% addition of various powders. From these figures, it is apparent that strains 1–11, 1–14, and 2–12 from DSW, strains 109 and H-6 from SSW, and strain R43 from FF were all identified as *L. plantarum*. However, except for strain 109, the α-GI activity of soy milk fermented with the addition of each powder was generally similar. In contrast, strains R4 and R8 from FF, both identified as *Lactobacillus sakei*, showed significantly different α-GI activity values for each powder.

Examining the results of soy milk fermented with the addition of each powder, it was observed that the addition of Guava leaf powder increased the α-GI activity of strains 116 from SS and R8, R4, R24, and R43 from FF, with the increase being particularly notable for R4 and R24. The addition of Persimmon leaf tea powder increased the α-GI activity of strains 1–14 from DSW, 116 from SSW, and R4, R24, and R43 from FF, with a significant increase observed for R4 and R24. Furthermore, the addition of *Cordyceps sinensis* powder increased the α-GI activity of strains 109 from SSW and R24 and R8 from FF. Additionally, the addition of Suginori powder increased the α-GI activity of strains KM-2 and 116 from SSW, with KM-2 showing significant α-GI activity only upon Suginori addition. Subsequently, this study focused on strain KM-2 for further research because the combination of strain KM-2 and Suginori exhibited the prominent appearance of α-GI activity.

Table 2 presents the physiological characteristics of this strain and its reference strains. As evident from the table, the strain exhibited α-GI activity in Suginori-added soy milk fermentation, whereas the reference strains did not show α-GI activity. Moreover, the strain was found to have negative saccharification activity for amygdalin, arbutin, and salicin, and it grew at a low temperature of 15℃. In contrast, the reference strain showed positive saccharification activity for these carbohydrates and did not grow at 15℃. Thus, even within the same species, some differences in physiological characteristics were observed depending on the isolation source.

Figure 3 presents the results of high-performance liq-

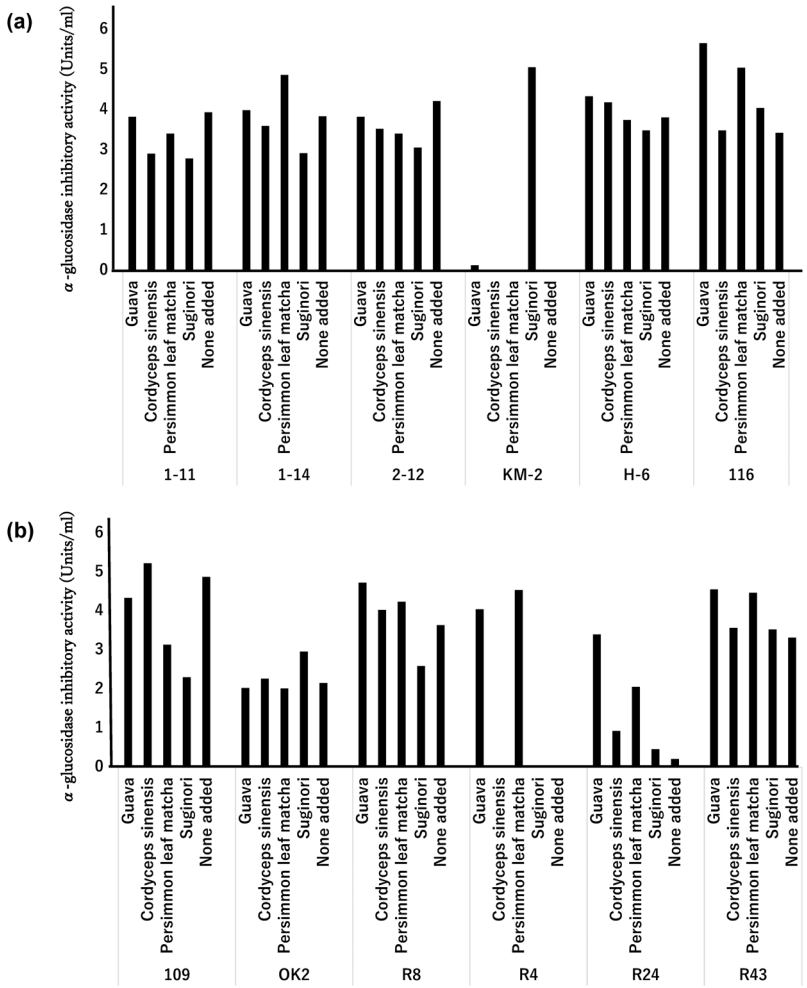

Fig. 2. (a) α-glucosidase inhibitory activity of fermented soymilk of lactic acid bacteria containing various plant and seaweed powders. (b) α-glucosidase inhibitory activity of fermented soymilk of lactic acid bacteria containing various plant and seaweed powders.

Table 2. Physiological characteristics of strain KM-2 and its standard strain.

Characters	Strain KM-2	Strain NBRC3202^{T}
a-GI activity	positive	negative
Utilization of amidagrine	negative	positive
Arbutin	negative	positive
Salicin	negative	positive
Growth at 15℃	positive	negative

uid chromatography (HPLC) analysis of Suginori-added and non-added soy milk fermentation products of strain KM-2. As evident from this figure, isoflavones in non-Suginori-added fermentation products were detected as glycoside forms of daidzin and genistin, while aglycone forms of daidzein and genistein were not detected. In contrast, Suginori-added fermentation products showed no detection of daidzin or genistin, indicating complete decomposition into daidzein and genistein.

Next, concentrated and dried samples of Suginori-added soy milk fermentation were dissolved in water (100 μL), analyzed by HPLC, and 11 fractions were collected, as shown in Fig. 4. The solvent used for HPLC elution (0.1% formic acid/acetonitrile) was removed from each fraction after freezing and drying (Fig. 4). After melting these samples in phosphate buffer, α-GI activity was measured using existing methods. As a result, strong α-GI activity was particularly observed in fractions ⑦ and ⑨, where daidzein and genistein were eluted. Therefore, it was inferred that daidzein and genistein are the main components of α-GI activity in Suginori-added KM-2 soy milk fermentation fluid. Although low α-GI activity was observed in other fractions, it was considerably lower than in fractions ⑦ and ⑨, suggesting

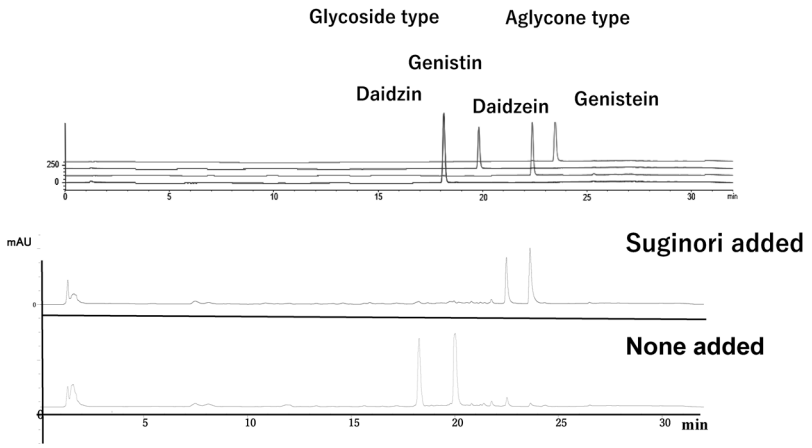

Fig. 3. HPLC analysis of fermented soy milk product of strain KM-2 with or without Suginori powder.

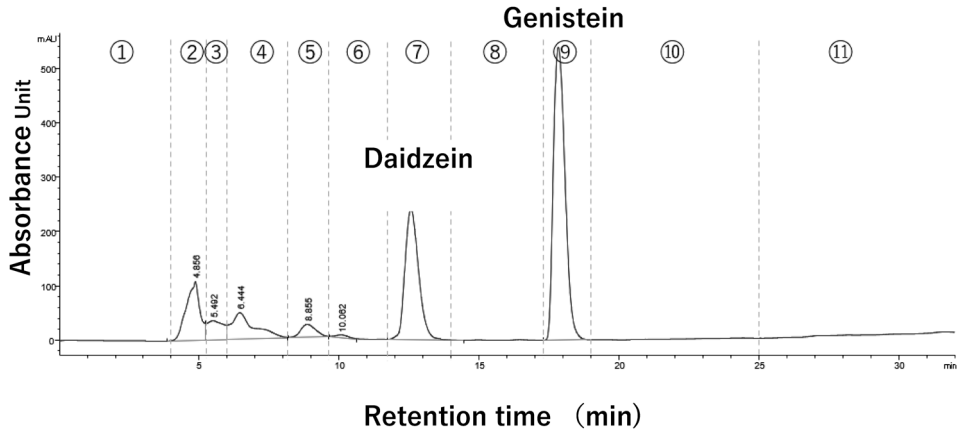

Fig. 4. Preparative HPLC elution pattern of fermented soy milk product of strain KM-2 with added Suginori powder.

that the solvent used in HPLC analysis may have affected α-glucosidase activity.

The sequence of 16S rDNA of strain KM-2 has been registered in the DDBJ database with the accession number LC685966.

4. Discussion

Lactic acid bacteria have been crucial microorganisms used in various fermented foods such as yogurt, miso, soy sauce, and pickles for a long time. While their traditional applications are well-established, recent exploration has focused on new uses for lactic acid bacteria, including fermentation of soy milk (Ito *et al.*, 2006). Fermented milk products, like yogurt, produced by fermenting milk with lactic acid bacteria, have been known for their diverse physiological functions, such as improving intestinal regulation, infection prevention, immune activation, anti-tumor effects, and hypertension inhibition, even when the bacteria are inactivated (Mitsuoka 2011). On the other hand, there is limited knowledge about fermented soy milk products, often referred to as soy milk yogurt or fermented soy milk, which are gaining attention not only as functional foods but also as raw materials for cosmetics (Chen *et al.,* 2013).

In this study, we explored the potential of soy milk by isolating lactic acid bacteria from less-studied sources such as DSW and SSW, and for comparison, from FF. The study aimed to investigate the functional properties of the fermentation products obtained by adding various plant, mushroom, and seaweed powders known for their potential health benefits to commercially available soy milk. We focused on α-GI activity as a functional component of the fermentation products and aimed to enhance

their functional properties by adding different powders. The results revealed strains with increased α-GI activity upon powder addition, as well as strains that showed no α-GI activity without powder but exhibited activity upon powder addition. Additionally, variations in α-GI activity were observed among strains of the same species, indicating strain-specific rather than species-specific α-GI production by lactic acid bacteria.

Guava leaf powder is reported to contain polyphenols that inhibit carbohydrate-degrading enzymes such as α -amylase and α-glucosidase (Yamauchi *et al.,* 2008). Persimmon leaf tea powder has been reported to exhibit α-GI activity (Masuda *et al.,* 2013), suggesting potential for controlling sugar absorption with regular consumption. Certain polysaccharides found in *Cordyceps sinensis* powder are reported to have α-GI activity (Lingran *et al.,* 2020). However, in this study, fermented soy milk products with added guava leaf, persimmon leaf tea, and *Cordyceps sinensis* powders did not show α-GI activity at a 0.1% concentration (Fig. 1). This could be attributed to changes in α-GI activity components during powder processing or variations in α-glucosidase types.

The addition of Guava leaf powder increased α-GI activity in fermentation products of strains H-6, 116 from SSW, and R8, R4, R24, and R43 from FF, particularly notable for R4 and R24. *Cordyceps sinensis* powder addition increased α-GI activity in strains 109, R24, and R8. Additionally, the seaweed powder, specifically Suginori, induced new α-GI activity in strain KM-2. This suggests that strain KM-2 produces β-glucosidase upon the addition of Suginori powder, leading to the cleavage of glycosidic bonds in glycoside-type isoflavones (daidzin and genistin) present in soy milk, resulting in the production of aglycone-type isoflavones, daidzein, and genistein.

Lactobacillus delbrueckii is an important lactic acid bacterium involved in the production of various fermented foods in both animals and plants. Four subspecies of *L. delbrueckii* have been reported, each with specific roles in different fermented products (Kudo *et al.,* 2012). *L. delbrueckii* subsp. *bulgaricus* is mainly isolated from yogurt (Michaylova *et al.*, 2007), *L. delbrueckii* subsp. *delbrueckii* is found in fermented plants (Germond *et al.*, 2003), *L. delbrueckii* subsp. *indicus* is isolated from fermented milk in India (Dellaglio *et al.,* 2005), and *L. delbrueckii* subsp. *lactis* is isolated from cheese (Weiss *et al.*, 1983).

Although strain KM-2 and its reference strains belong to the same species, differences were observed in several characteristics, such as the absence of α-GI activity in Suginori-added soy milk fermentation products, positive saccharification activity for carbohydrates, and the absence of growth at a low temperature in the reference strain. Strain KM-2, originally inhabiting terrestrial environments, might have adapted to marine environments over time, acquiring characteristics suitable for the ocean, such as growth at low temperatures. *Lactiplantibacillus plantarum*.is well-known terrestrial lactic acid bacterium. Yamada *et al.* (2022) reported that 4 lactic acid bacterial isolates from DSW at Izu-Akazawa and were identified as *L. plantarum*. However, there was a slight difference in physiological and biochemical characteristics between the isolates from DSW and the type strain of *L. plantarum*. Furthermore, there was also a little difference in enzyme production between the isolates and the type strain. This might suggest that some physiological changes have occurred in deep-sea environment.

Such changes in microbial products due to changes in the growth environment are intriguing and have been reported in other microorganisms, such as *Streptomyces aureofaciens*, which gained salt tolerance after repeated cultivation in seawater medium (Sato *et al.,* 1978).

Given that daidzein and genistein have been reported to have α-GI activity (Lee and Lee 2001, Park *et al.,* 2013), it is plausible that strain KM-2 did not show inhibitory activity without Suginori powder but exhibited α-GI activity upon its addition. Future studies on strain KM-2 should focus on investigating the productivity of β-glucosidase and isolating and purifying this enzyme. Additionally, identifying the active components in strains that showed increased α-GI activity with various powder ad-

ditions would be an interesting avenue for further research.

Acknowledgments

We would like to express our deep gratitude to Dr. Katsuhisa Yamada of DHC Corporation for providing the DSW bag filters for this research.

This research was funded by the Japan Society for Scientific Research Grants-in-Aid for Scientific Research (Grant-in-Aid for Scientific Research) Basic Research (C) (19K05786).

References

Chen, Y-M., T.-W. Shih. P. Chiu, T.-M. Pan and T.-Y. Tsai (2013) Effects of lactic acid bacteria-fermented soy milk on melanogenesis in B16F0 melanocytes. J. of Functional Foods 5, Issue 1, 395–405.

Dellaglio, F., Felis, G. E., Castioni, A., Torriani, S. and Germond, J. E. (2005) *Lactobacillus delbrueckii* subsp. *indicus* subsp. nov. isolated from Indian dairy products. Int. J. Syst. Evol. Microbiol., 55, 401–404.

Fujimoto, J. and M. Fukui (2005) 16S rDNA-clone library method. J. Intest. Microbiol., 19, 47–52 (in Japanese).

Franzmann, P. D., P. Hoepfl, N. Weiss and B. J. Tindall (1991) Psychrotrophic, lactic acid-producing bacteria from anoxic waters in Ace Lake, Antarctica; *Carnobacterium funditum* sp. nov. and *Carnobacterium alterfunditum* sp. nov. Arch. Microbiol., 155, 255–262.

Germond, J. E., Lapierre, L., Delley, M., Mollet, B., Felis, G. E. and Dellaglio, F. (2003) Evolution of the bacterial species *Lactobacillus delbrueckii* a partial genomic study with reflections on prokaryotic species concept. Mol. Biol. Evol., 20, 93–104.

Imada, C., J. Wakasugi, Y. Shibata and K. Yamada (2024) General properties of lactic acid bacteria from deep seawater and α-glucosidase inhibitory activity of fermented soymilk. Deep Ocean Water Res., 24, 33–41.

Ishikawa, M., K. Nakajima, Y. Itamiya, S. Furukawa, Y. Yamamoto and K. Yamasato (2005) *Halolactibacillus halophilus* gen. nov., and *Halolactibacillus miurensis* sp. nov., halophilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. Int. J. Syst. Evol. Microbiol., 55, 2427–2439.

Ito, M., M. Kojima, M. Yano and S. Naito (2006) Making novel fermented food from soy milk. Report from Aichi Prefecture Industrial Technology Research, 5, 144–145.

Jansen, P. R., T. J. Mincer P. G. Williams and W. Fenical (2005) Marine actinomycete diversity and natural product discovery. Antonie van Leeuwenhoek, 87, 43–48.

Kim, J. G., H. B. Chang, Y. I. Kwon, S. K. Moon, H. S. Chun, S. K. Ahn and C. I. Hong (2002) Novel α-glucosidase inhibitors, CKD-711 and CKD-711a produced by *Streptomyces* sp. ck-4416. I. Taxonomy, fermentation and isolation. J. Antibiot., 55, 457–461.

Koyama, J. (2003) Effect of *Morinda citrifolia* leaf extract on blood glucose levels in type II diabetes (2003) J. of the Jap. Soc. for Ningen Dock 9 42–44 (in Japanese).

Kuboyama, N., I. Hayashi and T. Yamaguchi (2006) Pharmacokinetic and pharmacological characteristics of miglitol (Sable tablets), a novel α-glucosidase inhibitor, and clinical trial results. Foli Pharmacol. Jpn., 127, 223–232 (in Japanese).

Kudo, Y., K. Oki and K. Watanabe (2012) *Lactobacillus delbrueckii* subsp. *sunkii* subsp. nov., isolated from sunki, a traditional Japanese pickle. Int. J. Syst. Evol. Microbiol., 62, 2643–2649.

Lee D. S. and S. H. Lee (2001) Genistein, a soy isoflavone, is a potent α-glucosidase inhibitor. Federat. Eur. Biochem. Soc., 501, 84–86.

Lingran, W., H. Sun, Y. Hao, X. Zheng, Q. Song, S. Dai and Z. hu (2020) Chemical structure and inhibition on α-glucosidase of the polysaccharides from *Cordyceps militaris* with different developmental stages. Int. J. Biol. Macromol. 148, 722–736.

Maeda, K., M. Sakagami, S. Ono and T. Ito (2016) Investigation of Adiponectin-boosting effects in Okinawan ingredients (Botanboufuu, Spring turmeric). J. Jpn. Soc. Integr. Med., 9, 1–5 (in Japanese).

Masuda, A., A. Fujimoto, T. Inouchi, C. Takahashi, M. Inai, Y. Miura, S. Imai and T. Masuda (2013) The functions of healthful tea utilized by the residents in Shikoku area, Part I. on their activities of radical scavenging, α-glucosidase inhibition, and xanthine oxidase inhibition. J. Integr. Stud. Diet. Habits, 24, 11–20 (in Japanese).

Matsuura, H., C. Asakawa, M. Kurimoto and J. Mizutani (2002) α-Glucosidase inhibitor from the seeds of balsam pear *(Momordica charanti*a) and the fruit bodies of *Grifola frondose*. Biosci. Biotech. Biochem., 66, 1576–1578. https://DOI.org/10.1271/bbb.66.1576.

Matsuura, T., Y. Yoshikawa, H. Masui and M. Sano (2004)

Suppression of glucose absorption by health tea in rats. Yakugaku Zasshi, 124, 217–233 (in Japanese).

Michaylova, M., S. Minkova, K. Kimura, T. Sasaki and K. Isawa (2007) Isolation and characterization of *Lactobacillus delbrueckii* sp. *bulgaricus* and *Streptococcus thermophiles* from plants in Bulgaria. FEMS Microbial Lett., 269, 160–169.

Mitsuoka, T. (2011) History and evolution of probiotics. J. Jpn. Lactic Acid Bacteria Soc., 22, 26–37 (in Japanese).

Nishizawa, K (1993) Seaweed and Prevention of Lifestyle Diseases. pp. 95–100 (Kenseisha).

Panwar, H., D. Calderwood, I. R. Grant, S. Grover and B. D. Green (2014) *Lactobacillus* strains isolated from infant faeces possess potent inhibitory activity against intestinal alpha and beta-glucosidases suggesting anti-diabetic potential. Eur. J. Nutr., 53, 1465–1474.

Park, M.-H., J.-W. Ju, M.-J. Park and J.-S. Han (2013) Daidzein inhibits carbohydrate digestive enzymes in vitro and alleviates postprandial hyperglycemia in diabetic mice. Eur. J. Pharmacol., 712, 48–52.

Sato, K., T. Okazaki, K. Maeda and Y. Okami (1978) New antibiotics, aplasmomycins B and C. J. Antibiot., 31, 632–635.

Weiss, N., U. Schillinger and O. Kandler (1983) *Lactobacillus lactis*, *Lactobacillus leichmannii* and *Lactobacillus bulgaricus* subjective synonyms of *Lactobacillus delbrueckii*, and description of *Lactobacillus delbrueckii* subsp. *bulgaricus* comb. nov. Syst. Appl. Microbiol., 4, 552–557.

Yamada, Y., T. Yamamoto, Y. Shibata, M. Nomura and C. Imada (2022) Characteristics of *Lactiplantibacillus plantarum* collected from deep seawater at Izu-Akazawa in Shizuoka prefecture. Deep Ocean Res., 23, 65–76 (in Japanese).

Yamauchi, A., R. Inai and M. Higashimoto (2008) Suppressive effect of guava leaf tea on the increase in blood sugar levels by its inhibition of saccharolytic enzyme activity, gastrointestinal transport and glucose absorption. Jpn. J. Nutr. Diet., 66, 25–29. (in Japanese).

Zhang, X, X. Kong, Y. Hao, X. Zhang and Z. Zhu (2020) Chemical structure and inhibition on α-glucosidase of polysaccharide with alkaline-extracted from glycyrrhiza inflata residue. Int. J. Biol. Macromol., 15: 147: 1125–1135. DOI:10.1016/j.ijbiomac.2019.10.081. Epub 2019 Nov 15.

Zhang, Y., Y. Chen, X. Liu, W. Wang, J. Wang, X. Li and S. Sun (2023) Preparation and identification of peptides with α-glucosidase inhibitory activity from Shiitake mushroom (*Lentinus edode*s). Protein Foods 2023, 12, 2534. https://DOI.org/10.3390/foods12132534; https://www.mdpi.com/journal/foodsFoods.

（Received: January 15, 2024; Received: March 21, 2024）