

Analysis of the physiological activities of green garlic fermented with QPS-designated *Bacillus* strains and lactic acid bacteria isolated from Sacheon

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Abstract

Lactic acid bacteria (LAB) and qualified presumption of safety (QPS)-designated *Bacillus* strains are widely applied to enhance the nutritional and functional properties of natural products. Green garlic (*Allium sativum* L.), a common Korean vegetable and recognized functional food, is valued for its antioxidant and immune-enhancing activities. In this study, we investigated the functional properties of green garlic fermented with LAB and QPS-designated *Bacillus* strains. A total of 450 strains were isolated from marine environments, livestock sources, and diverse agricultural and fishery products, including fermented derivatives, of which 191 originated from agricultural products. Enzyme assays identified 89 strains with strong protease and amylase activities. After excluding taxonomic duplicates, we obtained seven QPS-designated *Bacillus* strains and four LAB strains characterized by robust growth in media containing green garlic. Fermentation using Sacheon green garlic powder was conducted for 4 days, and compared with the control, we found that the antioxidant activity of green garlic fermented with *Latilactobacillus curvatus* GH-11-11 and *Bacillus amyloliquefaciens* JY-48-5 increased by 144.0% and 145.8%, respectively. In addition, relative to a non-fermented extract, a 2-day fermentation with JY-48-5 enhanced α -glucosidase inhibitory activity by over 189.4%. These findings indicate that cultures of selected LAB and QPS-designated *Bacillus* strains could serve as promising starters for enhancing the bioactive properties of foods, and also emphasize the importance of regional microbial resources.

Keywords: Fermentation, Green garlic, QPS-*Bacillus*, *Latilactobacillus*, Functional evaluation

Introduction

Agricultural products and their fermented derivatives have long been integral to human diets and are increasingly recognized as a rich source of functional ingredients that promote human health (Voidarou et al., 2020). In particular, agricultural products are not only rich in various dietary fibers, vitamins, minerals and phytochemicals, but also produce secondary metabolites such as organic acids, enzymes and bioactive peptides during fermentation. Such compounds are

known to exhibit a broad spectrum of physiological activities including immune modulation, antioxidant and anti-inflammatory effects, as well as the prevention of metabolic disorders (Wallace et al., 2020; Fabbri et al., 2024). Nevertheless, systematic and integrative investigations into the functional properties of fermented agricultural products remain limited, and the potential of region-specific crops and traditional fermentation resources has yet to be fully explored. South Korea, with its distinct four seasons and diverse climatic and soil conditions, is particularly rich in agricultural

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resources. Harnessing these resources for the development of tailored functional foods and bioactive ingredients is regarded as a promising avenue with high potential to generate substantial added value both domestically and globally (Jung et al., 2022; Kim et al., 2024).

Green garlic (*Allium sativum* L.) is a monocotyledonous vegetable of the genus *Allium* (family Amaryllidaceae, order Asparagales). It is cultivated worldwide, with numerous region-specific cultivars reported (Sönmez et al., 2025). In South Korea, green garlic is primarily grown in the mild climates of southern coastal and inland regions and is particularly renowned as a local specialty in areas such as Sacheon (Gyeongnam) and Goheung (Jeonnam), due to its abundance of bioactive compounds (Yang et al., 2005, 2025). Morphologically, green garlic varies in size and form depending on harvest time but generally consists of slender, tender green leaf sheaths measuring 30–50 cm in length and 1–2 cm in diameter (Sönmez et al., 2025). Traditional Korean medical texts, including the Dongui Bogam, describe green garlic as effective in dispelling internal cold and strengthening the immune system. In modern studies, various physiological activities such as antioxidant, anti-inflammatory and antiviral effects have been scientifically validated (Bozin et al., 2008). Furthermore, green garlic is particularly rich in allicin, γ -glutamyl cysteine compounds, and vitamin C, which contribute to immune enhancement, regulation of blood cholesterol levels, and prevention of metabolic disorders (Skoczylas et al., 2023).

Recent studies employing lactic acid bacteria (LAB) and *Bacillus* strains granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA) have demonstrated that bioactive compounds derived from fermented garlic exert beneficial effects on bone metabolism, growth promotion, and the enhancement of physical fitness and exercise performance (Ried et al., 2025; Lee et al., 2016; El Basuni et al., 2024; Qui et al., 2024). Furthermore, extensive research on protein hydrolysates and bioactive peptides produced through enzymatic hydrolysis of key proteins and sulfur-containing compounds in green garlic has revealed diverse biological activities. These include antioxidant, anti-inflammatory, anticancer, immunomodulatory, antibacterial, antifungal, skin-whitening, and anti-wrinkle effects, alongside high stability, underscoring their potential as functional food and health-promoting ingredients (Arzanlou et al., 2010; Kim et al., 2013; Ezeorba et al., 2024; Marrelli et al., 2025). Collectively, these findings highlight fermentation technology using LAB and QPS-designated strains, applied to regionally distinctive crops such as green garlic, as a

promising strategy for developing high-value functional ingredients.

In this study, green garlic with diverse bioactive properties was used to prepare a green garlic fermentation medium. LAB and QPS-designated *Bacillus* strains were isolated and identified from local specialties of the Sacheon region and subsequently inoculated into the green garlic fermentation medium to produce fermented green garlic. The antioxidant activity of the fermented products was evaluated, and the strain yielding the highest antioxidant activity was selected for further study. The resulting fermented green garlic extract was then assessed for antidiabetic bioactivities. Through this work, we aimed not only to demonstrate the industrial applicability of LAB and QPS-designated *Bacillus* strains isolated from Sacheon specialties but also to enhance the recognized value of these regional microbial resources.

Materials and Methods

Isolation and cultivation of LAB and QPS-designated bacillus strains

LAB and QPS-designated *Bacillus* strains were isolated (Table 1) from various local specialty products of the Sacheon region. Sources included Acacia syrup and soy sauce (In-Seok Cheon's household), pickled perilla leaves (Sang-Gwon Jang's household), donga makgeolli fermented concentrate (Si-young Seong's household, Sacheon-eup), ganppang meju doenjang (NH Yonghyeon Agricultural Cooperative, Yonghyeon-myeon), wild kiwi extract soy sauce (Jeong Wol Saem's farm, Jeongdong-myeon), and gochujang (Kongji Eun's farm, Sanam-myeon).

Each 1 g sample was suspended in sterile 0.85% saline solution and homogenized using a vortex mixer. One milliliter of the suspension was serially diluted (10^{-1} to 10^{-4}), and aliquots were spread onto MRS agar and NB agar (BD, USA), prepared as selective media for LAB and QPS-designated *Bacillus* strains, respectively. Plates were incubated at 37°C, after which colonies were examined for morphological characteristics, including size, shape, and color. Single colonies were then re-streaked on the same medium to obtain pure isolates.

Phylogenetic Analysis of 16S rRNA Gene Sequences

For molecular identification of LAB and QPS-designated *Bacillus* strains isolated from Sacheon local specialty products, the isolates

Table 1. Isolation and identification of lactic acid bacteria (LAB) and QPS-*Bacillus* strains from Sacheon

No.	Isolates	Closest match	Similarity (%)	Source	Ref.
1	jo 43-2	<i>Bacillus amyloliquefaciens</i>	99.9	Acacia syrup	Yang et al. (2025)
2	JS-13-5	<i>Bacillus amyloliquefaciens</i>	99.8	Ganppang meju doenjang	Yang et al. (2025)
3	JS-65-1	<i>Bacillus subtilis</i>	99.7	Wild kiwi extract soy sauce ¹⁾	
4	JS-66-3	<i>Bacillus subtilis</i>	99.9	Soy sauce ³	Yang et al. (2025)
5	JY-30-2	<i>Bacillus subtilis</i>	99.9	Pickled perilla leaves	Yang et al. (2025)
6	JY-48-5	<i>Bacillus amyloliquefaciens</i>	99.7	Gochujang ²⁾	
7	JY-65-8	<i>Bacillus subtilis</i>	99.9	Wild kiwi extract soy sauce ¹⁾	
8	GH-11-11	<i>Latilactobacillus curvatus</i>	100.0	Sacheon-eup donga makgeolli fermented concentrate	Park et al. (2025)
9	GH-11-12	<i>Latilactobacillus curvatus</i>	100.0	Sacheon-eup donga makgeolli fermented concentrate	Park et al. (2025)
10	GH-11-13	<i>Latilactobacillus curvatus</i>	100.0	Sacheon-eup donga makgeolli fermented concentrate	Park et al. (2025)
11	GH-118-24	<i>Latilactobacillus curvatus</i>	100.0	Sacheon-eup donga makgeolli products	Park et al. (2025)

¹⁾Jeong Wol Saem, Jeongdong-myeon, Sogok-gil Farm, 12 years.

²⁾Kongji Eun Farm, Sacheon-si, Sanam-myeon Farm, 7 years.

were cultured on MRS/NB agar plates. 16S rRNA gene sequencing was performed by Macrogen Co., Ltd. (Seoul, Korea). The resulting sequences were analyzed using the 16S-based ID service of EzBioCloud platform (<https://www.ezbiocloud.net/>), developed and operated by CJ Bioscience, for taxonomic identification.

Analysis of extracellular hydrolytic enzyme production

The production of extracellular hydrolytic enzymes (amylase, lipase, and protease) by the isolated LAB and QPS-designated *Bacillus* strains was evaluated using substrate-supplemented solid media. Amylase activity was tested on agar plates containing 0.2% soluble starch (BD, USA), lipase activity on plates containing 1% Tween 80 (BD, USA), and protease activity on plates containing 20% skim milk (BD, USA). Each substrate was incorporated individually into MRS/NB agar, which were then inoculated with the isolates and incubated at 37°C for 7 days. Enzyme activity was assessed by measuring the diameter of the clear hydrolysis zones surrounding the colonies. For each isolate, the hydrolytic zone was measured from the edge of the colony to the outer boundary of the clear zone in triplicate or more. The mean values were categorized as follows: +++, >7 mm; ++, 4-6 mm; +, 1-3 mm (Table 2).

Preparation of green garlic powder and fermented green garlic

Green garlic was wild-harvested in Miryong Village, Sacheon

City, and obtained from Il-Geon Lee's farm. To prepare green garlic powder suitable for fermentation, 500 g of fresh material was sterilized at 121°C for 15 min, dried at 45°C for 48 h, cooled to room temperature, ground into powder, and stored in a sealed container at room temperature until use.

For preparation of the green garlic fermentation medium, 2% (w/v) green garlic powder, 3% (w/v) NaCl, and 0.1% (w/v) yeast extract were mixed with distilled water and sterilized at 121°C for 15 min. Selected LAB and QPS-designated *Bacillus* strains were pre-cultured in MRS/NB broth, and 1% (v/v) of the pre-culture was inoculated into the green garlic medium. Cultures were incubated at 37°C with shaking at 180 rpm for 4 days, after which the culture supernatant was collected as the fermented green garlic product.

For the preparation of fermented green garlic extract, selected strains were pre-cultured as described above, and 1% (v/v) of the pre-culture was inoculated into 1 L of green garlic medium. Cultures were incubated under previously determined optimal conditions (37°C, 180 rpm, 24-96 h), during which highest antioxidant activity was observed. To extract bioactive compounds, 20 g of Amberlite resins (XAD7HP, XAD4, and XAD16N; Merck KGaA, Darmstadt, Germany) were mixed at a 1:1:1 ratio, washed three times with triple-distilled water, and added to the fully fermented green garlic culture broth. The mixture was shaken at 180 rpm for 2 h to allow adsorption of active compounds onto the resin. The resin was then filtered through cotton cloth and immersed in 150 mL of acetone, followed by shaking at 180 rpm at room temperature for >2 h to desorb the compounds. After filtration through Whatman No. 2 filter

Table 2. Hydrolytic enzyme activities of isolated lactic acid bacteria (LAB) and QPS-*Bacillus* strain

No.	Strains	Protease activity	Lipase activity	Amylase activity
1	<i>Bacillus amyloliquefaciens</i> jo 43-2	+++ ¹⁾	– ²⁾	+++
2	<i>Bacillus amyloliquefaciens</i> JS-13-5	+++	–	+++
3	<i>Bacillus subtilis</i> JS-65-1	+++	+ ³⁾	+++
4	<i>Bacillus subtilis</i> JS-66-3	+++	–	+++
5	<i>Bacillus subtilis</i> JY-30-2	+++	–	+++
6	<i>Bacillus amyloliquefaciens</i> JY-48-5	+++	+	+++
7	<i>Bacillus subtilis</i> JY-65-8	+++	+	+++
8	<i>Latilactobacillus curvatus</i> GH-11-11	+++	–	–
9	<i>Latilactobacillus curvatus</i> GH-11-12	+++	–	–
10	<i>Latilactobacillus curvatus</i> GH-11-13	+++	–	–
11	<i>Latilactobacillus curvatus</i> GH-118-24	+	–	–

¹⁾³⁾Clear zone size (+++¹⁾: >7 mm, ++: >4–6 mm, +³⁾: 1–3 mm).

²⁾No activity.

paper (Whatman International Ltd., Maidstone, UK), the acetone filtrate was concentrated using a rotary evaporator (Sunileyea, Seongnam, Korea) and finally dried into powder form using a nitrogen gas evaporator (Miulab, Hangzhou, China).

Antioxidant activity

The antioxidant effects of green garlic fermented products produced by each isolated LAB and QPS-designated *Bacillus* strain were evaluated using ABTS radical scavenging activity. The ABTS radical solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate (1:1, v/v) and allowing the mixture to stand in the dark for 16 h to generate ABTS radicals (OD=0.70±0.02). For the assay, 20 µL of fermented green garlic product was mixed with 180 µL of ABTS radical solution in a 96-well plate, followed by incubation at room temperature for 2 min. Absorbance was measured at 734 nm, and radical scavenging activity was calculated accordingly.

Antidiabetic activity

The antidiabetic effect of the green garlic fermented extract was evaluated by measuring α -glucosidase inhibitory activity using a chromogenic assay (Zhang et al., 2020). In a 96-well plate, phosphate buffer (containing 2 g/L bovine serum albumin and 0.2 g/L NaN₃) was mixed with either the green garlic fermented extract

or the positive control (acarbose) and incubated for 5 min. The concentrations of both acarbose and the JY-48-5 fermented green garlic sample (extract) used in the experiment were 500 µg/mL. Subsequently, a p-nitrophenyl- α -D-glucopyranoside substrate solution was added, and the mixture was incubated at 37°C for 5 min. The reaction was monitored by measuring absorbance at 405 nm using a microplate reader (Epoch, BioTek, USA). The α -glucosidase inhibitory activity of the samples was then determined from the absorbance values.

Statistical analyses

The data are represented as the mean±SD of triplicate experiments. The statistical analysis was performed using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The values were evaluated by one-way analysis of variance (ANOVA), followed by a post hoc Duncan's multiple range test, and values of $p<0.05$ or $p<0.01$ were considered statistically significant.

Results and Discussion

Isolation and identification of LAB and QPS-designated bacillus strains

LAB have long been used as beneficial microorganisms in fermented foods and continue to attract considerable research

attention. They remain widely utilized in the development of functional products aimed at enhancing physiological functions in humans (Wang et al., 2021). In recent years, research has expanded beyond probiotics to include postbiotics, particularly those derived from *Bacillus* strains recognized under the EFSA Qualified Presumption of Safety (QPS) framework (Hou et al., 2025). In line with these advances, the exploration and identification of novel LAB and QPS-designated *Bacillus* strains remain active areas of investigation.

In this study, LAB and QPS-designated *Bacillus* strains were isolated from local special products of the Sacheon region using MRS/NB agar media. A total of 450 strains were obtained from 81 samples of agricultural and marine products. Among these, 191 strains from 39 samples related to agricultural products and their fermented derivatives were identified and characterized (Supplementary Tables 1 and 2). Screening for extracellular enzyme activities identified seven QPS-designated *Bacillus* strains with strong protease and amylase activities, some of which also displayed lipase activity (Yang et al., 2025). In addition, four LAB strains previously shown to be effective in oyster fermentation (Park et al., 2025) were selected as candidate starter strains for green garlic fermentation.

In total, 11 strains were selected: seven QPS-designated *Bacillus* strains (from Acacia syrup, soy sauce, pickled perilla leaves, ganppang meju doenjang, wild kiwi extract soy sauce, and gochujang) and four LAB (from donga makgeolli). Based on 16S rRNA gene sequencing, phylogenetic analysis using EzBioCloud identified the isolates as four *Latilactobacillus curvatus*, three *Bacillus amyloliquefaciens*, and four *Bacillus subtilis* strains. These findings not only demonstrate the feasibility of isolating LAB and QPS-designated *Bacillus* strains from Sacheon specialty products but also highlight the potential of regional resources as valuable reservoirs for discovering diverse strains with safety assurance and multifunctional fermentation capabilities for food applications.

Analysis of hydrolytic enzyme activities

To assess the potential of LAB and QPS-designated *Bacillus* strains as fermentation starters for high-value food applications, extracellular hydrolytic enzyme activities including protease, amylase, and lipase were evaluated. As summarized in Table 2, all seven QPS-designated *Bacillus* strains exhibited strong (+++) protease and amylase activities, while three strains also demonstrated detectable

(+) lipase activity. Among the LAB strains isolated from donga makgeolli, three showed strong (+++) protease activity and one displayed weaker (+) activity.

Notably, *Latilactobacillus curvatus*, identified among the isolates, is a registered probiotic strain known to function as a biological protective agent by inhibiting pathogen growth during fermented meat production (Chen et al., 2020). The isolated *Bacillus amyloliquefaciens* and *Bacillus subtilis* strains are also included in the most recent EFSA QPS list (EFSA BIOHAZ Panel., 2025), confirming their safety for both food and feed applications. These species are already widely used as functional microorganisms in animal feed and agriculture and possess considerable industrial value, including enzyme production, antimicrobial peptide synthesis, biological control, and use as feed additives. Collectively, the QPS-designated *Bacillus* strains isolated in this study combine safety assurance with excellent extracellular enzyme activities, highlighting their potential as strategic, high-value microbial resources capable of providing both functional versatility and industrial applicability in food fermentation.

Physiological activity analysis of green garlic fermented products

Recently, an increasing number of studies have reported that fermentation of green garlic with LAB and QPS-designated *Bacillus* strains enhances its physiological activities. In this study, the isolated strains were evaluated as fermentation starters with potential to augment the inherent bioactivities of green garlic, which is naturally rich in allicin, niacin, β -carotene, and vitamins A, B1-B3, C, and K. These compounds are associated with antioxidant, anti-inflammatory, immune-enhancing, antibacterial, antifungal, skin-whitening, anti-wrinkle, and anti-aging effects (Arzanlou et al., 2010; Kim et al., 2013; El Basuini et al., 2024; Ezeorba et al., 2024; Qui et al., 2024; Marrelli et al., 2025; Ried et al., 2025). Green garlic was processed into powder and incorporated into the fermentation medium, after which selected strains with strong protease, amylase, and lipase activities were inoculated and cultured. Culture supernatants were collected daily as fermented products.

Antioxidant activity, measured by ABTS radical scavenging, revealed that green garlic fermented with *B. amyloliquefaciens* JY-48-5, *B. subtilis* JS-65-1, and *Latilactobacillus curvatus* GH-11-11 exhibited significantly higher activity compared to the non-fermented

control. The strongest effects were observed in JY-48-5 (145.8% at 2 days), JS-65-1 (144.9% at 2 days), and GH-11-11 (144.0% at 4 days) (Fig. 1).

Given these results, the strain with the highest antioxidant capacity, *B. amyloliquefaciens* JY-48-5 (2-day fermentation), was selected for further study. The resulting fermented extract was assessed for antidiabetic potential by measuring α -glucosidase inhibitory activity, a key mechanism for regulating blood glucose. The extract showed a 189.4% increase in inhibitory activity compared to the non-fermented extract. Notably, it exhibited approximately 59% of the inhibitory effect of acarbose, a clinically prescribed α -glucosidase inhibitor for type 2 diabetes (Fig. 2). These findings suggest that green garlic fermented extract can delay glucose release by inhibiting intestinal α -glucosidase, thereby contributing to reduced plasma glucose levels.

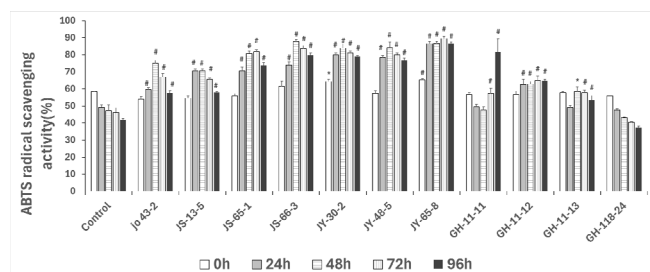


Fig. 1. ABTS radical scavenging activity of culture supernatants obtained from green garlic (*Allium sativum* L.) fermentation media inoculated with the isolated strains. The control represents the culture supernatant of green garlic medium without strain inoculation. Each value represents the mean \pm standard deviation ($n=3$). * $p<0.05$, vs. control; # $p<0.01$, vs. control.

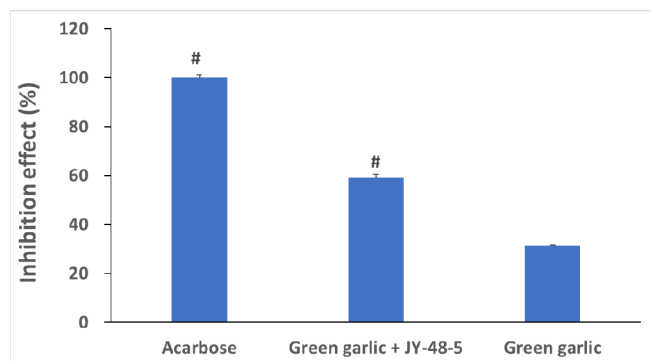


Fig. 2. Antidiabetic activity of fermented green garlic (*Allium sativum* L.) extracts prepared from the culture broth of *Bacillus amyloliquefaciens* JY-48-5. The control represents the extract from non-inoculated green garlic culture broth. Each value represents the mean \pm standard deviation ($n=3$). # $p<0.01$, vs. control.

This study provides the first report in Korea on the antioxidant and antidiabetic effects of green garlic fermented with LAB and QPS-designated *Bacillus* strains isolated from Sacheon local specialties. The results highlight the industrial potential of these fermented extracts as functional ingredients for food and cosmetic applications. Moreover, the strains identified in this study have been deposited in the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Biological Resource Center and the Sacheon Microbial Fermentation Foundation, ensuring their availability as valuable microbial resources.

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Conflict of interests

No potential conflict of interest relevant to this article was reported.

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Data availability

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authorship contribution statement

Conceptualization: Park MH, Ganbat D, Lee YJ, Lee SJ.

Data curation: Park MH, Ganbat S, Lee YJ, Lee SJ.

Formal analysis: Park MH, Ganbat S, Ganbat D, Lee YJ, Lee SJ.

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Validation: Ganbat S, Ganbat D, Lee YJ, Lee SJ.

Investigation: Park MH, Park JY.

Writing - original draft: Park MH, Lee SJ.

Writing - review & editing: Park MH, Ganbat S, Park JY,
Ganbat D, Lee YJ, Lee SJ.

Ethics approval

Not applicable.

Supplementary materials

Supplementary materials are only available online from: <https://doi.org/10.13050/foodengprog.2025.29.4.231>

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