

Microbial collection isolated from environmental marine samples and fermented seafood products in Sacheon region

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Abstract

A total of 128 halophilic and marine-associated bacterial strains were isolated from environmental marine samples and fermented seafood products collected in the Sacheon region of South Korea. The aim was to secure region-specific microbial resources and evaluate their potential for industrial use. The isolates belonged to 4 phyla, 15 families, 30 genera, and 61 species. The phylum Bacillota, particularly the family Bacillaceae, was the major taxonomic group (62.5%), comprising 14 genera and 36 species, such as *Alkalihalobacillus*, *Bacillus*, *Caldibacillus*, *Cytobacillus*, *Fictibacillus*, *Halobacillus*, *Lysinibacillus*, *Metabacillus*, *Neobacillus*, *Oceanobacillus*, *Priestia*, *Rosellomorea*, *Rummeliibacillus*, and *Ureibacillus*. Members of the phylum Pseudomonadota, primarily the family Alteromonadaceae, accounted for 4.7% of the isolates and included the genera *Marinobacter* and *Microbulbifer*. All isolates identified as members of the phylum Pseudomonadota were strong auxin producers or showed high extracellular enzyme activities. Functional characterization showed that 86 strains (67.2%) exhibited hydrolytic activity for at least one enzyme (protease, amylase, or lipase), and 32 strains (25%) produced auxin. The functions of the microbial resources in the Sacheon region are highly diverse. Collectively, the isolated strains show potential for application in managing marine environments, bioprocessing, and the fermented seafood industry, underscoring their value as genetic resources for future biotechnological use in South Korea.

Keywords: Sacheon region-specific microbial collection (SRSMC), Diversity, Characterization, Fermented seafood products, Marine environmental samples

Introduction

Sacheon City, located along the southern coast of Gyeongsangnam-do, South Korea, represents a unique convergence of diverse geographical and ecological environments within a relatively compact area, encompassing mountains, plains, coastlines, islands, rivers, and wetlands. The region exhibits a temperate oceanic climate typical of the southern coastal zone, with mild winters, hot, humid summers, and distinct seasonal transitions that support a dynamic,

productive natural ecosystem. The coastline forms a complex ria-type shoreline with extensive tidal flats-features rarely observed in inland environments-creating conditions highly favorable for the development of rich marine biodiversity. Owing to these geographical and climatic characteristics, Sacheon supports a vibrant marine ecosystem and has considerable potential for advancing the marine and fishery industries, including aquaculture, marine biotechnology, and the sustainable utilization of coastal biological resources. Furthermore, the integration of coastal agriculture,

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moderate-scale livestock and forestry industries, and advanced marine transportation infrastructure positions Sacheon as a strategically significant region where land, sea, and air-based industries coexist synergistically (Park et al., 2025; Yang et al., 2025). Despite this ecological richness, research on the exploration and industrial utilization of microbial resources in Sacheon remains limited. The implementation of the Nagoya Protocol under the Convention on Biological Diversity has intensified the need to secure, characterize, and utilize region-specific microbial biodiversity for potential bio-industrial development. In response, the Sacheon Microbial Fermentation Foundation, established in 2020, has initiated collaborative studies with the EF-Bio Laboratory at Silla University.

Marine microorganisms have evolved under high pressure, salinity, and low-temperature conditions, producing unique secondary metabolites that differ from those produced by terrestrial microbes (Zhang & Kim, 2010). As a maritime nation surrounded by three seas, South Korea possesses diverse marine bioresources, offering significant potential for the development of novel functional and medicinal foods (Moreno et al., 2013). Traditional Korean fermented seafood products, including salted fish sauces, seaweed pickles, and seasoned seafood dishes, are prepared through autolytic and microbial enzymatic processes under high-salinity conditions, in which halophilic bacteria play a crucial role in flavor development and texture modification (Sekar & Kim, 2020). Halophilic bacteria are known producers of industrially valuable hydrolytic enzymes such as proteases, amylases, and lipases, which are widely utilized in the food, detergent, textile, leather, and pharmaceutical industries, as well as in the recycling of fishery by-products (Barzkar et al., 2020). Moreover, several halophilic strains have demonstrated the ability to synthesize auxin (e.g., indole-3-acetic acid, IAA), thereby promoting plant growth and offering promise for use as eco-friendly biofertilizers (Etesami & Glick, 2024). For example, halophilic and halotolerant bacteria isolated from coastal soils have been shown to produce IAA alongside other plant growth-promoting traits (Reang et al., 2022). In this study, halophilic bacterial strains were isolated and characterized from marine environments and seafood (including fermented products) collected in Sacheon, South Korea. The enzymatic activities (amylase, lipase, and protease) and auxin production potential of the isolates were evaluated to explore their applicability in various industrial and agricultural sectors. These findings provide a fundamental basis for securing and utilizing halophilic microbial resources from the Sacheon coastal ecosystem.

Materials and Methods

Materials

Marine environmental samples and fermented seafood products were collected from diverse habitats in the Sacheon region, South Korea, including seawater, tidal flats, coastal sediments, marine mud, and traditional homemade fermented seafoods (Table 1). To minimize contamination, sterile gloves were worn during sampling, and sterilized spatulas were used to collect each sample. The samples were transferred into sterile 50 mL conical tubes and transported to the laboratory in an ice box under chilled conditions. Upon arrival, all samples were stored at 4°C until further processing. All microbial strains isolated during this study were deposited in the Microbial Value Enhancement Program, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, South Korea.

Isolation and cultivation of Sacheon-region-specific microorganisms

To isolate region-specific halophilic microorganisms, sixteen samples of marine environmental materials and fermented seafood products were collected from various sites in Sacheon, South Korea. Each sample (1 g) was suspended in 9 mL of sterile 0.85% (w/v) saline solution and homogenized using a magnetic stirrer to prepare a uniform suspension. The resulting suspension (1 mL) was serially diluted tenfold from 10^{-1} to 10^{-4} using sterile saline. Aliquots of each dilution were spread onto Marine Agar (MA; BD Difco, USA), a medium formulated for the cultivation of marine microorganisms. The inoculated MA plates were incubated aerobically at 37°C. Following incubation, colonies exhibiting distinct morphological characteristics (size, shape, color, and texture) were selected and streaked repeatedly on fresh MA plates to obtain pure isolates.

To evaluate the growth adaptability, each strain was cultivated on Nutrient Agar (NA; BD Difco, USA), Reasoner's 2A Agar (R2A; BD Difco, USA), and Tryptic Soy Agar (TSA; BD Difco, USA) at 37°C for seven days under static conditions. Salt tolerance and pH adaptability were assessed on MA supplemented with different NaCl concentrations (3% and 10%) and adjusted to pH 4, 7, and 9. Temperature tolerance was evaluated by incubating the strain on MA at temperatures ranging from 25°C to 60°C at 5°C intervals (Yang et al., 2025).

Table 1. List of marine environmental and fermented seafood samples collected from different locations in the Sacheon region, South Korea

No.	Sample name	Provider	Date	Sampling location (Sacheon-si)	Category	Maturation period
1	Hairtail viscera jeotgal	Mikyung Kim (Woorihoe Center)	Jan. 20, 21	Seopo-myeon	Restaurant	1 year
2	Seasoned oyster jeotgal					1 year
3	Unseasoned oyster jeotgal					1 year
4	Anchovy fish sauce 1					1 year
5	Anchovy fish sauce 2	Solmae Co., Ltd.	Aug. 23, 21	Namyang-dong	Company	6 months
6	Oyster shell	Myeongseung Raw Fish Restaurant	Jan. 20, 21	Seopo-myeon	Restaurant	1 week
7	Shell-on oyster					1 day
8	Shucked oysters					1 week
9	Anchovy extract	Ucheon AP Farm	Jan. 14, 21	Sanam-myeon	Farm	1 year
10	Anchovy jeotgal	Sang-Kwon Jang	Jan. 14, 21	Sacheon-eup	Household	3 years
11	Fermented salt	SMFF ¹⁾	Jun. 21, 21	Sacheon-eup	Foundation	6 months
12	<i>Sardinella zunasi</i> jeotgal	Inseok Cheon	Jan. 17, 21	Sacheon-eup	Household	3 years
13	Crushed oyster shell	Shell Recycling Facility	Jan. 20, 21	Seopo-myeon	Outdoor site	1 month
14	<i>Ruditapes philippinarum</i>	Jeodo Island MAF ²⁾	Jan. 20, 21	Silan Sea	Aquaculture	3 days
15	Near Bito coastal breakwater	34.982963, 127.963758	Jan. 20, 21	Seopo-myeon	Marine site	1 day
16	Near Geobukgyo bridge	34.982287, 127.965862	Jan. 20, 21			

All oyster are *Crassostrea gigas*.

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16S rRNA gene sequencing and phylogenetic analysis

For molecular identification, isolates obtained from the sixteen marine environmental and fermented seafood samples were subjected to 16S ribosomal RNA (16S rRNA) gene sequencing. Single colonies of each isolate were submitted to Macrogen Inc. (Seoul, South Korea) for PCR amplification and sequencing of the 16S rRNA gene. The 16S rRNA gene was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). The resulting sequences were compared against publicly available 16S rRNA gene sequences in the EzBioCloud database (<http://www.ezbiocloud.net>) to determine taxonomic identity based on sequence similarity. A circular phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987). Evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al., 2004). All evolutionary analyses were performed using MEGA version 12 (Kumar et al., 2024).

Analysis of extracellular hydrolytic enzyme production

The production of extracellular hydrolytic enzymes (protease,

amylase and lipase) by the isolated halophilic microorganisms was evaluated using solid media containing specific substrates for each enzyme. For the detection of protease activity, MA was supplemented with 20% (w/v) skim milk (BD, USA) as the substrate. Amylase activity was assessed on MA containing 0.2% (w/v) soluble starch (BD, USA), and lipase activity was evaluated using MA plates supplemented with 1% (v/v) Tween 80 (BD, USA). Each isolate was spot-inoculated onto the respective plates and incubated at 37°C for 7 days under aerobic conditions. After incubation, the diameter of the clear zone surrounding each colony was measured to determine enzyme activity. Hydrolytic activity was semi-quantitatively graded based on clear zone as follows: +++ (strong activity, >7 mm), ++ (moderate activity, 4–6 mm), and + (weak activity, 1–3 mm) (Yang et al., 2025).

Analysis of auxin production

The ability of the isolated strains to produce auxin (indole-3-acetic acid; IAA) was evaluated using a colorimetric assay. Each pure colony was inoculated into Marine Broth (MB; BD Difco, USA) supplemented with 0.1% (w/v) L-tryptophan (Sigma-Aldrich, USA) and incubated at 37°C for 5 days under aerobic conditions. After incubation, cultures were centrifuged at 10,000×g for 10 min to obtain

the cell-free supernatant. For IAA detection, 400 μ L of each culture supernatant was mixed with 800 μ L of Salkowski reagent (prepared by combining 50 mL of 35% HClO_4 and 1 mL of 0.5 M FeCl_3). The mixtures were incubated in the dark at room temperature for 30 min to allow color development, after which absorbance was measured at 535 nm using a spectrophotometer. IAA production was semi-quantitatively classified based on the absorbance value as follows: +++ (strong production, >1.0), ++ (moderate, 0.5–1.0), and + (weak, 0.3–0.5) (Yang et al., 2025).

Results and Discussion

Isolation and growth characteristics of halophilic bacteria

A total of sixteen samples, including seawater, tidal flat sediments, and various fermented seafoods, were collected from different sites in Sacheon City to isolate aerobic halophilic microorganisms with potential application in the food industry. Each sample was serially diluted and spread on MA, and colonies exhibiting distinct morphological characteristics (color, size, and texture) were selected. Pure cultures were obtained through repeated single-colony streaking on fresh MA plates. As shown in Fig. 1 and Supplementary Table 1, 36 strains were isolated from marine environmental samples, and

92 strains were obtained from fermented seafood samples, yielding a total of 128 halophilic isolates specific to the Sacheon region.

Because marine agar, composed primarily of inorganic salts, is well-suited for culturing marine halophiles, the growth ability of the isolates was further examined on complex media (NA, R2A, and TSA) commonly used for industrial-scale cultivation. Among the 128 isolates, 118 strains (including weak growth) were able to grow on at least one of these three media, indicating that medium composition affects the isolation efficiency of halophiles to some extent (Fig. 2). Based on these results, MA was confirmed to be the most suitable isolation medium for halophilic bacteria from marine and fermented seafood samples. To determine pH adaptability, isolates were cultivated on MA plates adjusted to pH 4, 7, and 9. All isolates exhibited growth (including weak growth) at pH 7, whereas 79 strains grew at pH 4 and 123 strains grew at pH 9. Notably, 75 isolates demonstrated tolerance across all three pH conditions (Fig. 2). Temperature tolerance tests revealed that all isolates grew optimally at 30–35°C, and 106 strains displayed growth at 45°C. Interestingly, six isolates grew at 50–55°C, and four strains of *Caldibacillus pasinlerensis* were capable of growth even at 60°C (Fig. 2). Salt tolerance tests showed that 126 isolates could grow in media containing $>3\%$ NaCl, and 90 isolates exhibited growth at NaCl concentrations exceeding 10% (Fig. 2).

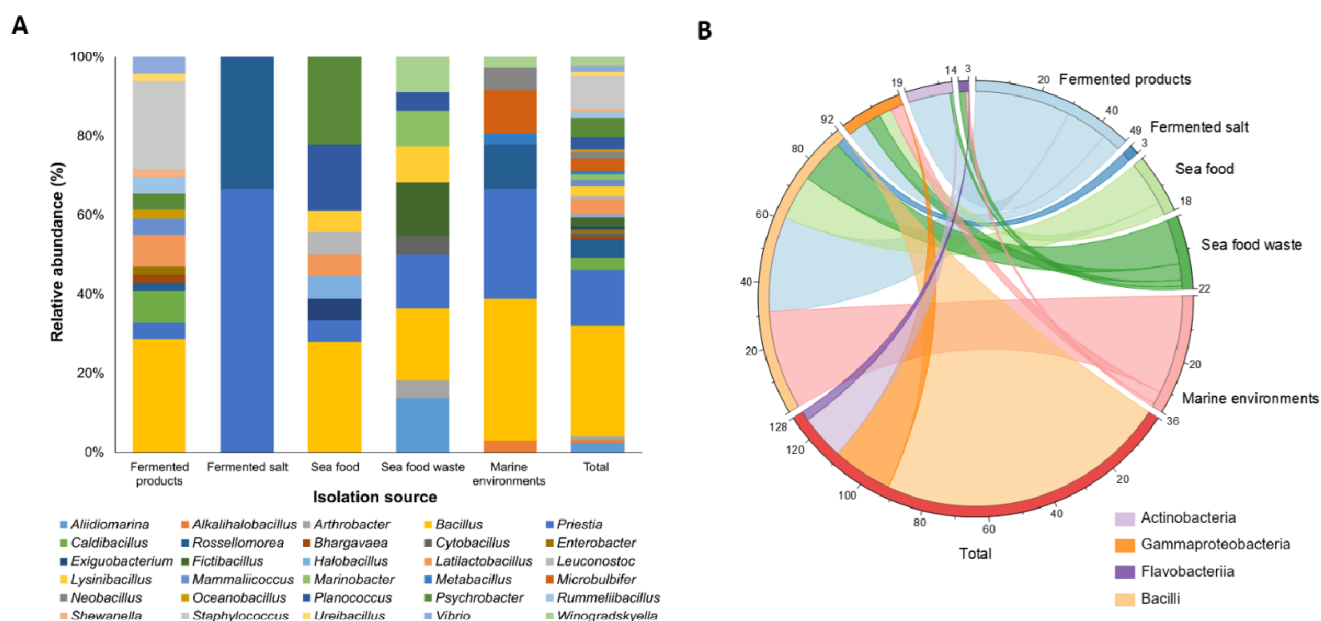


Fig. 1. Relative abundances and bacterial diversity in specific sources from the Sacheon Region. (A) Bacterial diversity at the genus level. Isolation sources are plotted on the horizontal axis, and the relative abundances of taxa are represented on the vertical axis, with each genus shown as a distinct color in the stacked columns. (B) Each arc represents the overlapping taxonomic composition among specific sources.

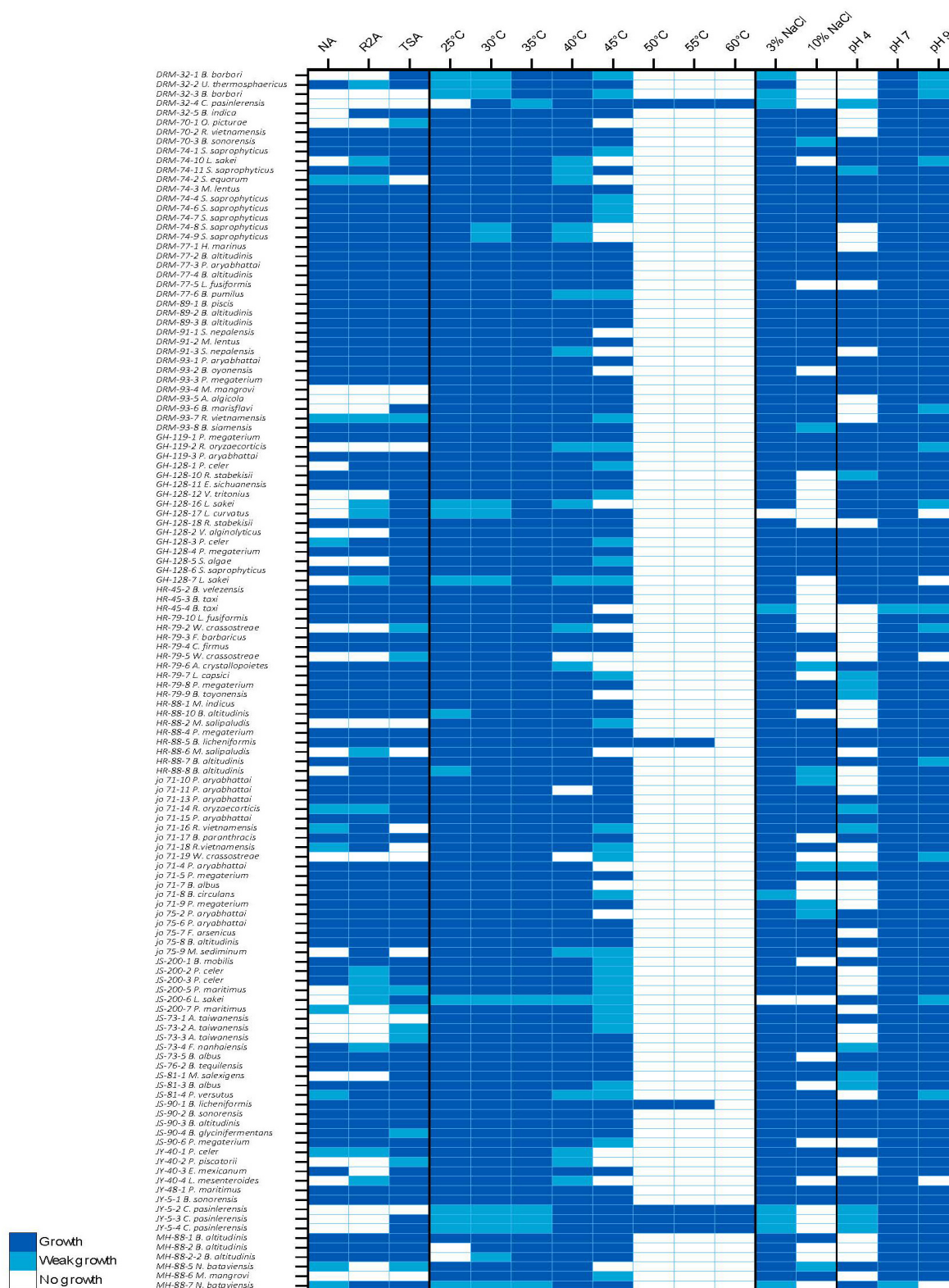


Fig. 2. Heatmap analysis of growth characteristics of bacterial isolates under different culture conditions. A heatmap illustrating the growth responses of bacterial isolates across various media (NA, R2A, and TSA), temperature ranges (25–60°C), NaCl concentrations (3% and 10% w/v), and pH values (4, 7, and 9). Color intensity indicates growth strength, where dark blue represents strong growth, light blue represents weak growth, and grey indicates no growth. Black boundary lines separate condition groups according to medium, temperature, salinity, and pH.

Although numerous studies have reported the isolation and identification of microorganisms from individual fermented seafoods such as anchovy sauce (myeolchi-aekjeot), sand lance (*bandengi*), oyster viscera, hairtail viscera, and clam (bajirak) products, comparative analyses of microbial distribution and characteristics among multiple fermented seafoods collected from a specific region remain limited. Likewise, studies isolating microorganisms from oyster shells, crushed shell debris, or tidal mud from small islands are also scarce. In recent years, however, regional initiatives in areas such as Sunchang, Jeju, and Sacheon have emphasized the exploration of region-specific microorganisms and their potential industrial applications. In Sunchang, key microbial strains involved in traditional fermented condiments (gochujang, doenjang, and ganjang) have been systematically characterized, while researchers have investigated the influence of unique environmental factors (volcanic soil, marine ecosystems) on microbial diversity in Jeju and published a three-volume Jeju Native Microorganism Atlas (Yang et al., 2025). In addition, microbial community analyses of various jeotgal types, including anchovy, sea squirt and shrimp fermentations (Song et al., 2018), studies on microbial profiling in oyster and clam samples (Kim et al., 2024), and reports of halophilic isolates from diverse marine environments such as sediments, seawater, shells, and tidal flats (Lee et al., 2023) have provided valuable foundations for expanding regional microbial resource studies. Following this trend, the Sacheon Microbial Fermentation Foundation has focused not only on the isolation and identification of microorganisms from regional fermented foods but also on the characterization of their industrially valuable traits. In this study, 128 halophilic bacterial strains exhibiting diverse media adaptability and strong extracellular enzymatic activities were secured from the marine and fermented seafood resources of Sacheon. These results provide a meaningful foundation for the industrial utilization of region-specific microbial resources.

Phylogenetic analysis based on 16S rRNA gene sequences

A total of 128 aerobic bacterial strains isolated from marine environmental samples and fermented seafood products collected in Sacheon City were identified using the EzBioCloud database based on 16S rRNA gene sequences. The isolates were classified into four phyla, 15 families, 30 genera, and 61 species (Fig. 1). Detailed information

regarding each isolate, its phylogenetically closest type strains, and sequence similarity values is provided in Supplementary Table 1. To elucidate evolutionary relationships among the isolates, a phylogenetic tree was constructed based on the 16S rRNA gene (Fig. 3). The phylum Bacillota was dominant, accounting for 82.0% of all isolates. Within Bacillota, the family Bacillaceae was the most abundant (76.2%), followed by Staphylococcaceae (12.4%), Lactobacillaceae (4.8%), Planococcaceae (3.8%), with minor contributions (~1% each) from Caryophanaceae, Exiguobacteraceae, and Leuconostocaceae. In total, 48 species across 21 genera belonging to seven families were identified.

Among the Bacillaceae family, which exhibited the highest isolation frequency, the genus *Bacillus* is widely known to be prevalent in marine and freshwater organisms and is frequently isolated from various traditional fermented foods, particularly those derived from marine environments, fermented agricultural materials, and jeotgal (salted seafood). Therefore, the predominance of Bacillaceae in the marine and fermented samples from Sacheon, a region characterized by a complex ria-type coastline and diverse coastal habitats, is well supported by previous findings (Yoon et al., 2001; Dharaneedharan & Heo, 2016). In this study, halophilic bacteria belonging to the Bacillaceae family were abundantly isolated from two marine environmental samples and 13 fermented seafood samples (including various types of jeotgal and oyster shell samples) among the 16 total sampling sites in Sacheon. Their predominance likely reflects the high-salinity conditions characteristic of fermented seafood environments, which favor the growth of *Bacillus* species known for robust pH regulation and antimicrobial potential (Kim et al., 2010; Kim et al., 2013).

Among the genus *Bacillus*, *Bacillus altitudinis* was identified in 12 isolates (33.3%) derived from five different marine and fermented seafood samples collected in the Sacheon region. As reported by Yang et al. (2025), *B. altitudinis* is considered a representative strain well adapted to and stable in the long-term fermentation environments of Sacheon, including both fermented agricultural and seafood products. Importantly, several isolates obtained in this study belonged to taxa designated as Generally Recognized As Safe (GRAS) or Qualified Presumption of Safety (QPS) by the European Food Safety Authority (EFSA BIOHAZ Panel, 2025), underscoring their potential safety and suitability for industrial use. Representative species include *Bacillus licheniformis*, *Bacillus sonorensis*, *Bacillus toyonensis*, *Latilactobacillus curvatus*, *Latilactobacillus sakei*, and *Leuconostoc mesenteroides*. Their presence highlights the value of

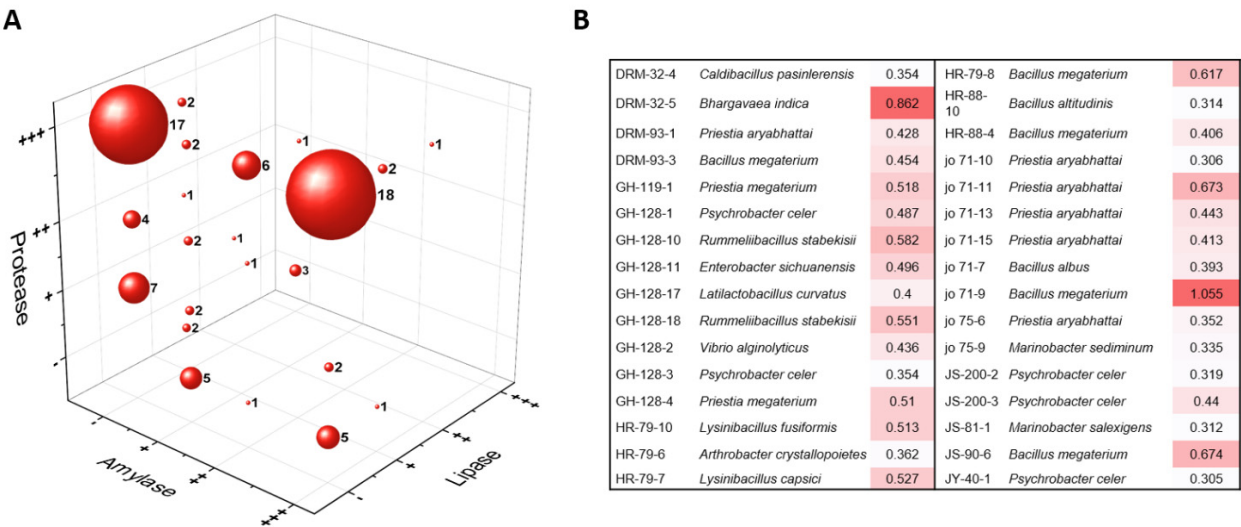


Fig. 4. Analysis of extracellular enzyme and indole-3-acetic acid (IAA) production activities among bacterial isolates. (A) A 3D bubble plot illustrating the extracellular hydrolase activities of the isolates. The X-, Y-, and Z-axes represent amylase, protease, and lipase activities, respectively. Bubble size indicates the relative number of isolates exhibiting the same enzyme activity pattern. Hydrolase activities were determined by the agar diffusion assay based on halo diameter (+++, >7 mm; ++, 4–6 mm; +, 1–3 mm; –, no activity). (B) Quantitative comparison of indole-3-acetic acid (IAA) production (OD₅₃₅ nm) by each isolate. Higher OD values indicate greater IAA production capacity.

strains exhibiting hydrolytic enzyme activity in this study (67.2%) is consistent with values typically reported for halophilic microorganisms in high-salinity habitats, although it is lower than the 92.2% recently documented for isolates from fermented agricultural products in the Sacheon region (Yang et al., 2025). Reported proportions from other salt-associated environments include 77.2% in strains from solar salt fields in Shinan and Gomso (Lee et al., 2020a), 77.6% in isolates from Jeju coastal ranch areas (Lee et al., 2020b), 64.0% in strains from South Sea and Tsushima seafoods (Jeong et al., 2021), and 67.4% in isolates from southwestern coastal soils of Jeju (Lee et al., 2022). These variations are likely attributable to differences in sample substrates and physicochemical conditions, as our study encompassed marine environmental and fermented seafood samples, whereas Yang et al. (2025) focused on carbohydrate-rich agricultural fermentations that selectively enrich strong protease- and amylase- producing taxa.

IAA, the primary form of microbial auxin, plays essential roles in plant growth and development, including cell elongation, root initiation, seed germination, fruit enlargement, photosynthetic enhancement, stress tolerance, and nitrogen fixation (Woo & Kim, 2007). A total of 32 isolates demonstrated IAA production capabilities (Fig. 4b). The highest IAA production activity was observed in *Bhargavaea indica* strain DRM-32-5, isolated from anchovy jeotgal, and *Priestia megaterium* (formerly *Bacillus megaterium*) strain JO71-9, isolated from near the Bito Coastal Breakwater. These findings highlight the

potential of isolated strains as microbial biofertilizers, particularly for coastal agriculture and saline soils where conventional plant growth-promoting microorganisms have limited survival (Etesami, 2020). In addition to their agricultural relevance, the inherent stress-tolerance of these halophiles positions them as promising candidates for environmental biotechnology, including the treatment of saline wastewater and the biodegradation of seafood-processing effluents (Sadeghi et al., 2019; Mainka et al., 2021). Halophilic bacteria are also known to harbor distinctive stress-adaptive biosynthetic pathways, offering valuable opportunities for genome mining, enzyme engineering, and metabolic pathway analysis aimed at discovering novel salt-stable enzymes and bioactive metabolites (DasSarma & DasSarma, 2015; Dutta et al., 2022).

The findings of this study contribute to expanding the diversity of indigenous Korean microbial resources derived from the marine environments and fermented seafoods of the Sacheon region. Collectively, the isolates secured in this study represent an important microbial resource with substantial potential for the development of industrial biocatalysts, environmentally friendly microbial formulations, and biofertilizer applications. All strains isolated in this study have been deposited in the Microbial Resource Division of the Korea Research Institute of Bioscience and Biotechnology (KRIBB) under the Microbial Value Enhancement Program (Supplementary Table 1), ensuring their long-term accessibility as region-specific biological 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: Ganbat S, Lee SJ.
 Formal analysis: Ganbat S, Park MH, Yang JY, Lee SJ.
 Methodology: Park MH, Lee YJ, Lee SJ.
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 Validation: Ganbat S, Park MH, Ganbat D, Lee YJ, Lee SJ.
 Investigation: Yang JY, Ganbat D.
 Writing - original draft: Ganbat S, Park MH, Lee SJ.
 Writing - review & editing: Ganbat S, Park MH, Yang JY, Ganbat D, Lee YJ, Lee SJ.

Supplementary materials

Supplementary materials are only available online from: <https://doi.org/10.13050/foodengprog.2025.29.4.248>

Ethics approval

Not applicable.

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