Research Article

Prospecting for Mannanolytic Bacteria from Cabbage kimchi for the Bioconversion of Glucomannan into Mannooligosaccharides

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Abstract | Despite the great potential of glucomannan as a biopolymer, its commercial uses are constrained by its highly viscous, poorly accessible molecular structure. To produce mannooligosaccharides (MOS), enzymatic hydrolysis is a desirable and eco-friendly alternative, which offers a high specificity and efficiency for the desired degrees of polymerization of the functional and industrially relevant oligomers, transferring them preferably from low- to medium-degree polymerized fractions. The objectives of this study were to isolate mannanolytic bacteria from cabbage kimchi, test their ability to use glucomannan as a sole carbon source, and identify the selected strain using morphological characterization and molecular 16S rRNA sequencing. In total, 13 bacterial isolates were isolated from cabbage kimchi. Among these, 8 isolates grew on the glucomannan solid medium, exhibiting a mannanolytic index (MI) ranging from 1.028 ± 0.006 to 1.717 ± 0.021, and showed variations in enzyme activity, ranging from 4.370 ± 0.494 U/ml to 34.125 ± 1.087 U/ml in a glucomannan broth medium. Thin-layer chromatography (TLC) showed that MOS products from all isolates exhibited distinct patterns. The KS7 isolate was thus selected for further analysis due to its strongest ability to degrade glucomannan among the isolates obtained. Liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis of KS7 hydrolysate showed the presence of MOS with a degree of polymerization 2-6. The highest enzyme activity was observed in the early-stationary phase (after 120 h) of cultivation. Molecular characterization of the selected isolate based on its 16S rRNA gene sequence confirmed the morphological characteristics and further established its identification as a member of the genus *Bacillus,* which showed 95.72% sequence homology to *Bacillus albus* MCCC 1A02146. These results displayed that cabbage kimchi is an alternative source of culturable β-mannanase-producing bacteria and that *Bacillus* sp. KS7 strain could be a potential and efficient biocatalyst for MOS production.

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Introduction

Glucomannan is a water-soluble polysaccharide composed of mannose and glucose residues linked primarily by β-1, 4-glycosidic linkages. It has diverse applications, particularly in the food, biomedical, and medicinal fields, but many of these applications remain under experimental investigation.

Glucomannan is widely distributed among several plant species and its multifunctional properties have been studied (Zhang *et al*., 2021; Karimi *et al*., 2025). However, glucomannan's broader commercial application remains limited by its poor processability and a relatively narrow molecular weight distribution (Aanisah *et al*., 2022). Controlled depolymerization therefore represents a rational strategy for reducing molecular size and viscosity while improving solubility and expanding the functional potential of glucomannan-derived products (Zhang *et al*., 2021; Zhao *et al*., 2024).

Mannooligosaccharides (MOS) are produced through enzymatic hydrolysis of glucomannan and other mannan-rich substrates, and have attracted substantial attention as functional oligosaccharides (Jana *et al*., 2021; Tripetch *et al*., 2023). MOS generally exhibit a decreased viscosity, increased solubility, and greater molecular accessibility than the native polymer, thereby facilitating their functional use (Zhao *et al*., 2024). The functional properties of MOS are determined by their molecular size, glycosidic linkage pattern, and degree of polymerization (DP), with low- to medium-DP oligosaccharides being particularly valuable, due to their enhanced microbial accessibility and potential for prebiotic or functional food applications (Jana *et al*., 2021; Suryawanshi and Kango, 2021; Tripetch *et al*., 2023). Enzymatic production of MOS from konjac glucomannan and other mannan-rich substrates has been widely reported, with hydrolysis efficiency and product composition depending on the substrate and catalytic properties of the mannanase employed (Jana *et al*., 2021; Suryawanshi and Kango, 2021; Cuong *et al*., 2024).

Glucomannan depolymerization can be achieved using chemical, physical, or enzymatic techniques. Chemical and physical treatments can reduce the molecular size of polymers but may offer a limited control over hydrolysis and product distribution. However, these approaches often require severe processing conditions, exhibit poor product selectivity, and may yield heterogeneous degradation products (Dawood and Ma, 2020; Jana *et al*., 2021). In contrast, enzymatic hydrolysis provides a more selective and environmentally acceptable technique. β-Mannanase enzyme catalyzes the breakage of β-1,4-mannosidic links in mannan-based polysaccharides to generate MOS (Dawood and Ma, 2020; Rana et al., 2023). However, β-mannanase properties and hydrolysis patterns can differ among microbial sources and substrates (Jana *et al*., 2021; Suryawanshi and Kango, 2021; Rana *et al*., 2023), making the investigation of novel β-mannanase-producing microorganisms crucial for optimizing MOS production.

During spontaneous fermentation, the microbial community changes substantially over time. Although lactic acid bacteria generally become dominant during the later stages of fermentation, the early stages are characterized by a larger and more diverse microbial population that is strongly influenced by processing conditions and ingredient formulation (Cha *et al*., 2024; Lee *et al*., 2024). Although kimchi has been extensively investigated for its probiotic potential, metabolite production, and fermentation quality, its use as a source of β-mannanase-producing bacteria for glucomannan hydrolysis remains largely unexplored. There is a serious information gap here, as bacteria associated with fermented plant matrices may have evolved extracellular polysaccharide-degrading enzymes to exploit plant-derived carbohydrates and cope with environmental stresses. In this study, kimchi was not used specifically to enrich lactic acid bacteria, but rather was considered a potential source of diverse culturable bacteria with carbohydrate-degrading capabilities, including mannanolytic activity, as previously reported for bacteria isolated from fermented vegetable-based foods (Oh *et al*., 2016; Regmi *et al*., 2016).

The present study aimed to isolate and screen culturable β-mannanase-producing bacteria obtained from cabbage kimchi, evaluate their ability to hydrolyze glucomannan to yield MOS-containing hydrolysates, and identify the promising isolate using morphological and molecular methods. The working hypothesis was that kimchi-derived culturable bacteria comprise non-specific isolates capable of generating extracellular β-mannanase and converting glucomannan into a low- to medium-DP MOS. This study further expected that functional variations across the microbial isolates would allow the selection of a superior candidate for preliminary MOS-oriented glucomannan bioconversion. The novelty of this work lies in the isolation and identification of *Bacillus* sp. KS7 strain from cabbage kimchi, as a promising β-mannanase-producing strain capable of forming MOS fractions from glucomannan, with its taxonomic identity and MOS profile conservatively

interpreted within the available data.

Materials and Methods

Materials

Cabbage kimchi was made in the laboratory for this study. Glucomannan (Chengdu Root Industry Co., Ltd., China) was used as the sole carbon source in the screening and production media. Mannobiose (M2) was obtained from Megazyme (Bray, Ireland), while D-mannose (M1) was obtained from Merck (Germany). Silica gel 60 F_{254} plates were obtained from Merck (Germany). All the components used in microbiological growth media were purchased from HiMedia Laboratories (India) and Merck (Germany). All chemicals used in this study were of good analytical grade. Genomic DNA was extracted using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan), and polymerase chain reaction (PCR) amplification was performed with reagents obtained from Bioline (UK).

Kimchi preparation and bacterial isolation

Cabbage kimchi was prepared following the method reported by Wadamori *et al.* (2014) with a minor modification, where refined sugar was replaced with apple and pear (as additional sources of plant-derived carbohydrates). Fermentation was conducted under controlled laboratory conditions for 5 d. Bacterial isolation was performed following the procedure described by Teul *et al.* (2023), with modifications. Briefly, 10 g of fermented kimchi were transferred into sterile saline solution. Subsequently, 1 ml of the homogenized suspension was serially diluted until a final dilution of 10^{-7} was obtained. For bacterial isolation, 1 ml aliquots from the 10^{-5}, 10^{-6}, and 10^{-7} dilutions was plated onto Nutrient Agar (NA) plates, spread using a sterile glass spreader, and incubated at 30 °C for 48 h. Distinctive macroscopic characteristics were used to select and isolate the bacterial colonies by the quadrant streaking method. Pure cultures were stored at 4 °C for short-term storage on NA slants and cryopreserved with 20% (v/v) glycerol at −80 °C until further analysis.

Qualitative screening for mannanolytic activity

The bacterial isolates were screened on a specific agar medium containing 1% (w/v) glucomannan as the primary carbon source and a mineral salts mixture consisting of 0.9% peptone, 0.1% yeast extract, 0.1% KH_2PO_4, 0.05% $MgSO_4$, 1.5% bacteriological agar, and 0.0002% MnSO4, following Muzaki *et al.* (2021) method with minor modifications. The method consisted of spot-inoculating a pure isolate onto the surface of medium using a pointed loop, incubation at 30 °C for 48 h, and flooding the medium surface with a 0.1% (w/v) Congo red solution for 15 min. Afterwards, a 1 M NaCl solution was used for 15 min. to de-stain. Appearance of a clear halo around the colony in relation to the hydrolysis of the glucomannan substrate was regarded as a mark of mannanolytic activity. The mannanolytic index (MI) was calculated using Equation 1 (Muzaki *et al.*, 2021).

$$MI = \frac{\text{Diameter of Clear Zone}}{\text{Diameter of Colony}} \quad (1)$$

This calculation is frequently employed in agar-based hydrolysis assays to normalize halo formation relative to colony size. Isolates exhibiting visible hydrolysis zones were subsequently selected for quantitative analysis of β-mannanase activity.

Quantitative β-mannanase activity and thin-layer chromatography analysis

Isolates with detectable mannanolytic activity on glucomannan agar medium (KS2, KS3, KS5, KS6, KS7, KS9, KS11, and KS13) were initially grown in Luria-Bertani (LB) broth for 24 h at 150 rpm and subsequently transferred to a glucomannan broth medium for 5 d at 30 °C. The culture was centrifuged at 10.000 g, 4 °C, for 10 min, and the cell-free supernatant was collected as a crude enzyme supernatant according to the study reported by Yopi *et al.* (2017), with slight modifications. The crude enzyme from each selected bacterial isolate was mixed with 1% glucomannan in phosphate buffer (50 mM, pH 7.0) at a 1:1 (v/v) ratio and incubated at 55 °C for 60 min (Dhawan, 2021). The reaction mixture was used for detection of β-mannanase activity and thin-layer chromatography (TLC) analysis.

The activity of β-mannanase was determined through the Nelson-Somogyi method using D-mannose as a standard (Nelson, 1944; Somogyi, 1952). Following the degradation of glucomannan, the reaction was terminated by adding the Somogyi reagent, and the mixture was heated to allow the reducing sugars to react with the copper reagent. A green-blue complex was formed after reaction of the sample with the arsenomolybdate reagent, and the absorbance was quantified at 540 nm using using a Jenway 7315 UV-

Vis Spectrophotometer (Fisher Scientific, UK). A standard curve was constructed using D-mannose solutions at different concentrations, and the relationship between absorbance and D-mannose concentration was determined by a linear regression using the equation (y = mx + c), where (y) represents the absorbance, (x) represents the D-mannose concentration, (m) represents the slope, and (c) represents the y-intercept of the D-mannose standard curve. One unit of mannanase activity (U) was defined as the amount of enzyme required to release 1 µmol of D-mannose equivalents per minute under the assay conditions. In Equation 2, the term (A-c)/m) represents the D-mannose-equivalent concentration calculated from the final absorbance of the sample using the D-mannose standard curve. Enzyme activity was evaluated using Equation 2 (Norizan *et al*., 2020).

$$\text{Mannanase activity (U/ml)} = \frac{(A-c)}{m} \times \frac{DF \times 1000}{Ve \times Rt \times MWmannose} \quad \ldots (2)$$

Where; A= final absorbance of the sample, c= y-intercept of the mannose standard curve, m= slope of the mannose standard curve, DF= dilution factor of the enzyme sample, V_e = volume of enzyme sample used in the reaction (ml), R_t = reaction time (min.), $MW_{mannose}$ = molecular weight of D-mannose, 1000 = conversion factor from mg to µg, U/ml = enzyme activity expressed as µmol of mannose equivalents released/ min./ ml of enzyme.

Thin-layer chromatography (TLC) was used to qualitatively assess the hydrolysis products obtained from glucomannan degradation. A 30 µl aliquot of each hydrolysate was spotted onto a silica gel plate, along with mannose and mannobiose standards. The plate was developed using a mobile phase of 1-butanol: ethyl acetate: distilled water (2:1:1, v/v/v). Afterwards, the plates were dried in air for 10 min. after being sprayed with 10% H_2SO_4 in 80% methanol, and finally visualized after heating in an oven at 110 °C for 10 min (Kim *et al*., 2018).

Liquid chromatography-tandem mass spectrometry analysis of the selected hydrolysate

The crude enzyme supernatant was obtained from the selected isolate after 5 d of fermentation in glucomannan-containing medium. The hydrolysate was subsequently analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following the analytical procedure reported by Akili *et al*. (2026). The hydrolysate was filtered using a 0.22 µm syringe filter before LC-MS/MS analysis. Ultra-Performance Liquid Chromatography (UPLC) (ACQUITY UPLC® H-Class, Waters Corporation, Milford, MA, USA) was coupled with a quadrupole time-of-flight mass spectrometer (Xevo G2-S QTof, Waters Corporation, Milford, MA, USA) for analysis. Chromatographic separation was performed on an ACQUITY UPLC® HSS C18 column (1.8 µm, 2.1 × 100 mm; Waters Corporation, Milford, MA, USA) maintained at 50 °C. The mobile phase comprised water with 5 mM ammonium formate (mobile phase A) and acetonitrile with 0.05% formic acid (mobile phase B), delivered at a flow rate of 0.2 ml/min. The total run time was 23 min., and the injection volume was 5 µl. Mass spectrometric detection employed electrospray ionization (ESI) in positive-ion mode over a mass range of 50–1200 m/z. The ion source temperature was 100 °C, the desolvation temperature was 350 °C, and the desolvation gas flow rate was 793 l/h. Collision energy levels of 4-60 eV were used for tandem mass spectrometric analysis. Data acquisition and processing were carried out using MassLynx version 4.1 software (Waters Corporation, Milford, MA, USA).

Morphological and molecular characterizations of the selected isolate

The selected isolate was characterized based on colony morphology and Gram-staining characteristics. Genomic DNA was extracted using a commercial DNA extraction kit according to the manufacturer's instructions. The bacterial 16S rRNA gene was amplified by PCR using two universal primers: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′), which target conserved regions of the bacterial 16S rRNA gene and amplify a nearly full-length 16S rRNA gene fragment (Frank *et al*., 2008). The PCR products were purified and submitted to 1st BASE for Sanger sequencing. The resulting sequences were edited using BioEdit (Hall, 1999). Sequence similarity was assessed using the Basic Local Alignment Search Tool for nucleotides (BLASTn) against the NCBI nucleotide database (Altschul *et al*., 1990). Reference sequences representing closely related taxa identified through BLASTn were retrieved from the NCBI nucleotide database and included in the subsequent phylogenetic analysis. Multiple sequence alignment and phylogenetic reconstruction were performed

using MEGA X (Kumar *et al.*, 2018). The phylogenetic tree was reconstructed using the selected nucleotide substitution model and the bootstrap analysis was performed to assess the reliability of the inferred phylogenetic relationships.

Growth and production profile of the selected isolate A single colony of the selected isolate was grown in LB broth at 30 °C for 24 h. An inoculum (8 ml) was aseptically transferred into 100 ml of glucomannan broth medium and incubated at 150 rpm, according to the method reported by Norizan *et al.* (2020). Samples were collected at 12 h and 24 h intervals to monitor bacterial growth by measuring the optical density at 600 nm (OD_{600}). To determine viable cell counts, the samples were serially diluted using 0.85% NaCl. 1 ml of the appropriate dilution was transferred onto nutrient agar (NA) plates and incubated at 30 °C for 24 h. Colonies were counted on the plates containing a suitable number of colonies, and viable cell density was expressed as colony-forming units per milliliter (cfu/ml) according to the following Equation 3 (Norizan *et al.*, 2020):

$$\text{cfu/ml} = \frac{\text{Number of Colonies}}{\text{Dilution x Volume Plated (ml)}} \quad \ldots (3)$$

The growth curve was generated by plotting $\log_{10}$ cfu/ml against cultivation time. The OD_{600} values were compared with the corresponding viable cell counts to establish the relationship between optical density (OD) and bacterial concentration under the experimental conditions. Based on the established correlation, an OD_{600} value of 1 corresponded to approximately 2.748×10^8 cfu/ml. The cell-free supernatant was collected at 24 h intervals throughout the incubation period and analyzed for β-mannanase activity using the Nelson–Somogyi method described above. The bacterial growth and β-mannanase production profiles were then compared over the cultivation period to determine the cultivation time associated with the highest enzyme activity.

Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation (±SD). Variation among isolates was assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test. Statistical significance was defined as $p < 0.05$.

Results

Isolation and qualitative screening of mannanolytic bacteria

Thirteen bacterial isolates were obtained from cabbage kimchi. Eight isolates produced visible clear halos after Congo red staining on glucomannan agar and were therefore classified as qualitatively mannanolytic bacterial isolates under the screening conditions. The present data showed a range of MI values ranging from 1.028 ± 0.006 to 1.717 ± 0.021. KS7 isolate demonstrated the highest MI (1.717 ± 0.021), followed by KS9 (1.565 ± 0.038), and KS11 (1.369 ± 0.049). KS2 (1.227 ± 0.025) and KS3 (1.129 ± 0.021) isolates displayed intermediate MI values, whereas KS5 (1.057 ± 0.007), KS6 (1.040 ± 0.017), and KS13 (1.028 ± 0.006) exhibited lower MI values. Significant differences were observed among the isolates (one-way ANOVA, $F = 287.20$, $p < 0.0001$). Tukey's HSD post hoc test showed that KS7 formed a distinct statistical group with a significantly higher MI than all other evaluated isolates. KS9, KS11, and KS2 also formed distinct statistical groups, whereas KS5 was not significantly different from KS3, KS6, or KS13, as indicated by Tukey's HSD grouping letters (Figure 1).

Quantitative screening of mannanolytic bacteria

The mannanolytic bacterial isolates examined in this study were capable of degrading glucomannan in broth medium, resulting in a measurable extracellular β-mannanase activity ranging from 4.370 ± 0.494 U/ml to 34.125 ± 1.087 U/ml (Figure 2). Significant differences were observed among the isolates (one-way ANOVA, $F = 321.45$, $p < 0.001$). Tukey's HSD post hoc test classified the isolates into five statistically distinct groups: group a (KS7), group b (KS9), group c (KS2 and KS11), group d (KS3), and group e (KS5, KS6, and KS13). Isolates sharing the same letter did not differ significantly, whereas isolates with different letters showed significant differences in β-mannanase activity.

Thin-layer chromatography profiles of glucomannan hydrolysates

Thin-layer chromatography exhibited multiple mannoligosaccharide spots in the hydrolysates of all the eight selected isolates (Figure 3). The hydrolysates of KS7, KS9, and KS11 showed prominent spots at migration positions corresponding to lower- DP products, particularly around the mannose (M1) and

mannobiose (M2) standards. Based on the obtained combined results of the screening assays, KS7 was selected for further LC-MS/MS analysis because it exhibited the highest MI and β-mannanase activity among the eight isolates and showed a pronounced hydrolysis profile towards lower-DP products.

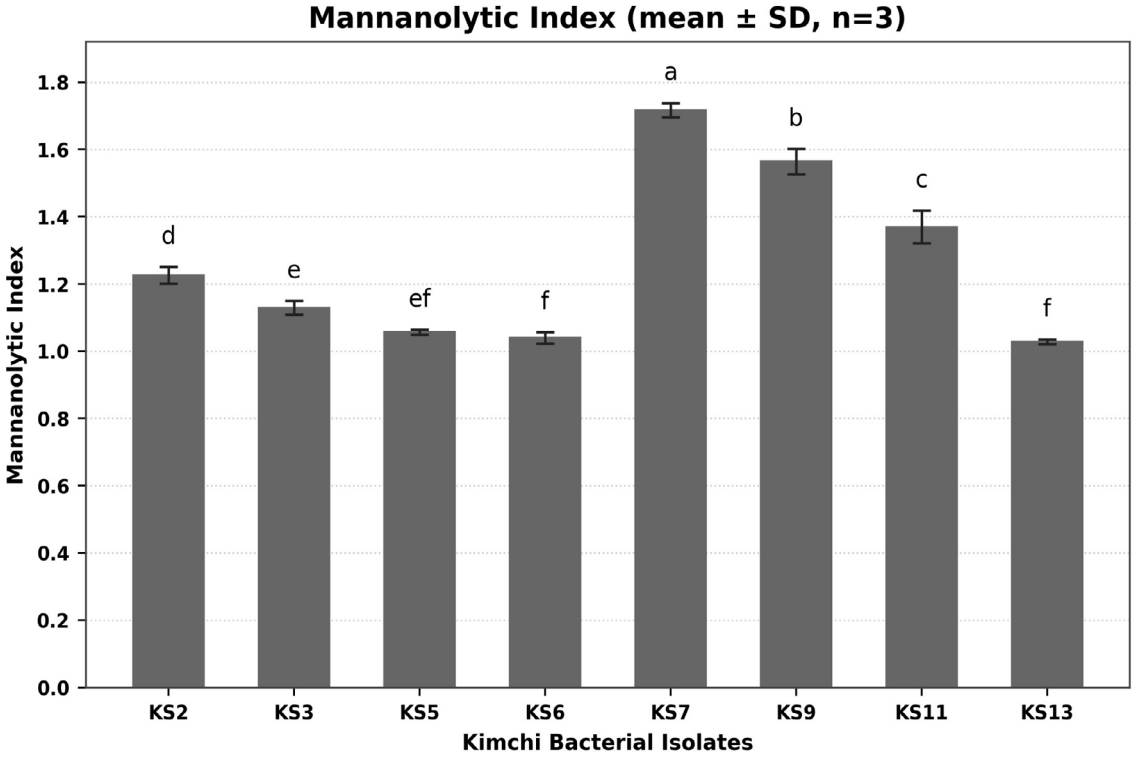

Figure 1: *Mannanolytic index (MI) of glucomannan-degrading bacterial isolates obtained from cabbage kimchi. Data are presented as mean ± SD (n = 3), with error bars representing standard deviation. Different lowercase letters indicate significant differences according to Tukey's HSD test (p < 0.05).*

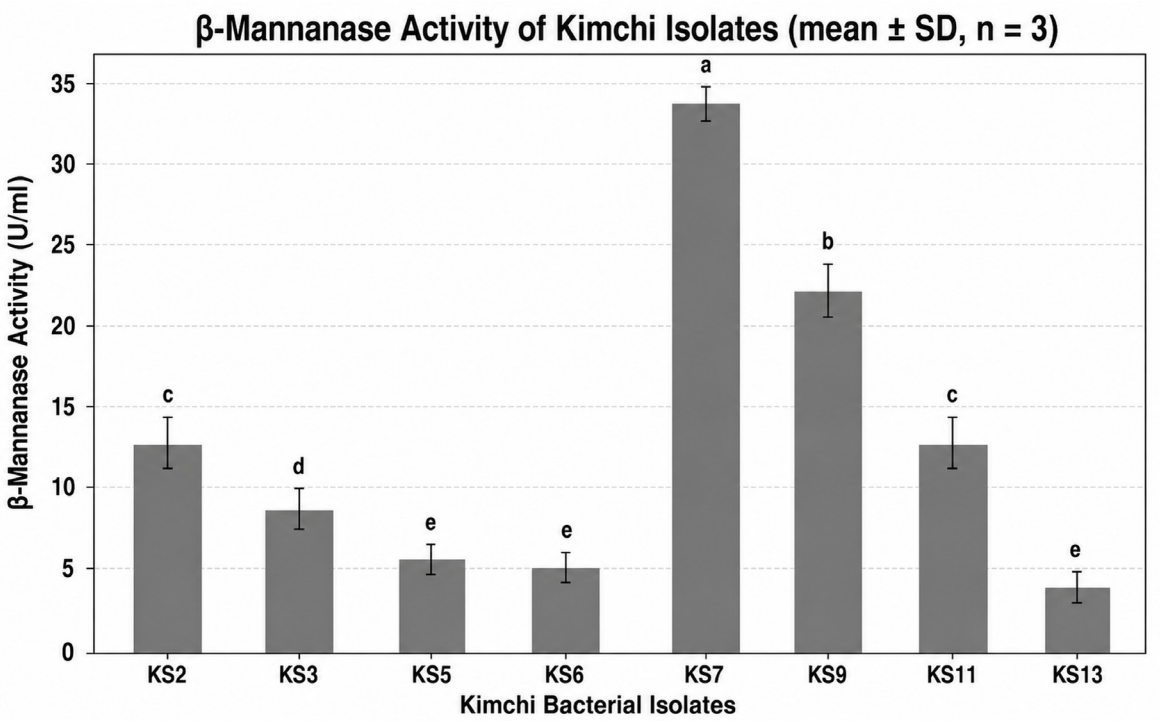

Figure 2: *Extracellular β-mannanase activity of cabbage-kimchi bacterial isolates. Data are presented as mean ± SD (n = 3), with error bars representing standard deviation. Different lowercase letters indicate significant differences according to Tukey's HSD test (p < 0.05).*

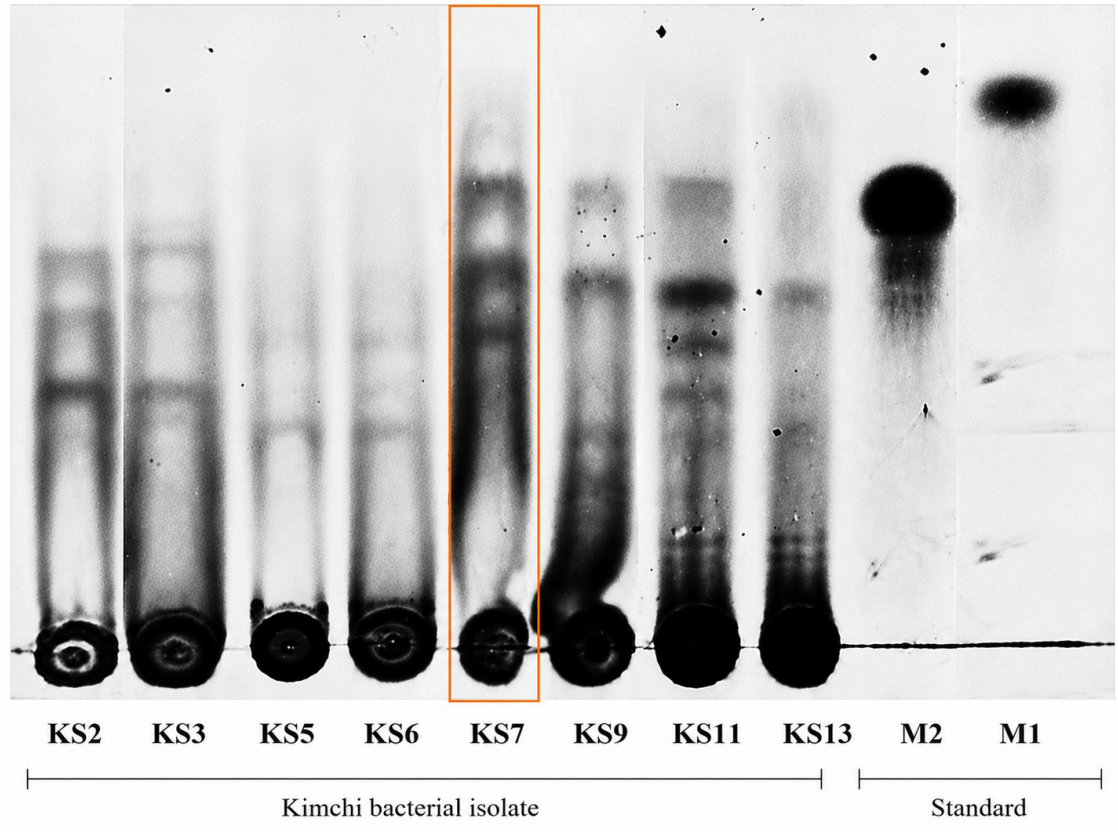

Figure 3: *Thin-layer chromatographic (TLC) profiles of glucomannan hydrolysis products produced by the eight selected bacterial isolates, with mannobiose (M2) and mannose (M1) used as standards.*

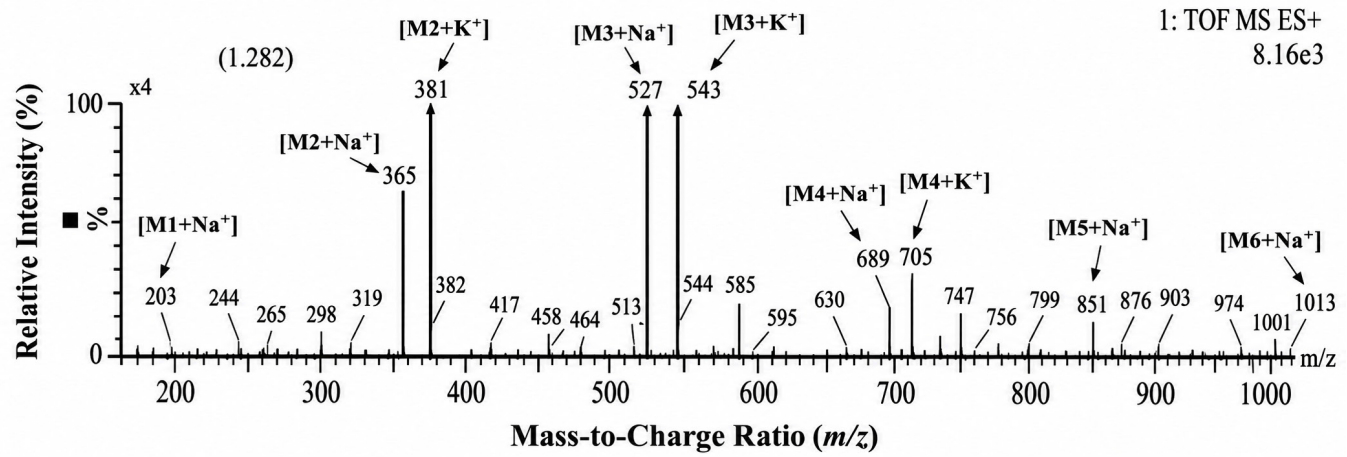

Figure 4: *Positive-ion LC-MS/MS analysis of the selected KS7 isolate hydrolysate. M1–M6 represent mannooligosaccharides with degrees of polymerization (DP) of 1–6, respectively. In the ion labels, M represents the neutral molecular form of the detected mannose and mannooligosaccharides (MOS), while Na and K represent sodium (Na^+) and potassium (K^+) adduct ions, respectively.*

LC-MS/MS assignments in the selected KS7 hydrolysate

Positive-ion LC-MS/MS analysis of the selected KS7 isolate hydrolysate yielded an ion series predominantly detected as sodium- and potassium-adducts of hexose-based oligomers (Figure 4). The ion at *m/z* 203 that matched the sodium-adducted hexose monomer was indicative of the mannose (M1) and matched the expected molecular weight of a neutral 180 Da hexose associated with Na^+. The sequential signals obtained from mass spectrometry at *m/z* 365/381, 527/543, and 689/705 corresponded to sodium and potassium adducts of mannobiose (M2), mannotriose (M3), and mannotetraose (M4), respectively. Mannopentaose (M5) and mannohexaose (M6) were also detected as sodium adducts at *m/z* 851 and 1013, respectively. Based on the precursor ion masses, the KS7 hydrolysate contained mannooligosaccharides with a DP ranging from 2 to 6 (DP2–DP6).

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Preliminary molecular identification of the selected KS7 isolate

Phylogenetic analysis based on 16S rRNA gene sequences positioned the selected isolate KS7 within the *Bacillus* lineage, forming a distinct branch separated from the reference strains with a bootstrap value of 85% (Figure 5). The reference strains included several *Bacillus* species: *B. albus* MCCC 1A02146, *B. cereus* ATCC 14579, *B. pacificus* MCCC 1A06182, *B. paranthracis* MCCC 1A00395, *B. paramycoides* MCCC 1A04098, *B. wiedmannii* FSL W8-0169, *B. proteolyticus* MCCC 1A00365, *B. sanguinis* MBL-B004, and *B. fungorum* 17-SMS-01. Among these, *B. albus* MCCC 1A02146 showed the highest 16S rRNA gene sequence similarity to KS7 (95.72%). This value remained below the threshold commonly used for species-level identification based solely on 16S rRNA gene sequences. Accordingly, isolate KS7 was tentatively classified as a *Bacillus* sp. PZ852543 based on the 16S rRNA gene analysis. Macroscopic examination showed circular, cream-colored colonies with entire margins and a flat elevation on NA. Microscopic analysis showed Gram-positive, rod-shaped cells. These phenotypic observations were considered complementary to the molecular characterization of KS7.

Growth and enzyme-production profile of the selected Bacillus sp. strain KS7

Viable *Bacillus* sp. strain KS7 cell counts increased progressively during the initial cultivation period, reaching a maximum of 8.66 $\log_{10}$ cfu/ml at 120 h (Figure 6). Cell density remained stable until 144 h, followed by a decline at 168 h. Concurrently, extracellular β-mannanase activity rose, peaking at 120 h. The temporal alignment of maximal enzyme production with the transition from exponential to stationary growth phases indicated that *Bacillus* sp. strain KS7 cells synthesized the highest levels of β-mannanase during the early stationary phase.

Discussion

In this study, 13 distinct bacterial isolates were recovered from cabbage kimchi, eight of which exhibited a mannanolytic activity. This result is consistent with the premise that kimchi is a dynamic fermentation matrix harboring a diverse culturable microbiota, which is heavily influenced by the ingredient composition and processing parameters (Kim *et al.*, 2021; Lee *et al.*, 2024). Previous culture-dependent studies have similarly shown that a wide variety of viable isolates can be recovered from kimchi under non-selective conditions (Kim *et al.*, 2021). In the present work, non-selective bacterial isolation followed by a substrate-specific screening enabled a functional selection without presuming a particular taxonomic group. This strategy is appropriate for enzyme prospecting, although it does not describe the abundance or ecological role of the isolates in the original fermentation. The extensive variance in MI values

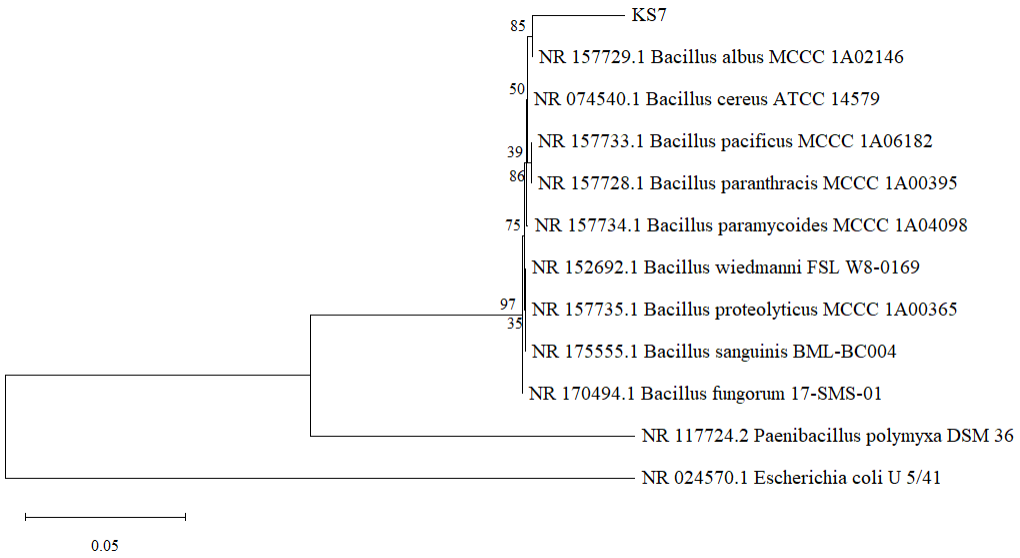

Figure 5: *Neighbor-joining phylogenetic tree of KS7 strain based on 16S rRNA gene sequences. The tree was constructed using 1,000 bootstrap replicates. Numbers at the nodes indicate bootstrap support values (%), and the scale bar represents the number of nucleotide substitutions per site. Paenibacillus polymyxa DSM 36 and Escherichia coli U 5/41 were used as outgroups.*

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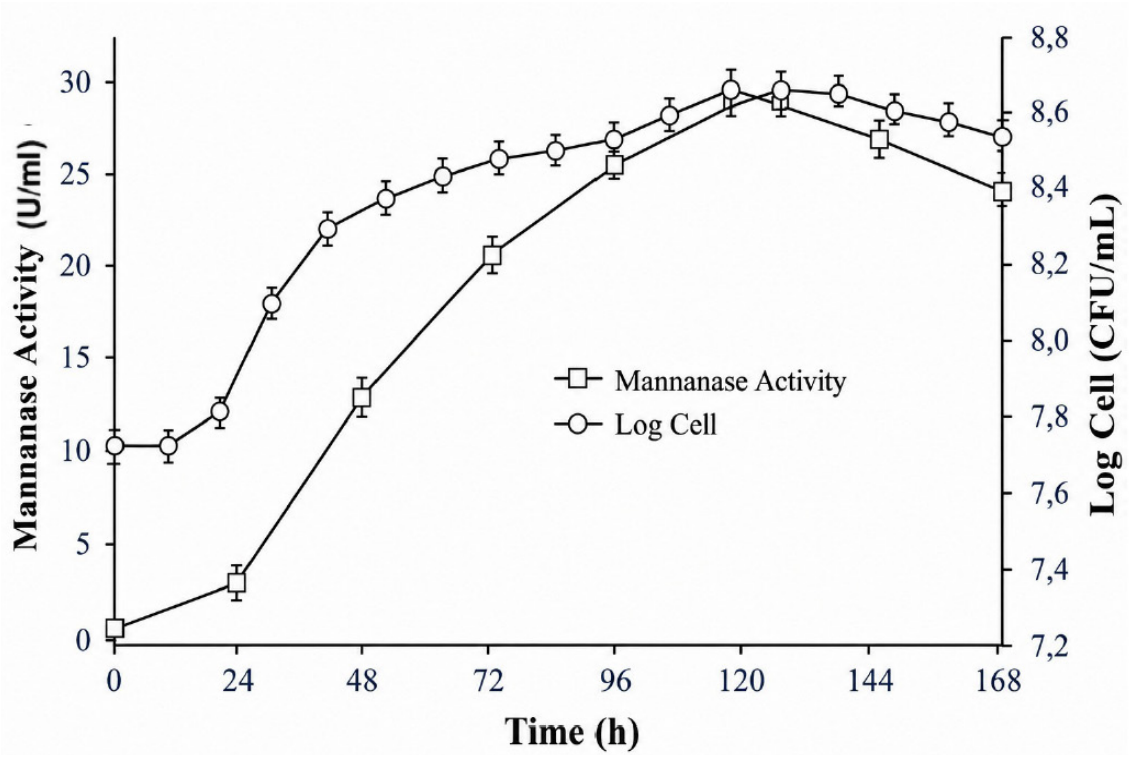

Figure 6: *Growth and extracellular β-mannanase production profile of the promising KS7strain in a glucomannan medium. Values are presented as mean ± SD of three independent replicate (n = 3), with error bars representing standard deviation.*

(1.028±0.006 to 1.717±0.021) indicated a considerable phenotypic variability in extracellular glucomannan hydrolysis. Although the MI provided a useful preliminary screening metric, it additionally integrated multiple factors, including enzyme secretion, colony expansion, substrate accessibility, and agar diffusion properties. Therefore, MI should not be interpreted as a direct measure of a catalytic efficiency. The observed positive correlation between elevated MI and enzyme activity in the KS7 isolate justified its selection for further studies. In the meantime, the lower concordance among the other isolates underscored the need for a quantitative secondary evaluation. A recent study on *B. licheniformis* indicated that glucomannan degradation often involves the synergistic functioning of diverse extracellular enzymes (Zhang *et al.*, 2024).

Microorganisms possessing diverse carbohydrate-active enzymes are better adapted to exploit specific polysaccharide resources, contributing to niche differentiation within the microbial communities (Sun *et al.*, 2023). Consequently, fermented foods have been considered potential sources for the isolation and characterization of microorganisms producing hydrolytic enzymes, including β-mannanase (Oh *et al.*, 2002; Regmi *et al.*, 2016). Quantitative screening revealed that crude supernatant enzyme production by some kimchi-derived isolates was relatively high (4.370±0.494 to 34.125±1.087 U/ml) when glucomannan was used as the substrate, suggesting a remarkable potential for glucomannan hydrolysis. In comparison, *Streptomyces violascens* BF 3.10 and *Aureobasidium pullulans* NRRL 58524 exhibited mannanase activities of 16.38 and 8.07 ± 0.12 U/ml with porang and konjac glucomannan, respectively (Safitri *et al.*, 2014; Ibrahim *et al.*, 2022). Ong *et al.* (2024) study reported a maximum activity of approximately 165 U/g palm kernel meal (PKM) using konjac glucomannan.

Thin-layer chromatography provided qualitative evidence that all the eight selected isolates converted glucomannan into smaller saccharide products, while the stronger patterns observed for KS7, KS9, and KS11 isolates paralleled their screening performance. From an enzymological perspective, the occurrence of sequential oligosaccharide fractions is anticipated, given that endo-β-mannanase hydrolyzes internal β-1,4-mannosidic bonds within the glucomannan backbone to yield a diverse mixture of mannan oligosaccharides (Couturier *et al.*, 2022; Ratnakomala *et al.*, 2022; Briganti *et al.*, 2024). The current observed product profile may be attributed to the concerted action of multiple enzymes targeting

the intermediate oligosaccharides. Furthermore, accessory enzymes, such as β-mannosidase and other exo-acting glycosidases may facilitate the subsequent degradation of these intermediates into smaller saccharide units (Bågenholm *et al*., 2017; Couturier *et al*., 2022). The present study did not purify the active protein or identify its family within the Carbohydrate-Active enzyme's (CAZy) classification; consequently, the hydrolysis phenotype cannot yet be attributed to a single β-mannanase.

The LC-MS/MS ion series is compatible with sodium- and potassium-adducted hexose oligomers with DP. This current ion distribution was comparable to previously reported ESI-MS profiles of enzymatically generated mannose and MOS (Moreira *et al*., 2011; Zhang *et al*., 2021). The predominance of sodium adduct formation was consistent with earlier studies reporting the preferential detection of MOS as sodium-associated ions under positive ESI conditions (Moreira *et al*., 2011; Bååth *et al*., 2018). Nevertheless, the production of a DP2-DP6 series was technologically relevant because contemporary MOS research increasingly targets controlled product distributions rather than indiscriminate polymer degradation. Several enzymatic systems have recently been used to generate MOS with defined DP ranges from different mannan-rich substrates. For example, a β-mannanase from *Bacillus* sp. was used to produce β-MOS from copra meal mannan (Cuong *et al*., 2024), while a β-mannanase from *Paenibacillus polymyxa* generated konjac glucomannan oligosaccharides with DP2–DP13 (Rahman *et al*., 2025).

Although the phylogenetic tree placed the selected isolate near the *B. cereus* group, a 95.72% identity was too low to support the claimed close relationship. This level of similarity does not provide a sufficient support for species-level assignment. A recent evaluation of 16S rRNA gene identity boundaries based on 19,556 prokaryotic type strains showed that in 90% of cases, strains belonging to the same species shared minimum sequence identities of 97.2–100%, whereas the corresponding range for genus-level classification was 90.1–99.0%, with a substantial overlap between the taxonomic boundaries (Hackmann, 2025). The 95.72% similarity observed for isolate KS7 should therefore be interpreted conservatively and supports its classification as *Bacillus* sp. based on the 16S rRNA gene data. Moreover, 16S rRNA sequences have insufficient resolution to distinguish among the closely related members of the *B. cereus* group; thus, whole-genome sequencing (WGS) with an average nucleotide identity and digital DNA-DNA hybridization is strongly recommended (Liu *et al*., 2015; Kowalska *et al*., 2024; Rutkowska *et al*., 2025).

Strains within the *B. cereus* group exhibit a considerable variation in carbohydrate utilization (Warda *et al*., 2016). Several members of this group and other *Bacillus* species have also been reported to harbor genes encoding glycoside hydrolases associated with plant polysaccharide utilization (Kim *et al*., 2018; Couturier *et al*., 2022; Briganti *et al*., 2024; Rutkowska *et al*., 2025). β-Mannanases are found in several glycoside hydrolase families, including GH5, GH26, GH113, and GH134 (Drula et al., 2022). The enzymatic basis of this activity remained ambiguous because neither enzyme purification nor CAZy families were defined in the current study. Therefore, it has not yet been confirmed whether glucomannan depolymerization was mediated by a single β-mannanase or by multiple glycoside hydrolases. This result remains to be investigated in future genomic and biochemical studies.

The maximum β-mannanase activity observed during the transition to the stationary phase suggested that enzyme production was growth-associated or early-stationary-phase enhanced under the tested conditions. In accordance with the current results, several previous studies had reported similar results for *Bacillus* sp. SWU60, *B. subtilis* ATCC 11774, and *Streptomyces violaceoruber*, which exhibited the highest mannanase activity in the stationary growth phase (Safitri *et al*., 2014; Seesom *et al*., 2017; Norizan *et al*., 2020). Mannanase activity continued to decrease gradually over an extended incubation period beyond 120 h. Similar post-peak reductions have been reported during *B. subtilis* fermentation, in which prolonged cultivation was accompanied by decreases in cell biomass and β-mannanase production, potentially reflecting entry into the death phase and degradation of the extracellular proteins (Norizan *et al*., 2020). The reduction in β-mannanase activity after its peak may be attributed to lower cell metabolic activity, degradation or instability of the enzyme during prolonged cultivation, and accumulation of products that can interfere with substrate-enzyme interactions and/or lead to repression of subsequent synthesis (Dhawan and Kaur, 2007; Srivastava and Kapoor, 2017). This study revealed that the stationary

growth phase plays a key regulatory role in mannanase biosynthesis in the mannan-degrading bacteria.

Conclusions and Recommendations

This study showed that cabbage kimchi can serve as a source of culturable bacteria with extracellular β-mannanase activity. A total of 13 isolates were obtained, of which 8 exhibited mannanolytic activity, with mannanolytic index ranging from 1.028 ± 0.006 to 1.717 ± 0.021 and β-mannanase activities ranging from 4.370 ± 0.494 to 34.125 ± 1.087 U/ml. Among them, KS7 isolate showed the strongest mannanolytic activity, with an index of 1.717 ± 0.021 and an enzyme activity of 34.125 ± 1.087 U/ml, and produced mannooligosaccharides with degrees of polymerization ranging from 2 to 6. Molecular characterization placed KS7 isolate within the genus *Bacillus*, with 95.72% sequence homology to *Bacillus albus* MCCC 1A02146. Overall, *Bacillus* sp. KS7 strain represents a promising candidate for further development as a β-mannanase-producing strain for mannooligosaccharide production. However, the taxonomic identity of the *Bacillus* sp. KS7 strain and the structural identities of the detected oligosaccharides remain provisional and require further confirmation. Further studies are recommended to confirm the taxonomic identity and biosafety of the selected strain, characterize and purify the β-mannanase, and verify the structures of the resulting oligosaccharides using complementary analytical methods. Evaluation under process-relevant conditions is also recommended before any practical or industrial application is considered.

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Novelty Statement

The novelty of this study lies in the discovery and characteristization of a culturable mannanolytic *Bacillus* sp. KS7 bacterial strain capable of highly degrading glucomannan and producing mannooligosaccharides. This study demonstrates the combined mannanolytic activity, glucomannan-degrading capability, and mannooligosaccharide-producing potential of this strain. These findings highlight the potential of kimchi-derived bacteria as sources of mannanolytic enzymes and provide a basis for further investigation of *Bacillus* sp. KS7 strain as a potential biocatalyst for glucomannan hydrolysis and mannooligosaccharide production.

Authors' Contributions

Shavira Amalia Rahim: Conceptualization, investigation, data curation, formal analysis, visualization, and writing - original draft, and writing review and editing.
Prayoga Suryadarma: Conceptualization, methodology, supervision, resources, validation, and writing - review and editing.
Nisa Rachmania Mubarik: Conceptualization, methodology, supervision, validation, and writing review and editing.
Dian Natalia Peni: Investigation and data curation.
All authors reviewed and approved the final manuscript.

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