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The Characterization of L-Asparaginase with Low L-Glutaminase Activity Produced by the Marine *Pseudomonas* sp. Strain GH-W2b

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Abstract: L-asparaginase (ASNase) hydrolyzes L-asparagine to L-aspartic acid and ammonia and has been used as an antitumor agent for the treatment of acute lymphoblastic leukemia. ASNase has also been used to mitigate the suspected carcinogenic effects of acrylamide in foods. Commercial ASNases currently used in the pharmaceutical and food industries are produced by microorganisms, such as bacteria and fungi. However, their toxicity and poor thermal stability limit their application. Therefore, identifying novel sources of ASNase is critical. In the present study, we identified an asparaginase-producing marine bacterial strain, GH-W2b, as a *Pseudomonas* species. Based on the plate assay results, GH-W2b produced ASNase with marginal L-glutaminase (GLNase) activity, which has been reported to cause adverse effects in clinical ASNases. The ASNase activity of GH-W2b was maximized at 50–65 °C and pH 7.0–8.5. Notably, the activities were consistent at a wide range of NaCl concentrations (0–15%) at 37 °C. In addition, compared to the control (no pre-incubation), ASNase activities were retained (>87%) by 2 h pre-incubation at 4–37 °C. Overall, our results suggest that GH-W2b ASNase has the potential to serve as a candidate for the development of salt-tolerant and/or alternative ASNases in pharmaceutical and food products.

Keywords: L-asparaginase; a marine bacterium; *Pseudomonas* sp.; L-glutaminase

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1. Introduction

L-Asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1, ASNase) catalyzes the hydrolysis of L-asparagine to aspartic acid and ammonia and concomitantly possesses glutaminase (GLNase) activity [1]. ASNase is clinically used to treat acute lymphoblastic leukemia (ALL) and lymphosarcoma [2]. As a therapeutic enzyme, clinical formulations of ASNase account for 40% of the global enzyme demand [3]. Additionally, ASNase is used to reduce the formation of acrylamide, a possible carcinogen, in heated foods such as potato chips, bread, and coffee [4]. ASNase is listed as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration (FDA).

ASNase is widely distributed in animals, plants, and microorganisms, including archaea, bacteria, actinomycetes, fungi, and microalgae [5]. Microorganisms are preferred over other sources for large-scale production because they are easier to cultivate, extract, and optimize in a cost-effective manner [3]. Currently, commercial forms of clinical ASNase

including Elspar®, Oncaspar®, Erwinase®, and Kidrolase® are originated from the bacteria, *Escherichia coli* and *Erwinia chrysanthemi* [6]. Moreover, ASNases used in the food industry under the trade names Acrylaway® and PreventAse® are produced by the fungi, *Aspergillus oryzae* and *Aspergillus niger* [4]. However, the application of currently used ASNases is limited because they have significant side effects, including toxicity and low thermal stability for chemotherapeutic and food applications, respectively [4,7]. Therefore, it is critical to explore various microbial resources to discover novel ASNases.

Marine microorganisms have a high potential to produce novel and unique bioactive compounds compared to terrestrial microorganisms because they can adapt to harsh ocean conditions, such as high salinity and a wide range of temperatures and pressures [8]. Microorganisms isolated from different marine substrates have been reported to produce various enzymes, including alginate lyase, amylase, cellulase, chitinase, ligninase, lipase, protease, and xylanase [9]. These enzymes possess more effective and stable activities than terrestrial enzymes under extreme temperature, pH, and salinity conditions [10].

ASNase activity has been characterized in several marine bacteria, actinomycetes, and fungi [11]. ASNases produced by the marine bacterium *Bacillus* sp. TVS55 and the marine actinomycete *Streptomyces canus* LA-29 exhibit anticancer activity [12,13]. Moreover, ASNases from *Paenibacillus barengoltzii* CAU904 and the marine fungus *Trichoderma viride* effectively reduce acrylamide formation in fried potatoes [14,15]. However, marine microbial ASNases remain largely unexplored.

In this study, we aim to explore alternative resources to develop novel ASNases with improved stability and fewer side effects. To address this, we characterized the ASNase activity of *Pseudomonas* sp. strain GH-W2b isolated from seawater. Using the crude enzyme extract, we examined the optimum temperature, pH, and NaCl concentration for GH-W2b ASNase activity. In addition, the thermostability of GH-W2b ASNase was investigated.

2. Materials and Methods

2.1. Sample Collection and Bacterial Isolation

Seawater was collected from Sacheon, Republic of Korea (34°55′43.5″ N, 128°03′24.8″ E), in August 2019; serially diluted to 10^{-3} with sterilized seawater; and spread onto marine agar (MA; BD, Franklin Lakes, NJ, USA) as described previously [16]. After incubation at 25 °C for 7 days, individual bacterial colonies were selected and transferred onto fresh MA to obtain pure cultures. After isolation, the bacterial strains were cultured on Luria–Bertani agar (LB agar; BD) at 25 °C unless described otherwise. GH-Wb2 was deposited in the Microbial Marine Bio Bank (MMBB) of the National Marine Biodiversity Institute of Korea (MABIK) (strain number MABIK MI00009254).

2.2. Examination of ASNase and GLNase Activities in Marine Bacteria

To examine L-asparaginase (ASNase) and/or L-glutaminase (GLNase) activities in marine bacterial strains, modified asparaginase dextrose salt agar (MADS) was used to culture the bacteria [17]. The composition of MADS was as follows: 2.0 g glucose, 10.0 g L-asparagine monohydrate (Sigma-Aldrich, St. Louis, MO, USA), 1.52 g KH_2PO_4, 0.52 g KCl, 0.52 g $MgSO_4 \cdot 7H_2O$, 0.05% (*w*/*v*) $CuNO_3 \cdot 3H_2O$, 0.05% $ZnSO_4 \cdot 7H_2O$, 0.05% $FeSO_4 \cdot 7H_2O$, and 15.0 g agar for 1 L media. Stock solutions of bromothymol blue (BTB) and phenol red (PR), as pH indicators of ammonia production, were each prepared at 2% (*w*/*v*) in ethanol and subsequently added to MADS agar at a final concentration of 0.005% (*v*/*v*). The final pH of the medium was adjusted to 6.0 using 1 M NaOH solution. Instead of L-asparagine, L-glutamine was added to MADS agar to assess GLNase activity. Sodium nitrate ($NaNO_3$) was used as the nitrogen source in the control media. After incubation at 25 °C for 2 to

3 days, the ASNase and GLNase activities were determined by the presence of blue (from BTB) or pink (from PR) zones around the colony.

To compare enzyme activities of bacterial strains, the enzymatic index (EI) was determined as follows:

EI = [diameter of the hydrolyzed zones/diameter of the colonies]

The hydrolyzed zones indicate the presence of blue or pink area.

2.3. DNA Extraction, PCR, and Phylogenetic Analysis

The genomic DNA of GH-W2b was extracted from 5 mL of culture grown overnight in LB broth using an Exgene DNA extraction kit (Gene All, Seoul, Republic of Korea) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was conducted using two primer sets: bacteria-specific universal primers 27F and 1492R to amplify the 16S rRNA gene [18] and 70Fs and 70Rs to amplify *rpoD* gene sequences (the sigma 70 factor subunit of the DNA polymerase) [19,20]. PCR products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and sequenced by Macrogen Inc. (Seoul, Republic of Korea). The amplified partial 16S rRNA and *rpoD* genes were assembled using Geneious program v9.0.5 to obtain a nearly full-length 16S rRNA gene sequence, which was deposited in the GenBank database under accession numbers PQ614850 and PQ633201 for the 16S rRNA and *rpoD* gene sequences, respectively. The 16S rRNA gene sequences (1340 nucleotides) of closely related taxa were searched using the EzBioCloud server (https://www.ezbiocloud.net/identify) (accessed on 22 October 2024). The *rpoD* gene sequences (844 nucleotides) were used as query sequences for the BLASTN search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (accessed on 15 November 2024) to assess sequence similarity. Phylogenetic analyses based on the 16S rRNA and *rpoD* gene sequences were conducted using the neighbor-joining (NJ) method with 1000 bootstrap resampled datasets in MEGA X version 11.0 [21].

2.4. Preparation of Crude Enzyme Extract

GH-W2b (100 µL of bacterial suspension at 10^7 CFU/mL) was inoculated in 200 mL of MADS broth without agar containing pH indicators and incubated at 25 °C for 2 days with shaking at 150 rpm. The cells were pelleted by centrifugation at 11,000 rpm for 15 min. The collected cells were washed twice with 50 mM Tris-HCl buffer (pH 8.0) and resuspended in 30 mL of buffer. The resuspended cells were placed on ice, sonicated for 20 min at 30 s intervals, and clarified by centrifugation at 11,000 rpm for 15 min. Supernatants were used as crude enzyme extracts to measure the ASNase activity of GH-W2b [22].

2.5. Quantitative Determination of ASNase Activity

ASNase activity was determined by measuring the amount of ammonia produced during the enzymatic reaction using the Nesslerization method [23,24]. The mixture containing 100 µL of 0.04 M L-asparagine prepared in 0.05 M Tris-HCl buffer (pH 8.0) and 50 µL of crude enzyme extract were reacted at 37 °C for 30 min. After 30 min, the reaction was terminated by 50 µL of 1.5 M trichloroacetic acid (TCA, Sigma-Aldrich). For the blank, the crude enzyme was treated with TCA to stop the enzymatic reaction prior to substrate addition to the reaction mixture.

Two sets (test and blank) of samples were centrifuged to remove the precipitated proteins, and the ammonia released in the supernatants was quantified colorimetrically by Nesslerization. One hundred microliters of clear supernatant was added to tubes containing 700 µL of distilled water, followed by the addition of 100 µL Nessler's reagent. The mixture was then incubated at room temperature for 20 min. The presence of ammonia is indicated by a yellow color change, whereas dark orange to brown precipitates may appear at higher ammonia concentrations. The absorbance of the reaction mixture was measured at 480 nm using a spectrophotometer (Hidex, Turku, Finland). The amount of ammonia released

was determined using an ammonium sulfate standard curve. The ASNase activity unit was defined as the amount of enzyme that releases 1 µmol of ammonia per hour under tested conditions.

2.6. Effect of Temperature, pH, NaCl Concentration, and Incubation Time on ASNase Activity

To study the effect of temperature on ASNase activity, 50 µL of GH-W2b crude enzyme extract was incubated with 100 µL of 0.04 M L-asparagine at 4, 15, 25, 37, 50, and 65 °C for 30 min. To examine the effect of pH on ASNase activity, the crude enzyme was incubated with 0.04 M L-asparagine prepared at various pH values (acetate buffer for pH 4.0 and pH 5.5, sodium phosphate buffer for pH 7, and glycine-NaOH buffer for pH 8.5 and pH 10) at 37 °C for 30 min. To assess the effect of incubation time on ASNase activity, the reaction mixture was incubated for varying durations: 15, 30, 45, 60, 75, 90, 105, and 120 min. The effect of 0, 2.5, 5, 10, and 15% (*w*/*v*) NaCl on ASNase activity was examined. The crude enzyme was incubated with 0.04 M L-asparagine solution supplemented with NaCl at 37 °C for 30 min. For the blank, the GH-W2b crude enzyme was first treated with TCA to stop the enzymatic activity and subsequently incubated with the substrate. ASNase activity was measured using the Nesslerization method described above.

2.7. Thermal Stability of ASNase

The thermal stability of ASNase was examined by pre-incubating the crude enzyme at different temperatures. Fifty microliters of the crude enzyme was pre-incubated at 4, 15, 25, 37, 50, and 65 °C for 2 h and promptly cooled in ice. After incubation with 0.04 M L-asparagine solution at 37 °C for 30 min, the ASNase activities of the test and blank samples were assessed as described above. Residual activities were calculated relative to the activity of the sample without pre-incubation, which was used as a control and set to 100%.

2.8. Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or *t*-test (GraphPad Prism software, version 5.0). All experiments were performed in triplicate unless stated otherwise.

3. Results

3.1. Examination of Activities of ASNase and GLNase by Plate Assay

We examined the ASNase and GLNase activities in marine bacteria using solid media supplemented with a pH indicator, phenol red (PR), or bromothymol blue (BTB). When cultured on the media containing asparagine at 25 °C for 2 days, GH-W2b formed a pink (from PR plates) and a blue (from BTB plates) zone around the colony (Figure 1). In the control plates containing $NaNO_3$, the pink and blue zones were much smaller and lighter in color compared to those on the asparagine plates (ASNase). On the glutamine plates (GLNase), the pink and blue zones were marginal and slightly larger than those on the control plates. Based on the EI values, the ASNase plates showed the highest activities (EI value = 3.35 ± 0.08 and 3.44 ± 0.05 on PR and BTB, respectively). The GLNase plates (EI value = 1.25 ± 0.01 and 1.45 ± 0.02 on PR and BTB) did not show significantly different activities from the control plates (EI value = 1.31 ± 0.06 and 1.50 ± 0.02) ($p > 0.05$). Overall, these results suggest that the marine bacterial strain GH-W2b produces ASNase with low glutaminase (GLNase) activity.

3.2. Identification of GH-W2b

The 16S rRNA and *rpoD* gene sequences of GH-W2b clearly clustered with species belonging to the genus *Pseudomonas* in neighbor-joining phylogenetic trees (Figure 2). The

comparative analysis of the 16S rRNA gene revealed that the GH-W2b strain was closely related to *Pseudomonas lactis* DSM 29167^T and *P. salmasensis* SWRI126^T (100% 16S rRNA gene sequence similarity) (Figure 2A). The *rpoD* sequences of GH-W2b were not assigned to a clade based on close references with a high bootstrap value (99%) (Figure 2B). A BLAST search using the *rpoD* sequence as a query indicated 97.87% and 97.46% similarities with *P. salmasensis* SWRI126^T and *P. lactis* DSM 29167^T, respectively. To distinguish between strains at the species level, the defined nucleotide identity threshold of the *rpoD* gene is 98% [25]. Therefore, GH-W2b could not be assigned to the species level and was designated as *Pseudomonas* sp.

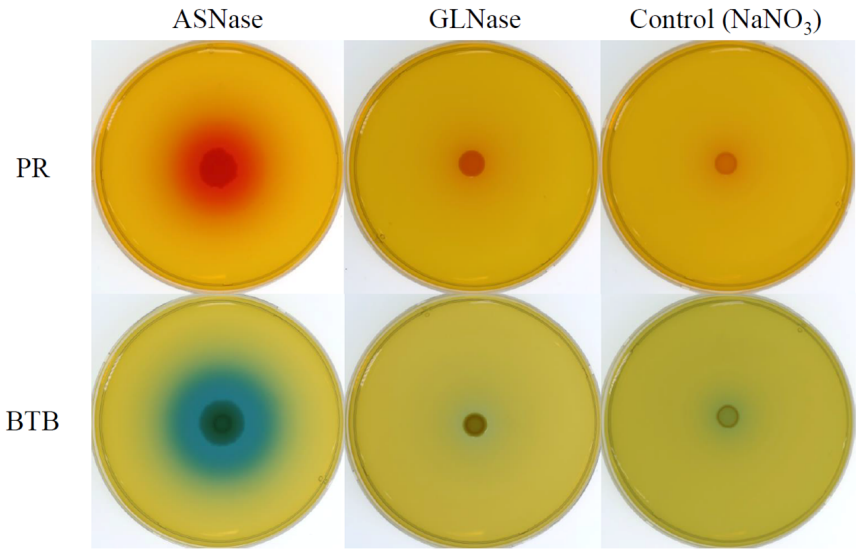


Figure 1. Detection of ASNase activity by plate assay. GH-W2b was inoculated onto a modified asparaginase dextrose salt agar supplemented with (L-asparagine (ASNase), L-glutamine (GLNase), or $NaNO_3$ (control)). Phenol red (PR) and bromothymol blue (BTB) were used as pH indicators. After incubation at 25 °C for 2 days, the presence of pink (PR plates) or blue (BTB plates) zones surrounding the colonies were observed.

3.3. Effect of Temperature on ASNase Activity

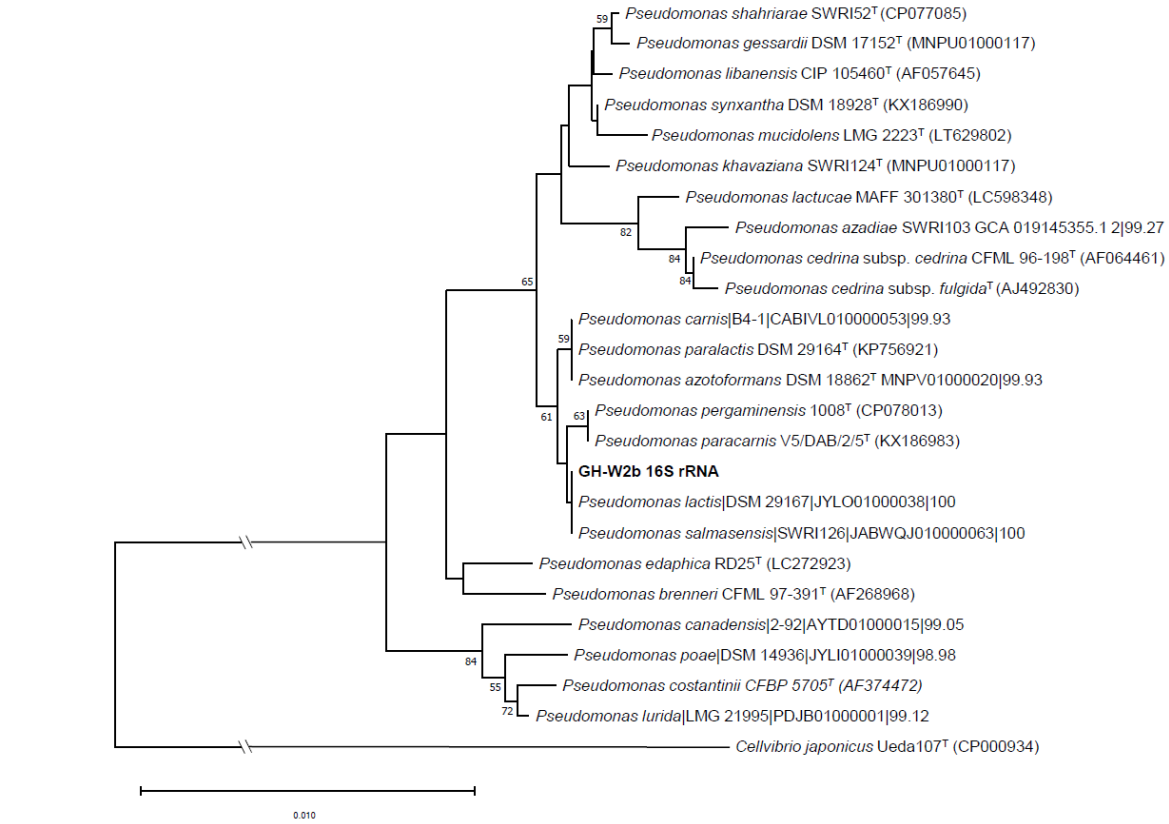
To assess the effect of temperature on the GH-W2b ASNase activity, the crude enzyme extract was incubated with asparagine at 4, 15, 25, 37, 50, and 65 °C for 30 min. ASNase activity indicated a tendency to gradually increase as the temperature increased, and the maximum activity was observed at 65 °C (Figure 3). ASNase activities at temperatures ranging from 4 °C to 37 °C and those between 50 °C and 65 °C were similar, respectively ($p > 0.05$).

3.4. Effect of pH on ASNase Activity

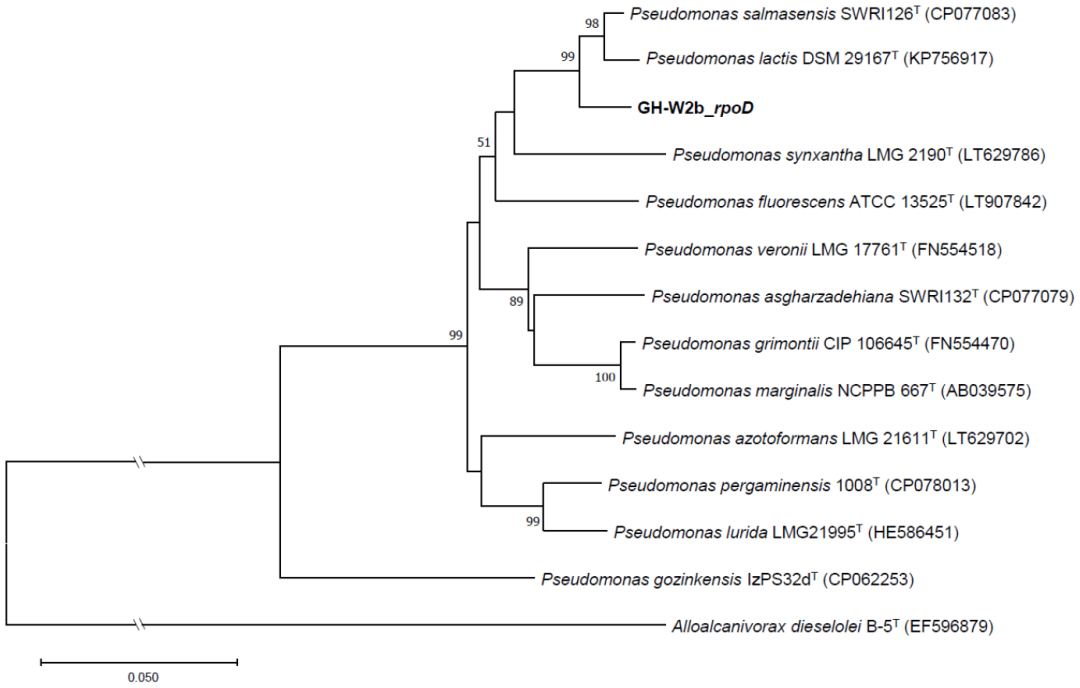
The effect of pH on ASNase activity was examined at pH 4.0, 5.5, 7.0, 8.5, and 10. The minimum activity was observed at pH 4.0 (2.71 units), approximately 2.7-fold lower than the activity at pH 8.5 (15.33 units) (Figure 4). Maximum activity was observed at pH 7 and pH 8.5 ($p > 0.05$). ASNase activities gradually increased from pH 4 to pH 7.0–8.5 and decreased at pH 10 ($p < 0.05$ between pH 7 and pH 10).

3.5. Effect of NaCl Concentration on ASNase Activity

The ASNase activity of GH-W2b was measured at 0, 2.5, 5.0, 10, and 15% (*w*/*v*) NaCl. Under all tested conditions, the ASNase of GH-W2b exhibited similar levels of activity ranging from 19.15 to 21.48 units ($p > 0.05$) (Figure 5).



(**A**)



(**B**)

Figure 2. The phylogenetic trees of a marine bacterial strain GH-W2b. Trees were constructed based on the (**A**) 16S rRNA and (**B**) *rpoD* gene sequences using the neighbor-joining (NJ) method. *Cellvibrio japonicus* Ueda107^T (CP000934) and *Alloalcanivorax dieselolei* B-5^T (EF596879) were included as outgroup taxa. The scale bar indicates the number of nucleotide substitutions per site. Numbers at the nodes indicate bootstrap values from 1000 replicates. Bootstrap values less than 50% are not shown.

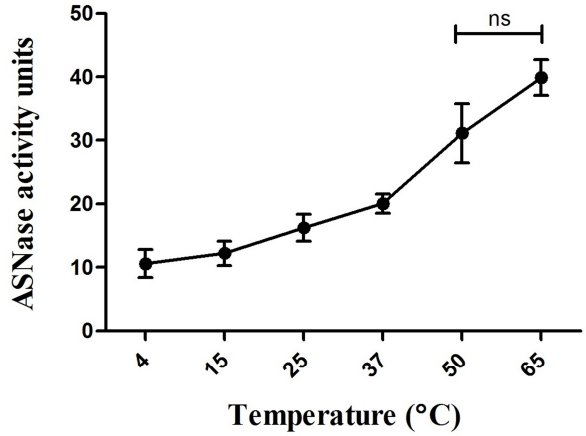


Figure 3. The effect of temperature on ASNase activity. The ASNase activity of the GH-W2b crude enzyme extract was examined at 4, 15, 25, 37, 50, and 65 °C. After incubation of the enzyme with L-asparagine solution for 30 min, activity was measured using the Nesslerization method. 'ns' indicates 'not significantly different ($p > 0.05$)'.

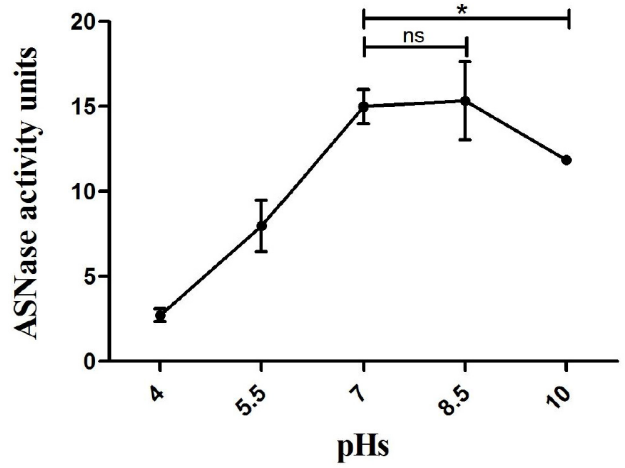


Figure 4. The effect of pH on ASNase activity. The ASNase activity of the GH-W2b crude enzyme extract was examined at pH 4.0, 5.5, 7.0, 8.5, and 10. After incubation of the enzyme with L-asparagine solution at 37 °C for 30 min, the activity was measured by the Nesslerization method. '*' and 'ns' indicate 'significantly different ($p < 0.05$)' and 'not significantly different ($p > 0.05$)', respectively.

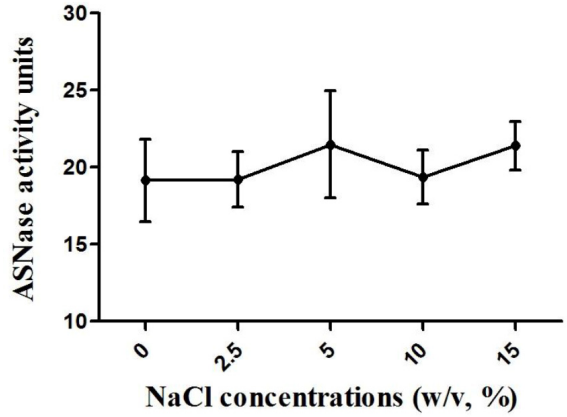


Figure 5. The effect of NaCl concentration on ASNase activity. The ASNase activity of the GH-W2b crude enzyme was examined at 0, 2.5, 5.0, 10, and 15% (w/v) NaCl. After incubation of the enzyme with L-asparagine solution at 37 °C for 30 min, the activity was measured by the Nesslerization method.

3.6. Effect of Incubation Time on ASNase Activity

The results on Figure 6 showed the effect of incubation time ranging from 15 to 120 min every 15 min. The maximum ASNase activity of GH-W2b was observed at 60 min (17.12 units), and the activity decreased with increasing incubating time after 60 min. ASNase activities at incubation time ranging from 30 to 75 min were similar ($p > 0.05$). ASNase activities between 15 and 30 min, and 15 and 60 min were significantly different, respectively ($p < 0.05$).

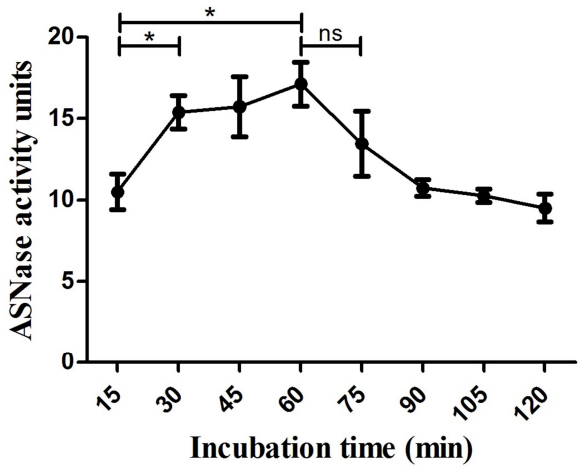


Figure 6. The effect of incubation time on ASNase activity. The enzyme and L-asparagine solution were incubated for 15, 30, 45, 60, 75, 90, 105, and 120 min. After incubation of the reaction mixture at 37 °C every 15 min, the activity was measured by the Nesslerization method. '*' and 'ns' indicate 'significantly different ($p < 0.05$)' and 'not significantly different ($p > 0.05$)', respectively.

3.7. Thermal Stability of GH-W2b ASNase

The crude enzyme extract of GH-W2b was pre-incubated at 4, 15, 25, 37, 50, and 65 °C for 2 h. When pre-incubated at 4, 15, 25, and 37 °C, more than 87% residual activities were observed relative to the control (no pre-incubation) (Figure 7). There were no differences in the residual activities at temperatures ranging from 4 °C to 37 °C ($p > 0.05$). ASNase activity significantly decreased after pre-incubation at 50 °C (30% residual activity), and no residual activity was detected after pre-incubation at 65 °C for 2 h.

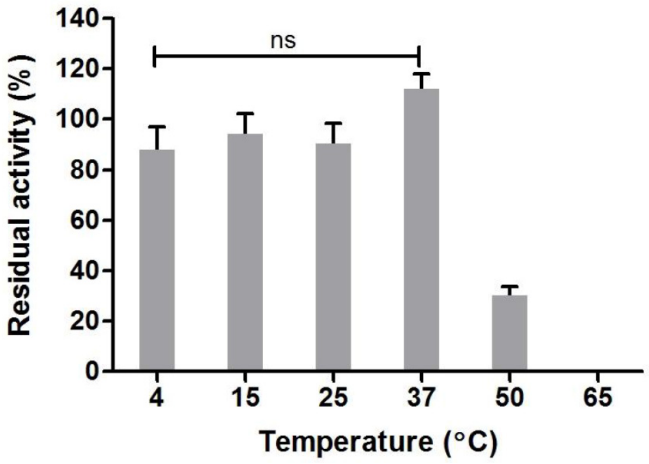


Figure 7. The thermal stability of the ASNase activity of GH-W2b. The crude enzyme was incubated at temperatures ranging from 4 °C to 65 °C for 2 h prior to the enzyme reaction. Residual activity was measured and compared with that of the control (no pre-incubation). 'ns' indicates 'not significantly different ($p > 0.05$)'.

4. Discussion

In the present study, we discovered an ASNase-producing marine bacterial strain, GH-W2b, belonging to the *Pseudomonas* species, closely related to *P. lactis* and *P. salmasensis*. A phylogenetic analysis using the 16S rRNA gene sequence is a widely used tool for bacterial identification; however, it often lacks the necessary discrimination to resolve relationships within the *Pseudomonas* genus owing to its very slow evolutionary rate [19]. Instead, analyses using protein-coding genes, specifically *gyrB* and *rpoD*, are used to distinguish between *Pseudomonas* species. These genes provide more detailed resolution than 16S rRNA gene sequences due to their much higher evolutionary rate [20]. The *rpoD* gene sequence alone is a reliable and affordable option, particularly for classifying environmental *Pseudomonas* isolates based on their taxonomic affiliation [25]. Based on the results of 16S rRNA and *rpoD* sequence analyses, we concluded that GH-W2b belongs to the *Pseudomonas* species.

Bacteria from the genus *Pseudomonas* inhabit a variety of terrestrial and aquatic environments and have been extensively studied for novel secondary metabolites, bioremediation, plant growth promotion, and biocontrol [26]. *Pseudomonas lactis* and *P. salmasensis* were first isolated from raw bovine milk and wheat rhizosphere in 2017 and 2004, respectively [27,28]. Several *Pseudomonas* species, including *P. acidovorans*, *P. aeruginosa*, *P. geniculata*, *P. fluorescens*, *P. otitidis*, *P. plecoglossicida*, and *P. stutzeri*, produce ASNases, some of which exhibit antitumor activity and/or the ability to reduce the acrylamide content in foods [29–35]. However, the ASNase activity has not been previously elucidated in *P. lactis* and *P. salmasensis.*

Based on plate assay results (Figure 1), GH-W2b appeared to produce ASNase with low GLNase activity. The toxicity of the currently used chemotherapeutic ASNases is generally attributed to their concomitant GLNase activity [1]. To address this issue, several researchers have attempted to discover novel sources of ASNases with low or no GLNase activity. Therefore, GH-W2b ASNases may be beneficial for the development of alternative clinical ASNases with reduced toxicity.

The ASNase activity of the GH-W2b crude enzyme extract was maximized at 50–65 °C, pH 7.0–8.5, and the incubation time of 30–75 min. The optimum temperature of GH-W2b ASNase activity was slightly higher than those of the majority of *Pseudomonas* ASNases that exhibit optimal activity at 37–45 °C [30,32,33,35–37]. The optimum pH of GH-W2b ASNase was similar to those of previously reported *Pseudomonas* ASNases (pH 7.5–9.5) [29–33,35–37].

Importantly, the ASNase activities of GH-W2b were consistent at a wide range of NaCl concentrations (0–15%) at 37 °C and pH 8.0 for 30 min incubation. The effect of the NaCl concentration on ASNase activity has been investigated in only a few bacterial ASNases. Halotolerant *Staphylococcus* spp. OJ82 produces ASNases that retain 61.2% of the control activity (at 0 M NaCl) in 2 M NaCl, which corresponds to 11.68% (*w*/*v*) NaCl [38]. GH-W2b ASNase activity was 100% retained at 15% NaCl (2.6 M) compared with that at 0% NaCl. This suggests that GH-W2b ASNase can be applied in industrial processes that require high-salt conditions.

GH-W2b ASNase exhibited significantly higher residual activities (more than 87%) after pre-incubation at 4–37 °C for 2 h. The thermal stability of ASNases has been evaluated in several bacterial strains. *Mycobacterium gordonae* ASNase shows high thermal stability at 35 °C for 50 min, but the activity is completely lost at 50 °C for 10 min [39]. Moreover, the half-life of *Acinetobacter soli* ASNase activity at 40 °C is approximately 9 min [40]. When foods are treated with ASNases for acrylamide reduction, poor thermal stability is one of the main reasons for limited application [4]. The addition of ASNase during the dough resting of biscuits or soaking blanched potato strips in ASNase solution resulted in a 92%

reduction in acrylamide content in the final products [41]. These processes were performed at temperatures ranging from 10 °C to 40 °C. Therefore, GH-W2b ASNase has the potential to mitigate acrylamide contamination in cooked foods.

Overall, GH-W2b ASNase could serve as a candidate ASNase in the medicinal and food industries. For biotechnological applications of GH-W2b ASNase, it will be critical for future studies to perform enzyme purification, optimize cultivation, and evaluate the anticancer activity and capability of acrylamide reduction.

Author Contributions: W.-J.Y., H.Y.L. and D.C. conducted the experiments. W.-J.Y. and D.C. designed the experiments, analyzed the data, and wrote the manuscript. Y.M.K. and S.S.B. assisted with the bacterial identification. G.C. and H.-J.H. conceived the study and supervised the project. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available in online repositories. The names of repositories, strain ID, and accession numbers can be found below: https://www.mbris.kr/biobank/, MABIK MI00009254 (strain identity); https://www.ncbi.nlm.nih.gov/genbank/, PQ614850 and PQ633201 (accession numbers) (accessed on 24 November 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

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