Original Article

Characterization of a Novel Microbial Source of L-Glutaminase Production by *Bacillus zanthoxyli* Jaberi, Isolated from Saline Soil in Shushtar City, Iran

Behnam Azizolahi, Reza Jaberi Manesh, Abbas Moridnia, Ladan Mafakher, Yasin Ahmadi, Maryam Gheibipour, Babak Elyasi Far

DOI: 10.34172/PS.026.43376

To appear in: Pharmaceutical Science (https://ps.tbzmed.ac.ir/)

Received date: 12 Oct 2025

Revised date: 21 Dec 2025

Accepted date: 23 Dec 2025

Please cite this article as: Azizolahi B, Jaberi Manesh R, Moridnia A, Mafakher L, Ahmadi Y, Gheibipour M, et al. Characterization of a Novel Microbial Source of L-glutaminase production by Bacillus zanthoxyli jaberi, isolated from saline soil in Shushtar City, Iran. Pharm Sci. 2026. doi: 10.34172/PS.026.43376

Characterization of a Novel Microbial Source of L-Glutaminase Production by *Bacillus zanthoxyli* Jaberi, Isolated from Saline Soil in Shushtar City, Iran

Behnam Azizolahi[1,2https://orcid.org/0000-0002-5852-6887], Reza Jaberi Manesh[3], Abbas Moridnia[1,4], Ladan Mafakher[5], Yasin Ahmadi[6], Maryam Gheibipour[7,] Babak Elyasi Far[1,8*https://orcid.org/0000-0003-2209-3095]

[1]Infectious and Tropical Diseases Research Center, Dezful University of Medical Sciences, Dezful, Iran.

[2]Department of Laboratory Sciences, School of Paramedicine, Dezful University of Medical Sciences, Dezful, Iran.

[3]Student Research Committee, Dezful University of Medical Sciences, Dezful, Iran.

[4]Department of Genetics and Molecular Medicine, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran.

[5]Thalassemia & Hemoglobinopathy Research center, Health research institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

[6]Department of Medical Laboratory Science, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq.

[7]Environmental Technologies Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

[8]Department of Immunology and Microbiology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran.

Abstract

Halophilic bacteria represent valuable sources of enzymes with exceptional stability under extreme conditions, making them promising candidates for industrial and therapeutic applications. This study aimed to isolate and characterize halophilic bacterial strains producing L-glutaminase, L-asparaginase, and L-methioninase from saline soils in Shushtar, Iran. Forty-four bacterial isolates were obtained through enrichment and selective culture techniques. Screening

for extracellular L-glutaminase, L-asparaginase, and L-methioninase production was performed using phenol red-based plate assays, followed by quantitative enzyme activity determination via the Nesslerization method. Enzyme activities were assessed under varying pH and temperature conditions. Two isolates exhibited significant activity: *Bacillus subtilis* Iran2024 produced L-asparaginase (3.55 ± 0.12 U/mL) and L-glutaminase (4.62 ± 0.18 U/mL), while *Bacillus zanthoxyli* Jaberi.Iran produced L-glutaminase (5.85 ± 0.21 U/mL). Optimal activity for both enzymes occurred at 40 °C and pH 8. Notably, this study reports for the first time the production of L-glutaminase by *Bacillus zanthoxyli*. The high stability and catalytic efficiency of these halophilic enzymes highlight their potential for biotechnological and therapeutic applications, particularly in enzyme-based cancer therapy.

Keywords: Halophilic bacteria, *Bacillus zanthoxyli*, *Bacillus subtilis*, L-glutaminase, L-asparaginase

1. Introduction

Biocatalysts are enzymes produced by living cells that accelerate biochemical reactions without being consumed. Their high catalytic efficiency and eco-friendly nature have made them valuable in industries such as food, agriculture, chemicals, cosmetics, and pharmaceuticals. Compared with animal or plant derived enzymes, microbial biocatalysts provide a sustainable, ethical, and non-toxic alternative .[1]

Recent advancements in cancer research have underscored the potential of microbial enzymes in targeting malignancies through metabolic disruption. Certain microbial enzymes have been demonstrated to influence nitrogen metabolism, selectively exploiting the metabolic vulnerabilities of cancer cells. By catalyzing the degradation of specific amino acids, these enzymes induce nutrient deprivation, ultimately inhibiting cancer cell proliferation and survival [2]. This strategy has led to the development of enzyme-based therapies, with several biocatalysts approved by the FDA for medical use. Among these, L-asparaginase, L-glutaminase, L-arginase, and L-methioninase have shown significant promise in cancer treatment, particularly in hematologic malignancies such as acute lymphoblastic leukemia.[3] These enzymes are derived

from a diverse range of microorganisms, including *Saccharomyces cerevisiae*, *Pichia pastoris*, *Pseudomonas pseudoalcaligenes*, *Escherichia coli*, and *Bacillus licheniformis*.[2,4-6]

Despite their potential, enzyme-based cancer therapies face several challenges, including immunogenicity, stability, and substrate affinity. In response, researchers are actively exploring novel sources of biocatalysts with improved enzymatic properties. Notably, halophilic bacteria have emerged as a promising source of salt-stable enzymes capable of functioning under extreme conditions, thereby enhancing therapeutic efficacy and industrial applications.[7]

Given the urgent need for more effective enzyme-based therapies, this study aims to isolate and characterize halophilic bacteria capable of producing L-glutaminase, L-asparaginase, and L-methioninase from saline soils in the vicinity of Shushtar city, Iran.

2. Materials and Methods:

2.1. Chemicals

The chemicals used for the isolation and identification of bacteria producing enzymes (L-glutaminase, L-asparaginase, and L-methioninase) were sourced from Merck Chemical Co. (Germany), with Nessler's reagent obtained from Fluka (Buchs, Switzerland). The ladder, MasterMix, and universal primers for molecular identification were purchased from Smobio (Taiwan) and Ampliqon (Denmark), respectively.

2.2. Sample Collection

Some of the microbial strains used in this study were previously isolated and described in a published research article.[8] In addition, new soil samples were collected between May and June 2024 from the Shushtar District, approximately 12 km northeast of Gotvand in Khuzestan Province, Iran (coordinates: 32°02'–42''N, 48°51'–34''E). A total of eight soil samples were collected using sterile containers and stored at 4°C until further processing.

2.3. Enrichment of Soil Sample

Enrichment culture medium was prepared by dissolving 150 g of NaCl, 9.7 g of $MgSO_4 \cdot 7H_2O$, 7.0 g of $MgCl_2 \cdot 6H_2O$, 3.6 g of $CaCl_2$, 2.0 g of KCl, 0.06 g of $NaHCO_3$, and 0.026 g of NaBr in 1000 mL of distilled water, adjusting the pH to 7.0. The prepared nutrient broth was supplemented with these ingredients and used for the enrichment of soil samples.[8]

2.4. Screening for Isolates Producing L-glutaminase, L-asparaginase, and L-methioninase

Halophilic isolates were screened for extracellular enzyme production on nutrient agar supplemented with varying NaCl concentrations (5%, 10%, 15%, 20%, and 25%, w/v) to determine their salt tolerance range. The isolates were incubated at 28°C for 48–72 hours to allow colony development under different salinity conditions. Screening for L-asparaginase, L-glutaminase, and L-methioninase activity was performed using phenol red-based selective media. Each plate contained 0.3 mL of 2.5% phenol red, 150 g NaCl, 9.7 g $MgSO_4 \cdot 7H_2O$, 7.0 g $MgCl_2 \cdot 6H_2O$, 3.6 g $CaCl_2$, 2.0 g KCl, 0.06 g $NaHCO_3$, 0.026 g NaBr, 1 g glucose, and 20 g agar in 1000 mL of distilled water (pH 7.0). The media were supplemented separately with 5 g/L of L-asparagine, L-glutamine, or L-methionine as substrate for the respective enzyme assays. After incubation at 28°C for 48 hours, enzyme-producing colonies were identified by the development of a pink coloration around colonies, indicating hydrolysis of the substrate and subsequent pH change due to ammonia release.[9]

2.5. Enzyme Activity Assay

The activity of the enzymes was measured using the Nesslerization method, as described in previous studies.[10,3] The isolated strains were cultured in modified M-9 broth medium at 37 °C for 24 hours. To obtain the cell-free crude enzyme, the culture was centrifuged at 5000×g for 20 minutes. A reaction mixture was prepared by combining 0.5 mL of the supernatant, 1.0 mL of 0.1 M sodium acetate buffer (pH 8.5), and 0.5 mL of 0.05 M L-asparagine, L-glutamine or L-methionine solution, followed by incubation at 37 °C for 10 minutes. The reaction was halted by adding trichloroacetic acid, and the precipitated proteins were separated by centrifugation at

10000×g for 20 minutes. Ammonia levels in the supernatant were quantified via nesslerization, with enzyme activity expressed in $U \cdot mL^{-1}$. Nessler's reagent was prepared by dissolving 45.5 g of HgI_2 and 35.0 g of KI in 1 liter of distilled water containing 112 g of KOH. Subsequently, 0.5 mL of the prepared reagent was added to the reaction supernatant, and the absorbance was recorded at 505 nm. One unit (U) of enzyme activity was defined as the amount of enzyme that generates 1 µmol of ammonia per minute under the specified assay conditions. Ammonium sulfate ($(NH_4)_2SO_4$) solutions of varying concentrations were used as calibration standards.[7,9]

2.6. Molecular Identification of Superior Isolates

The bacterial isolates were characterized morphologically, physiologically, and biochemically, and their characteristics were compared with data from *Bergey's Manual of Determinative Bacteriology*. Genomic DNA was obtained from bacteria cultured overnight in nutrient broth and used as a template for amplifying the 16S rRNA gene. Universal primers 27F (5'-CCA GCA GCC GCG GTA ATA CG 3') and 1492R (5' ATC GGCTAC CTT GTT ACG ACT TC 3') were employed in the PCR amplification. A 25-µL PCR mixture was prepared containing 12.5 µL of MasterMix 2X, 9.5 µL of deionized water, 1.5 µL of each primer, and 1 µL of DNA template. PCR conditions included an initial denaturation at 94°C for 10 minutes, followed by 35 cycles of 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C, with a final extension of 10 minutes at 72°C. The PCR products (5 µL) were analyzed on a 1% agarose gel in 1X Tris/Borate/EDTA (TBE) buffer containing a safe stain. The gel was run at 90 V for 40 minutes and examined under UV light. Bands of the expected size were purified using a gel DNA purification kit, sequenced (Macrogen, Korea), and the resulting sequences were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed using MEGA version 11 with the neighbor-joining algorithm and bootstrap analysis with 100 replicates.

2.7. Optimal Temperature and pH for Enzyme Activity

To determine the optimal pH for enzyme activity, enzyme solutions were incubated at pH values ranging from 4 to 10 for 60 minutes at 30 °C. Similarly, the effect of temperature on enzyme

activity was evaluated by incubating the enzymes at temperatures ranging from 20 to 60 °C for the same duration.

3. Results:

3.1. Microbial Isolation and Characterization

A total of 44 bacterial isolates were obtained from saline soil samples collected near the Shushtar region, representing a diverse range of bacterial species. Identification of these isolates was carried out using morphological, biochemical, and physiological characteristics, with particular emphasis on colony morphology, Gram staining, and various biochemical tests.

The bacterial isolates were predominantly Gram-positive (42 isolates), with only 2 identified as Gram-negative. Among the isolates, most exhibited a rod-shaped morphology (37 isolates), while 3 showed a spherical (coccus) shape (Fig 1).

Further details on the morphological features and biochemical test results for each isolate can be found in Table S1 and Table S2. These tables provide a comprehensive summary of the colony characteristics, Gram staining results, biochemical test outcomes, and environmental tolerance data for each bacterial isolate.

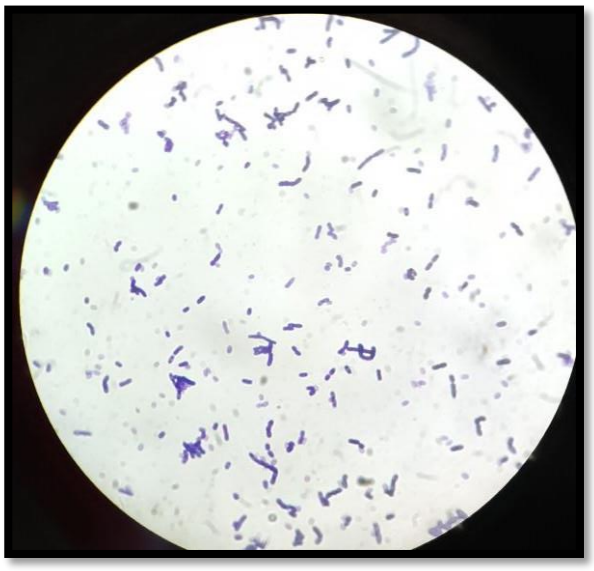

Figure 1. Microscopic morphology of representative halophilic bacterial isolates from saline soils of the Shushtar region, Iran.

3.2. Screening and assay of enzyme production

Among all the cultured samples, only the 5C and 4F isolates produce extracellular L-glutaminase and L-asparaginase. Based on this, these two isolates were selected for subsequent experiments and further analysis.

The enzyme production of these two bacterial isolates was assessed for L-glutaminase and L-asparaginase. The table1 summarizes the enzyme activity of isolates 4F and 5C.

Table 1. Enzyme production of 4F and 5C Isolates. Data represent mean ± SD of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Differences in enzyme activities between isolates were statistically significant ($p < 0.05$).

Isolate	**L-Glutaminase Production (U/mL)**	**L-Asparaginase Production (U/mL)**	**L-Methioninase Production (U/mL)**
4F	4.62 ± 0.18	3.55 ± 0.12	-
5C	5.85 ± 0.21	-	-

The highest enzyme production was observed in L-glutaminase-producing strain 5C, which showed maximum activity (5.85 U/mL). Strain 4F produced the enzyme at 4.62 U/mL. Additionally, strain 4F demonstrated L-asparaginase production at 3.55 U/mL.

3.3. Optimal pH and temperature for enzyme activity

Optimum enzyme activity was assessed under various pH conditions, with the highest activity observed at pH 8 (Fig. 2).

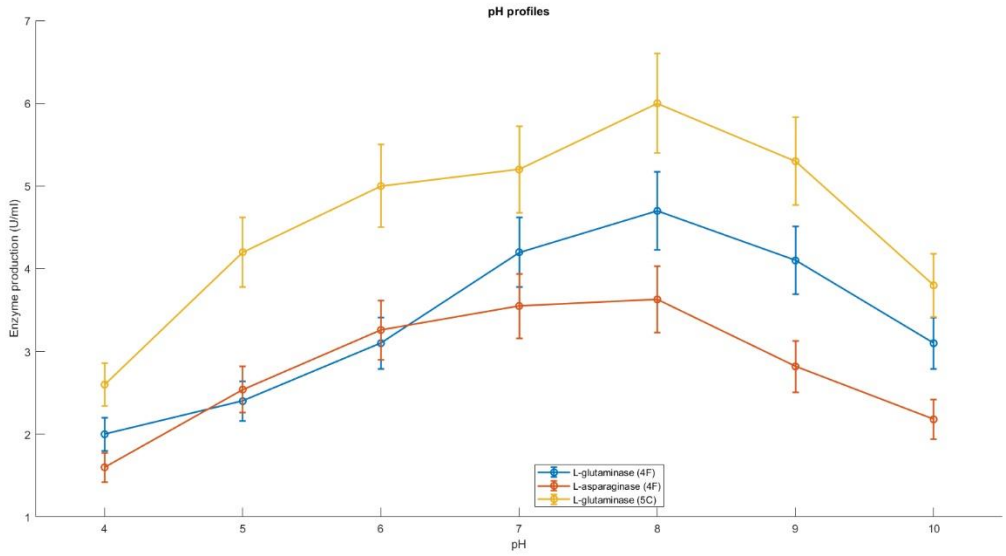

Figure 2. Effect of pH on the activity of L-glutaminase and L-asparaginase produced by *Bacillus zanthoxyli* isolate 5C and *Bacillus subtilis* isolate 4F. Enzyme activity was measured after incubation at pH values ranging from 4 to 10 at 30 °C for 60 minutes. Data represent mean ± SD of three independent experiments.

The results indicated that both enzymes exhibited optimal activity across temperature variations, with the highest activity observed at 40°C (Fig. 3).

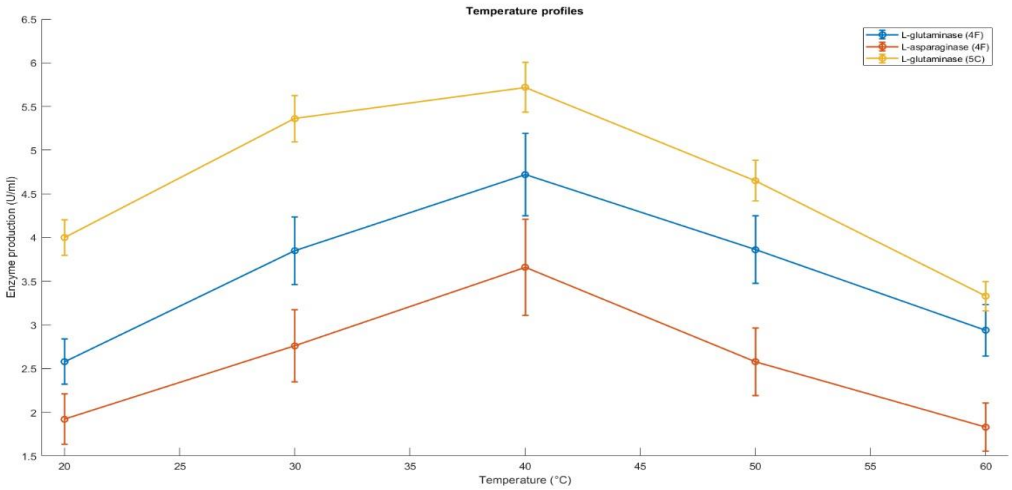

Figure 3. Effect of temperature on the activity of L-glutaminase and L-asparaginase produced by *Bacillus zanthoxyli* isolate 5C and *Bacillus subtilis* isolate 4F. Enzyme activity was determined at temperatures ranging from 20 to 60 °C at pH 8. Data represent mean ± SD of three independent experiments.

3.4. Identification of Producing Bacteria

After sequencing, the obtained sequences were compared against the NCBI database, revealing high similarity with specific bacterial strains. To further confirm the identification, the morphological characteristics of the bacteria were also used. These characteristics were identified and analyzed concerning the ABIS Encyclopedia, leading to the identification of the 5C isolate as *Bacillus zanthoxyli* (99% similarity) and the 4F isolate as *Bacillus subtilis* (99% similarity). Figure 4 shows Phylogenetic Tree by MEGA 11 software using neighbor-joining method.

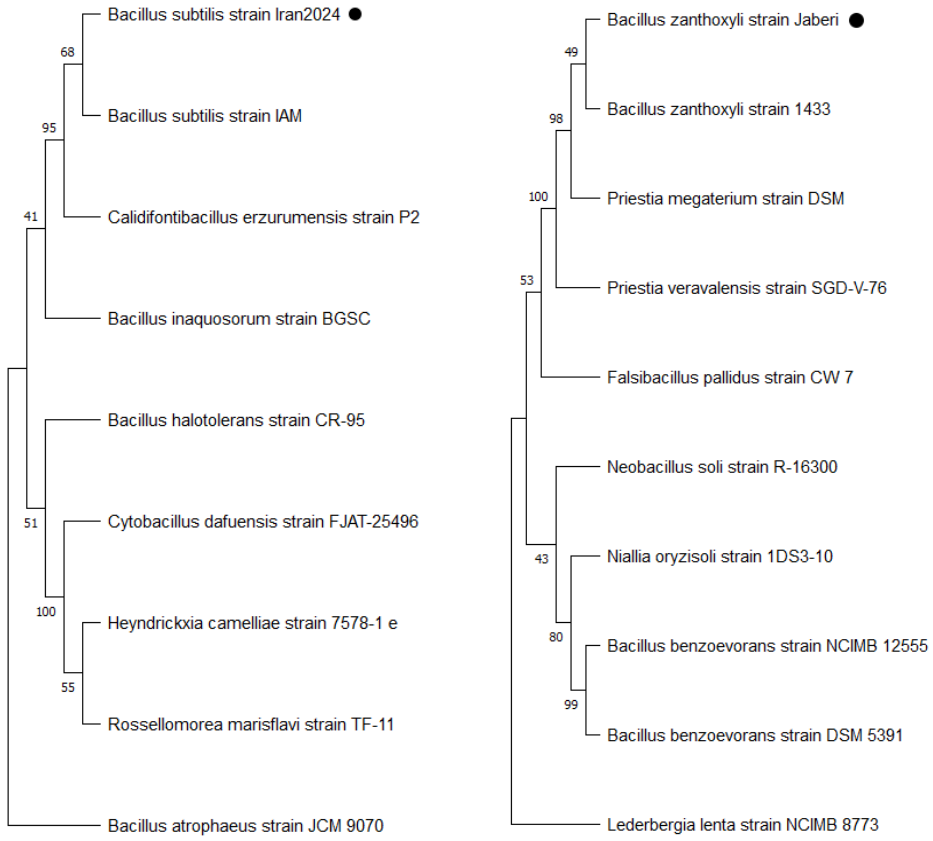

Figure 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of isolates 5C (*Bacillus zanthoxyli*) and 4F (*Bacillus subtilis*) with related taxa. Scale bar indicates 0.05 substitutions per site.

The NCBI accession numbers for the 5C strain (*Bacillus zanthoxyli* Jaberi) and the 4F strain (*Bacillus subtilis* Iran2024) are PQ301465 and PQ301466, respectively.

4. Discussion

This study identified *Bacillus zanthoxyli* isolate 5C as the most potent enzyme producer, yielding 5.85 U/mL of L-glutaminase. Similarly, *Bacillus subtilis* isolate 4F demonstrated enzymatic activity of 4.62 U/mL and L-asparaginase yield of 3.55 U/mL. The optimum enzyme activity assessments revealed that the enzymes exhibited peak activity at 40°C and pH 8.

Based on our results, *Bacillus zanthoxyli* Jaberi demonstrates the ability to produce L-glutaminase, constituting the first documented report of this species as a source of the enzyme. *Bacillus zanthoxy* is a rod-shaped bacterium, Gram-positive, non-spore-forming, aerobic, with motility facilitated by peritrichous flagella. The strain exhibits a broad tolerance for temperature and pH, thriving between 4–45 °C (with an optimal range of 28–32 °C) and pH 6.0–10.0 (optimal at pH 6.0–7.0). Additionally, it can grow in NaCl concentrations ranging from 0–7% (w/v), with optimal growth observed at 0–3% (w/v).[11] A study by Alisher Usmonov et al. demonstrated that the *Bacillus zanthoxyli* HS1 strain effectively suppresses disease progression and enhances salt stress tolerance in vegetable crops.[12]

Comparative analysis with existing literature highlights the significance of optimizing growth conditions to enhance enzyme production. Zolfaghar et al. (2019) reported L-glutaminase activity of 0.6 U/mL in *Rhodococcus* sp. and L-asparaginase activity of 1 U/mL in *Vibrio* sp. [13], values considerably lower than those observed in our study. These discrepancies may be attributed to variations in cultivation parameters, including culture medium composition and incubation conditions, emphasizing the necessity of tailored optimization strategies.

Kassab (2024) investigated L-glutaminase production in *Bacillus cereus*, reporting 5.2 U/mL at pH 7.4 and 37°C. However, genetic engineering approaches significantly enhanced enzyme production to 42.96 U/mL.[14] These findings closely align with our results, reinforcing the enzymatic potential of *Bacillus* species. The enhanced production observed following recombinant DNA technology highlights the necessity of genetic modifications for industrial-scale enzyme synthesis.

A study by Mustafa et al. reported an L-glutaminase activity of 23.20 U/mL in *Halomonas meridiana,* with optimum activity observed at 37°C and pH 8.[15] The markedly higher activity compared to our isolates may be attributed to species-specific metabolic adaptations or cultivation differences. These variations highlight the importance of selecting optimal bacterial strains for enhanced enzyme production. Additionally, the optimum pH and temperature reported in their study align with our findings.

Similarly, Gholami et al. (2022) reported L-asparaginase activity of approximately 7.8 U/mL in *Bacillus subtilis*, a value consistent with our findings, suggesting conserved metabolic pathways in these strains. However, their study did not evaluate enzyme activity under varying pH and temperature conditions, a crucial factor for industrial applications.[16] In contrast, Amin et al. (2020) observed significantly higher L-asparaginase activity (50–60 U/mL) in *Bacillus subtilis*.[17] The substantial differences in enzymatic yield may be attributed to strain-specific genetic variations and physiological differences.

Lakshmi et al. (2020) examined L-glutaminase and L-asparaginase production in *Pseudoalteromonas arabiensis*, reporting enzyme yields of 6.707 U/mL and 7.445 U/mL, respectively.[18] The slightly higher production levels compared to our isolates may result from species-specific metabolic capabilities and cultivation conditions. These results highlight the enzymatic potential of halophilic microorganisms and the necessity of further exploration into their biotechnological applications.

Orabi et al. (2019) reported an L-glutaminase activity of 89.78 U/mg in *Bacillus subtilis*, with maximum enzyme activity at pH 8.2 and 40°C.[19] The higher enzymatic activity observed compared to our findings may be attributed to strain-specific genetic variations and environmental factors influencing enzyme yield. Additionally, the optimum pH and temperature for enzyme activity reported in their study are similar to our results.

Gouma (2022) examined L-glutaminase production in *Bacillus* sp. DV2-37, reporting initial activity of 5.16 U/mL, which increased to 12.47 U/mL following process optimization.[5] These findings align closely with our results and emphasize the importance of optimization strategies for maximizing enzyme production.

Nocardiopsis alba exhibited enzyme activity of 6.73 U/mL, with optimization strategies, including the addition of 1.5% maltose as a carbon source, enhancing yield to 18.47 U/mL. Its optimum enzyme activity was observed at 37°C and pH 8.[20] These findings are highly similar to our results and demonstrate potential for enhancing enzyme production.

Ibrahim et al. (2020) reported L-glutaminase activity of 5.3 U/mL in *Klebsiella oxytoca* [21], closely approximating our findings. However, enzyme activity under varying pH and temperature conditions was not assessed in their study, limiting comparative evaluation.

In other research, Zolfaghar et al. [13] reported significantly lower enzymatic activity (0.6–1 U/mL), likely due to genetic variations or differences in culture media composition. Similarly, studies on *Bacillus cereus* revealed comparable enzymatic activity (5.2 U/mL) to our *Bacillus zanthoxyli* isolate 5C. However, several studies have documented fluctuations in enzymatic yield by microorganisms in response to changes in environmental conditions, such as pH and temperature.

When compared with previous studies (Table 2), the enzyme activity observed in *B. zanthoxyli* Jaberi (5.85 U/mL) and *B. subtilis* Iran2024 (4.62 U/mL for L-glutaminase and 3.55 U/mL for L-asparaginase) demonstrates a reasonably good catalytic performance under moderate temperature and alkaline pH conditions.

Table 2: Summary of previous studies on the production of L-glutaminase and L-asparaginase.

Microorganisms	**Enzyme type**	**Enzyme activity**	**References**
Rhodococcus sp	L-glutaminase	0.6 U/mL	[13]
Vibrio sp.	L-asparaginase	1 U/mL	[13]
Bacillus cereus	L-glutaminase	5.2 U/mL	[14]
Halomonas meridiana	L-glutaminase	23.20 U/mL	[15]
Klebsiella oxytoca	L-glutaminase	5.3 U/mL	[21]
Pseudoalteromonas arabiensis	L-glutaminase	6.707 U/mL	[18]
Pseudoalteromonas arabiensis	L-asparaginase	7.445 U/mL	[18]
Klebsiella oxytoca	L-glutaminase	5.3 U/mL	[21]

In addition to their enzymatic behavior, both enzymes exhibited stable activity across a range of pH and temperature values, suggesting potential use in industrial and therapeutic processes. Since L-glutaminase and L-asparaginase play key roles in cancer therapy, their effectiveness at 40 °C and pH 8 supports their suitability for stable formulations and possible inclusion in pharmaceutical or food-grade applications. The halophilic characteristics of these strains may also help reduce microbial contamination and enhance enzyme durability during fermentation.

Altogether, the findings expand our understanding of halophilic enzyme producers and highlight the practical importance of extremophilic bacteria for enzyme-based cancer treatments and industrial biotechnology.

5. Conclusion

This study identified two halophilic bacterial isolates with the capacity to produce enzymes of potential biomedical relevance. *Bacillus zanthoxyli* isolate 5C exhibited the highest L-glutaminase activity (5.85 U/mL), while *Bacillus subtilis* isolate 4F produced both L-glutaminase (4.62 U/mL) and L-asparaginase (3.55 U/mL). To our knowledge, supported by comparative literature analysis, this is the first report of L-glutaminase production by *Bacillus zanthoxyli*. Both isolates demonstrated optimal enzyme activity at 40 °C and pH 8, indicating favorable biochemical properties for biotechnological applications.

While these results highlight the potential of halophilic bacteria as novel sources of clinically relevant enzymes, the present study did not evaluate direct anticancer effects on cell models. Therefore, the therapeutic relevance of these enzymes should be considered preliminary. Future work will focus on functional validation to substantiate their anticancer potential.

Ethical Approval

The Ethics Committee of Dezful University of Medical Science approved this study (ethical code: IR.DUMS.REC.1402.008).

Acknowledgements

This study was financially supported by Dezful University of Medical Sciences, Iran.

Authors' contributions

Conceptualization: Babak Elyasi Far, Behnam Azizolahi

Methodology: Reza Jaberi Manesh, Babak Elyasi Far, Behnam Azizolahi, Maryam Gheibipour,

Software: Babak Elyasi Far

Investigation: Reza Jaberi Manesh, Babak Elyasi Far, Abbas Moridnia, Maryam Gheibipour

Data curation: Babak Elyasi Far, Yasin Ahmadi

Formal analysis: Babak Elyasi Far, Yasin Ahmadi, Ladan Mafakher, Behnam Azizolahi

Writing – original draft: Babak Elyasi Far, Yasin Ahmadi, Ladan Mafakher, Maryam Gheibipour

Writing – review & editing: All authors

Supervision: Behnam Azizolahi, Babak Elyasi Far

Project Administration: Babak Elyasi Far

Funding

This article is a part of a thesis of the Corresponding author Babak Elyasi Far, which was financially supported by the Infectious and Tropical Diseases Research Center of Dezful University of Medical Sciences. In this article, we have received funding from vice chancellor of research from Dezful University of Medical Sciences.

Availability of data and materials

The datasets of the current study are available from the corresponding author on reasonable request.

Grammar check

The English grammar of this work was checked by Artificial intelligence and Grammarly software.

References

1. Saleem R, Ahmed S. Characterization of a new L-glutaminase produced by Achromobacter xylosoxidans RSHG1, isolated from an expired hydrolyzed L-glutamine sample. Catalysts. 2021;11(11):1262. Doi: org/10.3390/catal11111262.

2. Alrumman S, Mostafa Y, Al-Izran KA, Alfaifi M, Taha T, Elbehairi S. Production and anticancer activity of an L-asparaginase from Bacillus licheniformis isolated from the Red Sea, Saudi Arabia. Sci Rep. 2019;9(1):3756. Doi: org/10.1186/s40360-018-0242-1.

3. Roberts J, MacAllister TW, Sethuraman N, Freeman AG. Genetically engineered glutaminase and its use in antiviral and anticancer therapy. Google Patents; 2001.

4. Qoura LA, Balakin KV, Hoffman RM, Pokrovsky VS. The potential of methioninase for cancer treatment. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2024:189122. Doi: org/10.1016/j.bbcan.2024.189122.

5. Gomaa EZ. Production, characterization, and antitumor efficiency of l-glutaminase from halophilic bacteria. Bull Natl Res Cent. 2022;46(1):10. Doi:org/10.1186/s42269-021-00693-w.

6. Badoei-Dalfard A. L-asparaginase production in the Pseudomonas pseudoalcaligenes strain JHS-71 isolated from Jooshan Hot-spring. Mol Biol Res Commun. 2016;5(1):1.

7. Selim MS, Mounier MM, Abdelhamid SA, Hamed AA, Abo Elsoud MM, Mohamed SS. Characterization, modeling, and anticancer activity of L. arginase production from marine Bacillus licheniformis OF2. BMC biotechnology. 2024;24(1):6. Doi:org/10.1186/s12896-024-00829-6.

8. Moridnia A, Hoseinitabar L, Khosropanah A, Mafakher L, Anaam M, Far BE. Isolation and Antimicrobial Activity of Halophilic Bacteria Isolated from Saline Soil of Shushtar City, Iran. Pharm Sci. 2024;31(1):89-95. doi:10.34172/PS.024.40785.

9. Shirazian P, Asad S, Amoozegar MA. The potential of halophilic and halotolerant bacteria for the production of antineoplastic enzymes: L-asparaginase and L-glutaminase. EXCLI journal. 2016;15:268. doi: 10.17179/excli2016-146.

10. Gulati R, Saxena R, Gupta R. A rapid plate assay for screening l-asparaginase producing micro-organisms. Lett Appl Microbiol. 1997;24(1):23-6. Doi:org/10.1046/j.1472-765X.1997.00331.x.

11. Li M, Hong CY, Yan WX, Chao ZS, Gang YC, Ling DJ, et al. Bacillus zanthoxyli sp. nov., a novel nematicidal bacterium isolated from Chinese red pepper (Zanthoxylum bungeanum Maxim) leaves in China. Antonie van Leeuwenhoek. 2017;110:1179-87. Doi:org/10.1007/s10482-017-0890-y.

12. Usmonov A, Yoo S-J, Kim ST, Yang JS, Sang MK, Jung HW. The Bacillus zanthoxyli HS1 strain renders vegetable plants resistant and tolerant against pathogen infection and high salinity stress. Plant Pathol J. 2021;37(1):72. doi: 10.5423/PPJ.NT.12.2020.0219.

13. Zolfaghar M, Amoozegar MA, Khajeh K, Babavalian H, Tebyanian H. Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran. Mol Biol Rep. 2019;46:3275-86. doi.org/10.14715/cmb/2016.62.12.6.

14. Kassab, M.M.S. Screening and industrial production of bacterial L-glutaminase as an anticancer agent from different soil environments in Egypt. J Biotechnol Res.2024;45(3), 120–129. Doi: 10.20944/preprints202405.1087.v1.

15. Mostafa YS, Alamri SA, Alfaifi MY, Alrumman SA, Elbehairi SEI, Taha TH, et al. L-glutaminase synthesis by marine Halomonas meridiana isolated from the red sea and its efficiency against colorectal cancer cell lines. Molecules. 2021;26(7):1963. Doi:org/10.3390/molecules26071963.

16. Gholami N, Ebrahimipour Gh, Yaghoubi Avini M. A Study of the Optimization of Anti-Tumor L-Asparaginase Production Using Bacillus subtilis Isolated from the Soil of East Azerbaijan Province. J Microb Biol. 2022;11(44):1-12. doi: 10.22108/bjm.2021.129050.1393.

17. Ameen F, Alshehri WA, Al-Enazi NM, Almansob A. L-Asparaginase activity analysis, ansZ gene identification and anticancer activity of a new Bacillus subtilis isolated from sponges of the Red Sea. Bioscience, Biotechnology, and Biochemistry. 2020;84(12):2576-84. Doi: 10.22108/bjm.2021.129050.1393.

18. Aishwariyaa Lakshmi V, Sheeja L, Devi Mari S. Screening and identification of asparginase and glutaminase producing halophilic bacteria from natural saline habitats. Int J Recent Adv. Biotechnol Nanotechnol. 2020;3(1):34-53.

19. Orabi H, El-Fakharany E, Abdelkhalek E, Sidkey N. Production, optimization, purification, characterization, and anti-cancer application of extracellular L-glutaminase produced from the marine bacterial isolate. Preparative Biochemistry & Biotechnology. 2020;50(4):408-18. Doi:org/10.1080/10826068.2019.1703193.

20. Meena B, Anburajan L, Dheenan PS, Begum M, Vinithkumar NV, Dharani G, et al. Novel glutaminase free L-asparaginase from Nocardiopsis alba NIOT-VKMA08: production, optimization, functional and molecular characterization. Bioprocess Biosyst Eng. 2015;38:373-88. Doi:org/10.1007/s00449-014-1277-3.

21. Ibrahim ZKM, Ahmed TTM, Salah MG. Isolation, Screening and Characterization of L-Glutaminase Producing Bacteria from Brackish Water. Int J Progress Sci Technol. 2020;22(1):227-31.