Marine bacteria producing L-Asparaginase with low glutaminase and urease co-activity from Pangandaran East Coast Indonesia

Wulan Pertiwi*, Azmy J. Namira, Maelita R. Moeis, Luthfia H. Muharram, Nelis Hernahadini

OPEN ACCESS

[1] Universitas Muhammadiyah Bandung, Bandung 40614, West Java, Indonesia

*Corresponding Author:
wulanpertiwi@umbandung.ac.id

Received: 23 January 2024
Accepted: 17 May 2025
Published: 30 May 2025
Academic Editor: Dr. Dandy Yusuf

 Accreditation Number:148/M/KPT/2020. ISSN: 2089-5690, e-ISSN: 2406-9272. https://doi.org/ 10.15578/squalen.908

Abstract

Bacterial L-asparaginase is a therapeutic enzyme widely used in the treatment of Acute Lymphoblastic Leukemia (ALL). Although L-asparaginase is prominent in treating ALL, its use is limited due to its side effects caused by its dual substrate specificity towards both asparagine and glutamine. This study aimed to isolate and identify marine bacteria from the East Coast of Pangandaran capable of producing L-asparaginase with low glutaminase and urease co-activities. A semi-quantitative approach was employed, involving the isolation and screening of seawater bacteria using Zobell Marine media supplemented with L-asparagine, glutamine or urea and phenol red as a pH indicator to determine the enzymatic activity. Molecular identification was performed by amplifying and sequencing the 16S rRNA gene, followed by phylogenetic analysis using the neighbor-joining method with 1,000 bootstrap replicates. The results indicated that the bacterial isolate designated PT3 exhibited a high enzymatic index of 4.2 for L-asparaginase, surpassing that of the positive control (*E. coli*), which had an index of 1.4. Sequence analysis revealed that PT3 shared 99.58% identity with *Marinobacterium georgiense* strain NBRC 102606, an earlier synonym of *Marinobacterium iners*. Therefore, PT3 was identified as a strain of *Marinobacterium iners*, with potential as a novel source of L-asparaginase and displayed significant L-asparaginase activity with minimal co-activity of glutaminase and urease, highlighting its potential as a safer alternative for therapeutic enzyme development.

Keywords: enzymatic index, *Marinobacterium iners*, , molecular identification, therapeutic enzyme 16S rRNA.

Introduction

Cancer remains a leading cause of morbidity and mortality worldwide. According to Globocan 2020, approximately 19.3 million new cancer cases and nearly 10 million cancer-related deaths occurred globally in that year (Bray *et al*., 2021; Sung *et al*., 2021). By 2040, cancer is expected to remain a major cause of death (GCO, 2022). Leukemia ranked as the tenth deadliest cancer in 2020 (WHO, 2020). Leukemia is a type of cancer that originates from mutations in progenitor cells within the bone marrow. These malignant cells proliferate uncontrollably, infiltrate the bloodstream, and increase the white blood cell count, often spreading to other organs and impairing their function (Cancer Council, 2018).

L-asparaginase (L-ASNase) (L-asparagine amidohydrolase, EC 3.5.1.1) is a therapeutic enzyme that catalyzes the conversion of L-asparagine (L-Asn) to L-aspartate and ammonia (Vimal & Kumar, 2018). L-Asn is an essential amino acid for cancer cells, particularly lymphoblastic leukemia cells. L-ASNase depletes L-Asn levels in the bloodstream, thereby inhibiting protein synthesis and inducing apoptosis in cancer cells (Nagarethinam *et al*., 2012; Piatkowska-Jakubas *et al*., 2008). The general mechanism of L-Asn hydrolysis by L-ASNase is illustrated in Figure 1.

L-ASNase is an intracellular enzyme produced by microorganisms, animals, and plants (Bahraman & Alemzadeh, 2016). It has been widely applied in chemotherapy treatment of Acute Lymphoblastic Leukemia (ALL), particularly in pediatric patients. In

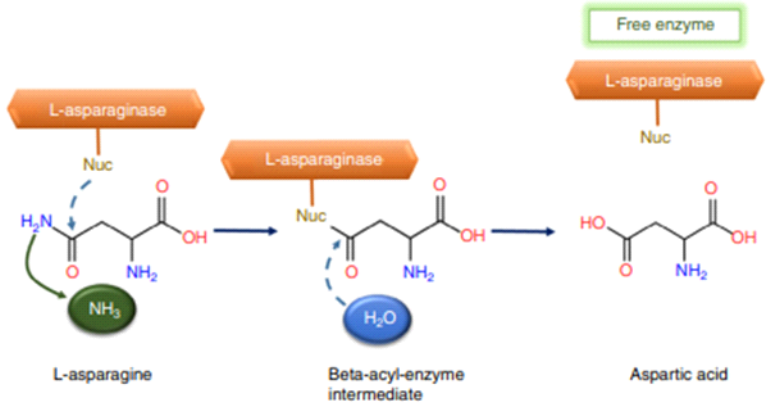

Figure 1. L-Asn hydrolysis reaction by L-ASNase (Cachumba *et al*., 2016)

addition to ALL, L-ASNase has also been used in the treatment of other malignancies such as Hodgkin's cancer, lymphosarcoma, Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), reticulosarcoma, and melanosarcoma (Lopes *et al*., 2017).

Despite its therapeutic efficacy, the clinical use of L-ASNase is limited by significant side effects, including liver and pancreatic dysfunction, central nervous system toxicity, edema, skin rash, fever, diabetes, leukopenia, bleeding, and some hypersensitivity reactions. These adverse effects are largely attributed to the enzyme's dual substrate specificity, as it catalyzes both L-asparagine and L-glutamine hydrolysis (Athanassiado *et al*., 2004; Batool *et al*., 2016; Howard & Carpenter, 1972; Ramya *et al*., 2012). In addition, the presence of glutaminase destroys glutamine which is necessary for normal cell growth (Greenberg *et al*., 1964). Additionally, urease activity can result in the hydrolysis of urea, releasing ammonia and carbon dioxide, which contribute to systemic toxicity (Ashok *et al*., 2019; Bano & Sivaramakrishnan, 1980; Doriya & Kumar, 2016a). The commercial L-ASNase used in the treatment of lymphoblastic leukemia and lymphosarcoma originated from bacteria that are glutaminase free. L-ASNases from *E. coli* (EcaII) and *Erwinia chrysanthemi* (ErA), typically exhibit glutaminase activity that can reach up to 9% of their L-asparaginase activity. Therefore, many studies have been carried out to obtain L-ASNase with low glutaminase activity, such as bioprospecting other microbial sources or modifying commercial L-ASNase with activated Polyethylene Glycol (PEG) (Loureiro *et al*., 2012) and L-ASNase II engineering from *E.coli*, to obtain mutants that have low glutaminase activity through molecular tethering, molecular dynamics, and QM-MM simulations (Ardalan *et al*., 2018). Notably, glutaminase-free L-ASNases have been successfully isolated from *Streptomyces cyaneus*, *Streptomyces exfoliates*, and *Streptomyces phaeochromogenes* (Saxena *et al*., 2015). Enzymes lacking both glutaminase and urease activities hold significant promise as safer alternatives for cancer therapy (Ashok *et al*., 2019).

Clinical studies have shown that L-ASNase derived from terrestrial microorganisms can induce toxicity and immunosuppression, which may contribute to treatment resistance. In contrast, marine-derived L-ASNase have emerged as promising alternatives for both clinical and industrial applications, owing to their distinct characteristics such as novel structural features, lower molecular weight, and higher substrate specificity (Qeshmi *et al*., 2018). In light of these advantages, this study aimed to isolate and characterize marine bacteria from the East Coast of Pangandaran as potential sources of L-ASNase with reduced glutaminase and urease co-activities, and improved therapeutic properties

Material and Methods

Sampling

Seawater sampling was conducted on the East Coast of Pangandaran (coordinates: -7.7020578,108.658508) at 08.00 Western Indonesian Time (WIB), approximately 5 meters from the shoreline at a depth of 0.5–1.0 meters below sea level. The collected seawater sample exhibited a salinity of 3.5% and a pH of 7.27. The sampling location is depicted in Figure 2.

Media Preparation

For bacterial isolation, Zobell Marine Broth (ZMB) (Himedia) and Zobell Marine Agar (ZMA) (Himedia) were used, and sterilized by autoclaving at 121 °C (15 psi) for 15 minutes. For enzymatic screening, modified ZMA media were prepared by supplementing the base

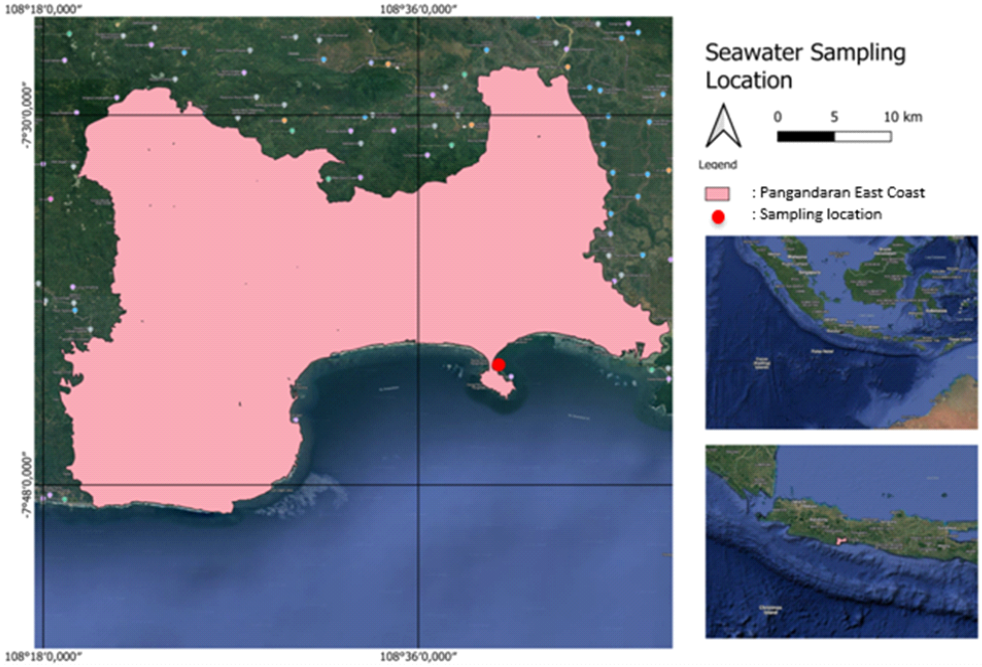

Figure 2. Sampling location.

medium with either L-asparagine, glutamine, or urea at a final concentration of 5 g/L, along with 1.8 ppm phenol red as a pH indicator. The pH of the media was adjusted to 5.8 using HCl or NaOH (Ashok *et al*., 2019)

Isolation and Purification of Marine Bacteria

Isolation was performed using the spread plate method, by spreading 100 µL of seawater onto ZMA plates, followed by incubation at 30 °C for 16 hours (Sanz-Sáez *et al*., 2020). Morphologically distinct colonies were sub-cultured separately on new ZMA medium to obtain single colonies.

Bacterial Characterization

Colony morphology was characterized based on criteria from Leboffe and Pierce (Leboffe & Pierce, 2012), including colony shape (circular, irregular, and punctiform or dots), shape of margins or colonial rim shape (entire or smooth and flat, undulate or wavy, filamentous, rhizoid or branches like roots, and lobate or grooved edges), colony elevation (raised, convex, umbonate, plateau, raised, raised spreading edge, flat raised margin, and growth into), the color of the colonies formed can also be determined by the appearance of colors such as: dull, shiny, translucent, and opaque.

Screening for Bacteria Producing L-asparaginase with Low Glutaminase and Urease Activities

Each bacterial isolate was screened in triplicate using modified ZMA media supplemented with either L-asparagine, glutamine, or urea to assess enzymatic activity. *Escherichia coli* ATCC served as the positive control, while sterile distilled water was used as the negative control.

The cultures were incubated at 30 °C for 16 hours. Enzymatic activity was inferred from the presence of a red halo surrounding bacterial colonies, resulting from pH elevation due to ammonia production. The diameter of both the colonies and the red zones were measured and the enzymatic index or zone index was calculated using the formula described by Ashok et al. (Ashok *et al*., 2019) as shown in Equation 1.

$$Zone\ Index = \frac{red\ zone\ diameter}{colony\ diameter} \quad (1)$$

Molecular Identification of Bacteria Producing L-asparaginase

DNA isolation was performed using the Thermo Scientific GeneJET Genomic DNA Purification kit from 5 ml of bacterial culture grown in ZMB medium. The 16S rRNA gene in the DNA was amplified using DreamTaq Master Mix with primers BactF1(5'-AGAGTTTTGATC(A/C)TGGCTCAG-3') and UniB1 (5'-GGTTAC(G/C)TTGTTACGACTT-3'), yielding

amplicons approximately 1,400 bp in length (Baker *et al*., 2001; Shabarni Gaffar *et al*., 2014). The PCR profile used was initial denaturation at 95 °C for two minutes, followed by 25 cycles of denaturation at 95 °C for one minute, annealing at 50°C for one minute, elongation at 72 °C for one minute and ending with a final elongation at 75 °C for ten minutes. Electrophoresis of the amplicons was performed in 1% agarose gel in 1x TAE buffer. To each 5 µL sample, 1 µL loading dye and 1 µL GelRed solution were added. Electrophoresis was carried out at a voltage of 75 mV for 45 minutes. DNA bands were visualized under a UV transilluminator.

The PCR amplicons were then sequenced by the Sanger method at 1st BASE (Malaysia). Analysis of the 16S rRNA sequence was carried out using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/) (Altschul *et al*., 1990). The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates using the MEGA 11 application (Tamura *et al*., 2021).

Results and Discussion

Isolation of Bacteria from the East Coast of Pangandaran

Three distinct colonies (PT1, PT2, and PT3) with differing morphologies were isolated and purified from seawater samples collected from the East Coast of Pangandaran (Figure 3). PT1 exhibited circular colonies with entire margins, convex elevation, pale white color, and medium size. PT2 had similar shape and color but was flat and smaller in size. PT3 showed a punctiform shape with entire margins, convex elevation, and a pale white color. These results align with earlier studies, such as those by Jamaluddin et al. (Jamaluddin *et al*., 2018), who identified thermohalophilic L-asparaginase-producing bacteria from Wawolesea hot springs, and Sulistiyani & Kusumawati (Sulistiyani & Kusumawati, 2019) who isolated glutaminase-free L-asparaginase-producing endophytes from medicinal plants in East Sumba. Similar work by Alrumman et al. (Alrumman *et al*., 2019) demonstrated that marine bacteria from the Red Sea could also serve as a source of L-asparaginase.

Screening for Bacteria Producing L-asparaginase, Glutaminase, and Urease

All three isolates (PT1, PT2, and PT3) tested positive for L-asparaginase, glutaminase, and urease activity, indicated by red zones around colonies on modified ZMA media (Figure 4). These color changes resulted from ammonia production, which increases the pH, altering the phenol red indicator from yellow to red. The enzymatic index (EI) was calculated as the ratio of red zone diameter to colony diameter.

L-asparaginase hydrolyzes L-asparagine to produce aspartic acid and ammonia; glutaminase hydrolyzes glutamine to yield glutamic acid and ammonia; and urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia. The ammonia (NH_f) released in the medium reacts with water to form ammonium hydroxide ($NH_{,,}$ OH), leading to an increase in pH. Phenol red, a pH indicator, appears yellow under acidic conditions and red in alkaline environments. Enzymatic activity was assessed semi-quantitatively using the enzymatic index (EI), calculated as the ratio of the red zone diameter to the colony diameter (Doriya & Kumar, 2016a). Enzyme production was visualized through a qualitative plate assay, enabling rapid screening of enzyme-producing bacterial isolates via direct observation (Hankin & Anagnostakis, 1975). Gulati *et al*. (1997) established a correlation between the enzymatic index and actual enzyme activity. In this study, the L-asparaginase indices of the isolates ranged from 3.0 to 4.2, glutaminase indices from 1.8 to 3.6, and urease indices from 1.4 to 4.0 (Table 1). These values are consistent with previous findings by Doriya & Kumar (2016b), who reported microbial enzymatic indices ranging from 0.8 to 4.0. Among the isolates,

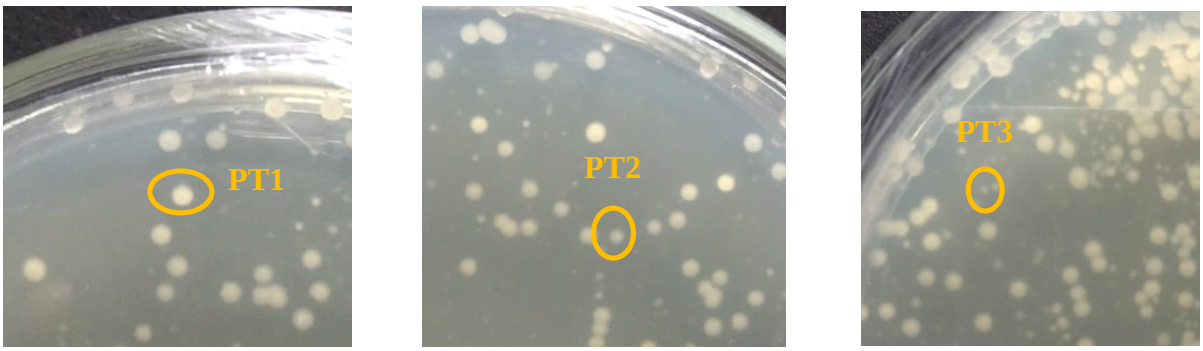

Figure 3. PT1, PT2 and PT3 colonies.

Table 1. Measurement of L-asparaginase, glutaminase and urease indices after 16 hours of incubation

Potential Enzyme	Isolate	Red zone diameter (mm)	Colony diameter (mm)	Enzymatic Index
L-ASPARAGINASE	*E. coli*	7.5	5.5	1.4
	PT1	15	5	3.0
	PT2	20	5	4.0
	PT3	25	6	4.2
GLUTAMINASE	*E. coli*	14	5.5	2.5
	PT1	17	6	2.8
	PT2	18	5	3.6
	PT3	9	5	1.8
UREASE	*E. coli*	13	6	2.2
	PT1	14	5	2.8
	PT2	20	5	4.0
	PT3	8	5.5	1.4

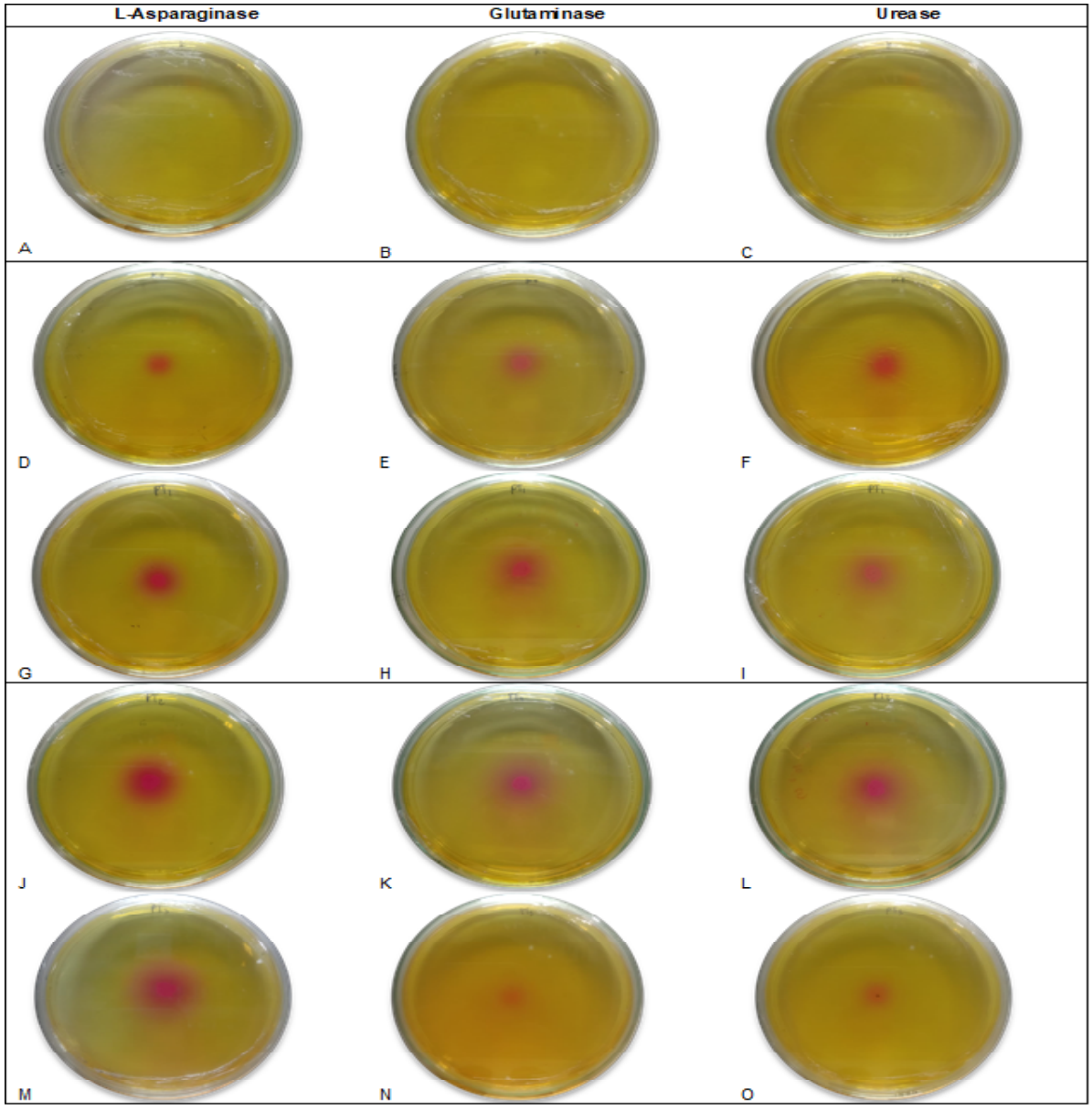

Figure 4. Representative results of bacterial screening for L-asparaginase production with low glutaminase and urease activities, using modified ZMA medium supplemented with either L-asparagine, glutamine or urea, and phenol red as a pH indicator. A-C: negative control (sterile water). D-F: positive control (*E. coli*). G-I: Isolate PT1. J-L: Isolate PT2. M-O: Isolate PT3.

PT3 exhibited the highest L-asparaginase activity, with comparatively lower glutaminase and urease co-activities. PT3 had a colony diameter of 6 mm and a red zone diameter of 25 mm, resulting in an L-asparaginase index of 4.16—substantially higher than that of the positive control (*E. coli*), which was 1.4. For glutaminase activity, PT3 displayed a colony diameter of 5 mm and a red zone diameter of 9 mm, yielding a low glutaminase index of 1.8—again lower than *E. coli* (2.5). Similarly, PT3 had the lowest urease index of 1.4 (colony diameter: 5.5 mm; red zone diameter: 8 mm), compared to *E. coli*, which had a urease index of 2.2. These results suggest that PT3 is a promising candidate as a marine bacterial source of L-asparaginase with minimal glutaminase and urease co-activities.

Molecular identification of PT3

The genomic DNA of PT3 was used to amplify its 16S rRNA gene (~1500 bp), a widely accepted marker for bacterial identification and phylogenetic analysis (Figure 5). The 16S rRNA gene is one of the most common house-keeping genes used for bacterial identification and phylogenetic studies. The 16S rRNA gene contains conserved and variable regions with a size of around 1500 bp (Srinivasa *et al*., 2015).

The sequenced fragment (1443 bp) was analyzed using BLAST, showing 99.58% identity with *Marinobacterium georgiense* strain NBRC 102606 (Table 2).

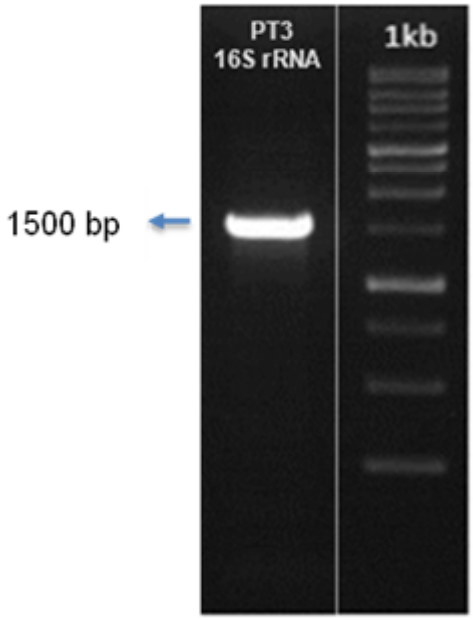

Figure 5. Electropherogram of 16S rRNA gene amplification from PT3 isolate.

Table 2. Bacterial strains showing the highest partial 16S rRNA gene sequence homology with the PT3 isolate

Species	Max Score	Total Score	Query Cover	E Value	Percent Identity	Acc. Len	Accession
Marinobacterium georgiense strain NBRC 102606	2619	2619	98%	0.0	99.58%	1468	NR_114163.1
Marinobacterium georgiense strain KW-40	2553	2553	97%	0.0	99.29%	1435	NR_042980.1
Marinobacterium halophilum strain mano11	2416	2416	96%	0.0	98.06%	1400	NR_042980.1
Marinobacterium maritimum strain AR-11	2329	2329	98%	0.0	96.28%	1425	NR_116301.1
Marinobacterium stanieri strain ATCC 27130	2294	2294	99%	0.0	95.48%	1517	NR_024699.1
Marinobacterium sediminicola strain CN47	2257	2257	99%	0.0	95.06%	1464	NR_044529.1
Marinobacterium coralli strain R-40509	2148	2148	93%	0.0	95.53%	1349	NR_117449.1
Marinobacterium profundum strain PAMC 27536	2139	2139	99%	0.0	93.62%	1474	NR_148801.1
Marinobacterium aestuarii strain ST58-10	2128	2128	99%	0.0	93.48%	1541	NR_159348.1

Phylogenetic analysis confirmed that PT3 clustered closely with this strain, supported by a high bootstrap value of 100, indicating a strong relationship. Their placement on the same branch indicates a close phylogenetic relationship. A bootstrap value of 100 reflects a highly robust phylogenetic grouping. Generally, bootstrap values ranging from 70 to 100 suggest strong support for the stability of clade positions, implying minimal likelihood of topological changes. In contrast, bootstrap values below 70 indicate weak support, suggesting greater potential for variation in the branching pattern (Anafarida & Badruzsaufari, 2020).

The minimum full-length 16S rRNA gene sequence identity required to consider two bacterial isolates as belonging to the same species has been proposed to be approximately 99% (Johnson *et al*., 2019). Chun *et al*. (2018) further recommended a threshold of 98.7% identity with the full-length 16S rRNA gene sequence of a type strain for species-level classification. *Marinobacterium georgiense* strain NBRC 102606 is the designated type strain for this species, and it shares 99.58% identity with the 16S rRNA gene sequence of the PT3 isolate. *Marinobacterium georgiense* was originally described by González et al. (1997). Satomi *et al*. (2002) compared the 16S rDNA and *gyr*B sequences of *Marinobacterium georgiense* and *Pseudomonas iners*, finding sequence similarities of 99.7% for 16S rDNA and 98.5% for *gyrB*. Additionally, the amino acid sequences of GyrB in both species were found to be identical. Based on these findings, Satomi *et al*. (2002) concluded that *P.iners* was a strain of *M.georgiense*. Tindall (2019) later revised the nomenclature, renaming the species *Marinobacterium iners*. Therefore, PT3 is classified as a strain of *Marinobacterium iners*, belonging to the family Oceanospirillaceae, order Oceanospirillales, class Gammaproteobacteria, and phylum Proteobacteria. The discovery of marine bacteria producing L-asparaginase from the East Coast of highlights a promising avenue for developing novel therapeutic enzymes. The phylogenetic tree supporting this identification is shown in Figure 6.

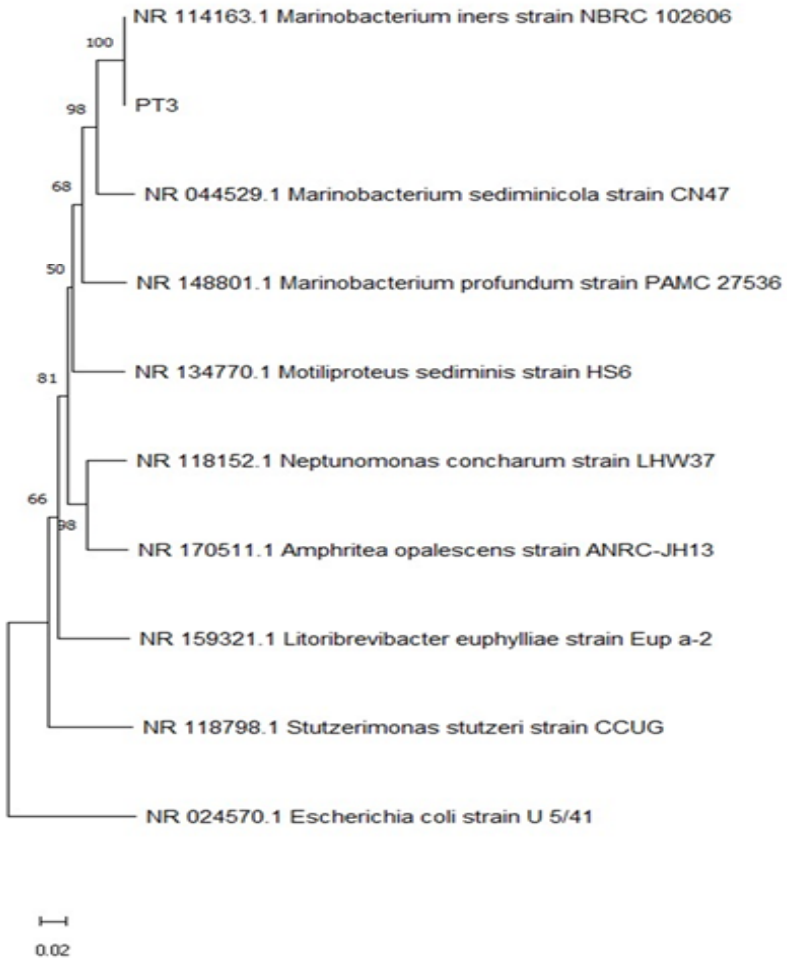

Figure 6. Phylogenetic tree of PT3 isolate constructed using the Neighbor-Joining method with 1000 bootstrap replications.

Marinobacterium georgiense or *Marinobacterium iners* is a Gram-negative species commonly found in marine environments and oil reservoirs. It is halophilic and characterized by short, straight rod-shaped cells measuring 1.3–2 µm in length and 0.6–0.8 µm in width. The species is aerobic or facultatively aerobic, reproduces by binary fission, and typically exists as solitary cells without aggregation. Colonies generally form within 1–3 days at an optimal temperature of 25–30 °C. Morphologically, colonies are circular with convex elevation, entire margins, and a yellowish cream color, typically measuring 1–3 mm in diameter (González & Buchan, 2021). PT3 colonies exhibited similar shape, elevation, margin, and color characteristics but were slightly larger in size.

Although specific pathogenicity studies on *M. georgiense* are lacking, there is no evidence to suggest it poses a risk to humans, animals, or plants. Furthermore, this species is not listed among pathogenic bacteria by the Centers for Disease Control and Prevention or the National Institutes of Health (Meechan & Potts, 2020). In the absence of reports linking this species to disease, *M. georgiense* is generally considered non-pathogenic. Nevertheless, standard biosafety protocols should be observed when handling any microorganism. Research indicates that certain strains within the Marinobacterium genus such as *Marinobacterium lutimaris* produce hydrolytic enzymes capable of degrading Tween 80 and *Marinobacterium rhizophilum* is known to hydrolyze starch and Tween 20 (González, J. M., & Buchan, 2021). These findings suggest that *Marinobacterium georgiense*, as an earlier heterotypic synonym of *Marinobacterium iners*, could be a valuable source of L-asparaginase enzyme for large-scale applications.

Conclusions

In this study, PT3 was identified as a strain of *Marinobacterium iners*, isolated from seawater off the East Coast of Pangandaran. It exhibited high L-asparaginase activity with minimal glutaminase and urease co-activity, making it a promising candidate for the development of safer therapeutic enzymes. Future research should focus on optimizing fermentation conditions, scaling up enzyme production, and exploring recombinant expression systems for PT3-derived L-asparaginase in clinical applications, particularly for Acute Lymphoblastic Leukemia therapy.

Acknowledgement

The authors gratefully acknowledge the Faculty of Science and Technology, Muhammadiyah University of Bandung for its support through an internal grant during the 2022/2023 academic year (Grant Number: 001/DEK/KTR/II.3.AU/2023).

References

Alrumman, S. A., Mostafa, Y. S., Al-Izran, K. A., Alfaifi, M. Y., Taha, T. H., & Elbehairi, S. E. (2019). Production and anticancer activity of an L-asparaginase from Bacillus licheniformis isolated from the Red Sea, Saudi Arabia. *Scientific Reports*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-40512-x

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/https://doi.org/10.1016/S0022-2836(05)80360-2

Anafarida, O., & Badruzsaufari, B. (2020). Analisis Filogenetik Mangga (Mangifera Spp.) Berdasarkan Gen 5,8S Rrna. *Ziraa'Ah Majalah Ilmiah Pertanian*, *45*(2), 120. https://doi.org/10.31602/zmip.v45i2.3001

Ardalan, N., Mirzaie, S., Sepahi, A. A., & Khavari-Nejad, R. A. (2018). Novel mutant of Escherichia coli asparaginase II to reduction of the glutaminase activity in treatment of acute lymphocytic leukemia by molecular dynamics simulations and QM-MM studies. *Medical Hypotheses*, *112*(January), 7–17. https://doi.org/10.1016/j.mehy.2018.01.004

Ashok, A., Doriya, K., Rao, J. V., Qureshi, A., Tiwari, A. K., & Kumar, D. S. (2019). Microbes Producing L-Asparaginase free of Glutaminase and Urease isolated from Extreme Locations of Antarctic Soil and Moss. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-018-38094-1

Athanassiado, F., M, K., T, P., M, S., & A, M. (2004). Severe hyperlipidemia in a child with acute lymphoblastic leukemia treated with L-asparaginase and prednisone. *Pediatr. Int.*, *46*(6), 743–744.

Bahraman, F., & Alemzadeh, I. (2016). Optimization of L-Asparaginase Immobilization onto Calcium Alginate Beads. *Chemical Engineering Communications*, *204*(2), 216–220. https://doi.org/10.1080/00986445.2015.1065821

Baker, G. C., Gaffar, S., Cowan, D. A., & Suharto, A. R. (2001). Bacterial community analysis of Indonesian hot springs. *FEMS Microbiology Letters*, *200*(1), 103–109. https://doi.org/https://doi.org/10.1111/j.1574-6968.2001.tb10700.x

Bano, M., & Sivaramakrishnan, V. M. (1980). Preparation and properties of L-asparaginase from green chillies (*Capsicum annum* L.). *Journal of Biosciences*, *2*(4), 291–297.

Batool, T., Makky, E. A., Jalal, M., & Yusoff, M. . (2016). A Comprehensive review on Lasparaginase and its applications. *Applied Biochemistry and Biotechnology*, *178*(5), 900–923. https://doi.org/10.1007/s12010-015-1917-3

Bray, F., M, L., E, W., & I., S. (2021). The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer, as a leading cause of premature death worldwide. *Cancer*, *127*(16), 3029–3030. https://doi.org/DOI 10.1002/cncr.33587.

Cachumba, J. J. M., Antunes, F. A. F., Peres, G. F. D., Brumano, L. P., Santos, J. C. Dos, & Da Silva, S. S. (2016). Current applications and different approaches for microbial L-asparaginase production. *Brazilian Journal of Microbiology*, *47*, 77–85. https://doi.org/10.1016/j.bjm.2016.10.004

Cancer Council. (2018). *Understanding Acute Leukaemia* (J. Bruce (ed.)). The University of Newcastle.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D. R., da Costa, M. S., Rooney, A. P., Yi, H., Xu, X. W., De Meyer, S., & Trujillo, M. E. (2018). Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, *68*(1), 461–466. https://doi.org/10.1099/ijsem.0.002516

Doriya, K., & Kumar, D. S. (2016a). Isolation and screening of l-asparaginase free of glutaminase and urease from fungal sp. *3 Biotech*, *6*(2). https://doi.org/10.1007/s13205-016-0544-1

Doriya, K., & Kumar, D. S. (2016b). Isolation and screening of L-asparaginase free of glutaminase and urease from fungal sp. *3 Biotech*, *6*(2), 1–10. https://doi.org/10.1007/s13205-016-0544-1

GCO. (2022). *Cancer Tomorrow*. International Agency for Research on Cancer.

González, J. M., & Buchan, A. (2021). Marinobacterium. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–20).

González, J. M., Mayer, F., Moran, M. A., Hodson, R. E., & Whitman, W. B. (1997). Microbulbifer hydrolyticus gen. nov., sp. nov., and Marinobacterium georgiense gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *International Journal of Systematic Bacteriology*, *47*(2), 369–376. https://doi.org/10.1099/00207713-47-2-369

Greenberg, D., Blumenthal, G., & Ramadan, M. (1964). Effect of Administrationof The Enzyme Glutaminase on The Growth of Cancer Cells. *Cancer Res*, *24*, 957–963.

Gulati, R., Saxena, R. K., & Gupta, R. (1997). A rapid plate assay for screening L-asparaginase producing micro-organisms. *Letters in Applied Microbiology*, *24*(1), 23–26. https://doi.org/10.1046/j.1472-765X.1997.00331.x

Hankin, L., & Anagnostakis, S. L. (1975). The Use of Solid Media for Detection of Enzyme Production by Fungi. *Mycologia*, *67*(3), 597. https://doi.org/10.2307/3758395

Howard, J. B., & Carpenter, F. H. (1972). l-Asparaginase from Erwinia carotovora. *Journal of Biological Chemistry*, *247*(4), 1020–1030. https://doi.org/10.1016/s0021-9258(19)45610-x

Jamaluddin, Alfin, Muzuni, & Yanti, N. A. (2018). Eksplorasi Bakteri Termohalofilik Potensial Penghasil L-Asparaginase Sebagai Antikanker di Sumber Air Panas Wawolesea. *BioWallacea (Journal of Biological Research)*, *5*(1), 716–725.

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 1–11. https://doi.org/10.1038/s41467-019-13036-1

Leboffe, M. J., & Pierce, B. E. (2012). Brief Microbiology Laboratory Theory & Application. In *Englewood: Morton Publishing* (2nd Editio).

Lopes, A. M., Oliveira-Nascimento, L. de, Ribeiro, A., Tairum, C. A., Breyer, C. A., Oliveira, M. A. de, Monteiro, G., Souza-Motta, C. M. de, Magalhães, P. de O., Avendaño, J. G. F., Cavaco-Paulo, A. M., Mazzola, P. G., Rangel-Yagui, C. de O., Sette, L. D., Converti, A., & Pessoa, A. (2017). Therapeutic l-asparaginase: upstream, downstream and beyond. *Critical Reviews in Biotechnology*, *37*(1), 82–99. https://doi.org/10.3109/07388551.2015.1120705

Loureiro, C. B., Borges, K. S., Andrade, A. F., Tone, L. G., & Said, S. (2012). *Purification and Biochemical Characterization of Native and Pegylated Form of L-Asparaginase from Aspergillus terreus and Evaluation of Its Antiproliferative Activity*. *2012*(June), 138–145.

Meechan, P. J., & Potts, J. (2020). Biosafety in Microbiological and Biomedical Laboratories. In *Centers for Disease Control and Prevention National Institutes of Health* (6th ed.). U.S. Department of Health and Human Services. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

Nagarethinam, S., Nagappa, A. N., Udupa, N., Rao, V. J., & Balashanmugam, M. (2012). Microbial L-Asparaginase and its future prospects. *Asian Journal of Medical Research*, *1*(4), 159–160.

Piatkowska-Jakubas, B., Krawczyk-Kuliœ, M., Giebel, S., Adamczyk-Cioch, M., Czyz, A., Marañda, E. L., Paluszewska, M., Pal³ynyczko, G., Piszcz, J., & Ho³owiecki, J. (2008). Use of L-asparaginase in acute lymphoblastic leukemia: Recommendations of the Polish adult leukemia group. *Polskie Archiwum Medycyny Wewnetrznej*, *118*(11), 664–669. https://doi.org/10.20452/pamw.518

Qeshmi, F. I., Homaei, A., Fernandes, P., & Javadpour, S. (2018). Marine microbial L-asparaginase: biochemistry, molecular approaches and applications in tumor therapy and in food industry. *Microbiological Research*, *208*, 99–112. https://doi.org/10.1016/j.micres.2018.01.011

Ramya, L. N., Doble, M., Rekha, V. P. B., & Pulicherla, K. K. (2012). L-asparaginase as potent anti-leukemic agent and its significance of having reduced glutaminase side activity for better treatment of acute lymphoblastic leukaemia. *Applied Biochemistry and Biotechnology*, *167*(8), 2144–2159. https://doi.org/10.1007/s12010-012-9755-z

Sanz-Sáez, I., Salazar, G., Sánchez, P., Lara, E., Royo-Llonch, M., Sà, E. L., Lucena, T., Pujalte, M. J., Vaqué, D., Duarte, C. M., Gasol, J. M., Pedrós-Alió, C., Sánchez, O., & Acinas, S. G. (2020). Diversity and distribution of marine heterotrophic bacteria from a large culture collection. *BMC Microbiology*, *20*(1), 1–16. https://doi.org/10.1186/s12866-020-01884-7

Satomi, M., Kimura, B., Hamada, T., Harayama, S., & Fujii, T. (2002). Phylogenetic study of the genus Oceanospirillum based on 16S rRNA and gyrB genes: Emended description of the genus Oceanospirillum, description of Pseudospirillum gen. nov., Oceanobacter gen. nov. and Terasakiella gen. nov. and transfer of Oceanospirillum. *International Journal of Systematic and Evolutionary Microbiology*, *52*(3), 739–747. https://doi.org/10.1099/ijs.0.01427-0

Saxena, A., R, U., & N, K. (2015). Isolation and identification of actinomycetes for production of novel extracellular glutaminase free L-asparaginase. *Indian J Exp Biol*, *53*(12), 786–793.

Shabarni Gaffar, Maksum, I. P., & Julaeha, E. (2014). Identifikasi populasi bakteri dalam spons pencuci piring dengan metode Pcr-Rflp Identification of bacteria population in dishwashing sponge using pcr-rflp method. *Chimica et Natura Acta*, *2*(2), 120–125. http://journal.unpad.ac.id/jcena/article/view/9154

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L., & Lynch, S. V. (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS ONE*, *10*(2), 1–22. https://doi.org/10.1371/journal.pone.0117617

Sulistiyani, T., & Kusumawati, D. (2019). Keragaman Bakteri Endofit Penghasil L-Asparaginase Bebas L-Glutaminase. *Jurnal Kefarmasian Indonesia*, *9*(1), 28–39. https://doi.org/9.DOI: 10.22435/jki.v9i1.1302.

Sung, H., J, F., RL, S., M, L., I, S., Jema, A., & F., B. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*, *71*(3), 209–249. https://doi.org/Doi: 10.3322/caac.21660

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tindall, B. J. (2019). Marinobacterium iners (Iizuka and Komagata 1964) comb. nov. arising from the Synonymy of Marinobacterium georgiense González et al. 1997 and Pseudomonas iners Iizuka and Komagata 1964 (Approved Lists 1980). *Current Microbiology*, *76*(10), 1128–1129. https://doi.org/10.1007/s00284-019-01732-6

Vimal, A., & Kumar, A. (2018). l-Asparaginase: a feasible therapeutic molecule for multiple diseases. *3 Biotech*, *8*(6), 1–3. https://doi.org/10.1007/s13205-018-1282-3

WHO. (2020). *Healthy Diet*.