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Enhancement of Halophilic Glutaminase Producing by *Tetragenococcus muriaticus* FF5302 in Bioreactor

Sawitree Dueramae[1], Safiah Saah[2], Sirinan Shompoosang[3] and Patthinan Varichanan[3,*]

[1]Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand
[2]Department of Nutrition and Health, Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand
[3]Department of Applied Microbiology, Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand

([*]Corresponding author's e-mail: ifrptn@ku.ac.th)

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Abstract

From Thai fermented fish sauce (Nam-pla), 59 bacterial isolates of halophilic glutaminase-producing bacteria were isolated. The hydrolysis of glutamine served as the primary screening procedure. It was discovered that strain FF5302 was an influential producer of the extracellular halophilic glutaminase enzyme. The moderately halophilic bacterium *Tetragenoccus muriaticus* FF5302 was identified through sequence analysis of the 16S rRNA gene, phylogenetic tree analysis, and phenotypic identification before it was possible to determine the optimal nutritional and culture conditions for its halophilic glutaminase activity. The purpose of this research was to determine the optimal nutritional and cultural conditions for producing halophilic glutaminase activity in a stirred tank bioreactor with a volume capacity of 3 L. The production of halophilic glutaminase from strain FF5302 was investigated by optimizing various physicochemical parameters. Seven potential factors are generally considered in halophilic glutaminase production, namely NaCl concentration, initial pH, temperature, incubation time, nitrogen sources, carbon sources, and inoculum size. According to the findings, the amount of halophilic glutaminase in the inoculum had an effect on the growth and activity of the enzyme when it was present at a concentration of 5 % (v/v). It was also found that halophilic glutaminase showed the highest activity (87.4 U mL^{-1}) of strain FF5302 in SGC liquid medium containing NaCl 20 % (w/v), pH 8.0, agitation at 200 rpm, and an aeration rate of 0.05 VVM at 37 °C for 120 h. The size of the inoculum influenced both the proliferation and activity of halophilic glutaminase in the inoculum. Consequently, *T. muriaticus* FF5302 possessed an exceptional capacity to synthesize halophilic glutaminase. Furthermore, the halophilic glutaminase enzyme from halophilic bacteria is a prospective option for usage in the food industry as an aroma and flavor enhancer.

Keywords: Bioreactor, Enhancement, Fish sauce, Glutaminase, Halophilic, Optimization, *Tetragenoccus muriaticus*

Introduction

L-Glutaminase is also known as L-glutamine amidohydrolase (EC 3.5.1.2). L-Glutaminase is the potential enzyme that hydrolyzes the amide bond of L-glutamine to yield ammonium ions and L-glutamic acid [1]. The prospective applications of L-glutaminase in the medical profession, the pharmaceutical industry, and the food industry have garnered significant attention in recent years. It demonstrates its potential as an anticancer drug while also acting as an antileukemic agent. Additionally, it has been proven efficient against the human immunodeficiency virus (HIV) [2]. In addition, L-glutaminase is utilized as a flavor and fragrance enhancer in the manufacture of fermented foods, giving them a particular flavor (umami taste). Umami is mostly constituted of glutamic acid in the form of glutamine in traditional fermented foods. Traditional Chinese fermented foods, such as fermented beans and soy sauce, frequently contain glutaminase [3]. Another application of glutaminase is as a crucial monitoring agent in biosensors for measuring glutamine levels [4]. Numerous types of animals, such as animal tissues, plant tissues, and microbes, such as actinomycetes, bacteria, fungi, and yeasts, are capable of producing glutaminolytic enzymes. Glutaminases are produced by a variety of microorganisms, including *Aeromonas veronii* [5], *Alcaligenes faecalis* [6], *Aspergillus flavus* [7], *A. niger* [8], *A. oryzae* [9], *Bacillus subtilis* [10], *B. pasteurii*

[11], *Clostridium welchii* [12], *Escherichia coli* [13], *Halomonas meridiana* [14], *Micrococcus luteus* [15], *Lactobacillus rhamnosus* [16], *Pseudomonas stutzeri* [17], *Rhizobium etli* [18], *Streptomyces canarius* [19], *Vibrio costicola* [20], and *Zygosaccharomyces rouxii* [6], were isolated and well-studied. As expected, the glutaminases of these microorganisms differ in their biochemical and physicochemical properties Microorganisms are regarded as an essential source of glutaminase since the vast majority of glutaminases sold in commercial settings are derived from bacteria. In most cases, bacterial glutaminases are expelled from the cells into the surrounding medium [21,22]. However, intracellular synthesis of glutaminases has been documented [10]. The primary microorganisms responsible for fermentation, primarily lactic acid bacteria (LAB), are usually considered safe for consumption by humans. *Tetragenococcus muriaticus* is a member of the LAB family, including bacteria that can withstand the high levels of salt in fermented foods. *T. muriaticus* is an essential component in the production of nutrients and tastes in a wide variety of fermented foods and beverages [23].

Numerous publications have been published over time about the optimization of various microbiological factors and fermentation techniques for glutaminase production. Nevertheless, as the vast majority of industrial processes work under harsh circumstances, it is essential to discover the types of enzymes that maintain their optimal activity at varying pH, temperature, and salt concentrations [24]. However, the quest for novel microbiological sources with enhanced production conditions that produce glutaminases with increased catalytic efficiency must continue indefinitely. Halophilic bacteria and the enzymes they produce are receiving growing attention in the quest for additional enzymes to enhance existing biotechnological uses or to develop whole new applications. Interestingly, researchers are looking at Archaea as a source of enzymes that have halophilic properties [25]. Because halophilic enzymes are exceptionally resistant and well-adapted to hostile environments, these enzymes could be used in various industrial operations. Halophilic enzymes are functional and stable in hypersaline environments or media with low water activity because, even under these circumstances, there is enough water to maintain an appropriate charge distribution at the active site and to preserve the conformation of the enzyme [26,27]. Because of this feature, they are interesting for applications in biotechnology because they are able to maintain their activity in environments with low levels of water. These changes prove useful in demanding industrial processes in organic solvents and allow enzymatic reactions to occur [28].

The purpose of this investigation was to selectively isolate halophilic glutaminase-producing bacteria from Thai samples of fermented fish sauce (Nam-pla). In addition, several physicochemical parameters needed for optimal synthesis of halophilic glutaminase in a stirred bioreactor of 3 L by the selected isolate were improved.

Materials and methods

Chemicals

All chemicals, solvents, and media components utilized in this investigation were of analytical quality. HiMedia Laboratories was the source for the L-glutamine, yeast extract, and potassium chloride (KCl) (Mumbai, India). Purchased casamino acid from Difco Laboratories (Becton Dickinson, Sparks, MD USA). Merck supplied Hydrochloric acid (HCl), Sodium chloride (NaCl), and sodium hydroxide (NaOH) (Darmstadt, Germany). Ajax Finechem supplied iron (II) chloride 4-hydrate ($FeCl_2.4H_2O$) and magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) (Taren Point, NSW, Australia).

Screening of halophilic glutaminase production

For the preliminary screening, 1 g of fish sauce was suspended in 10 mL of sterile saline (9 g L^{-1} NaCl), uniformly mixed, and allowed to settle. Then, an aliquot of 100 µL of the clear suspension of the fish sauce sample was applied to Sehgal and Gibbons complex (SGC) agar comprising (g L^{-1}): Casamino acid 7.5, $FeCl_2.4H_2O$ 0.01, KCl 2, $MgSO_4.7H_2O$ 20, NaCl 200, trisodium citrate 3, yeast extract 10, agar 15 in 1,000 mL distilled water pH 6.8 - 7.0 [29]. To examine halophilic glutaminase production, isolates were spotted on SGC agar enriched with 1 % (v/v) glutamine using various salt concentrations in the agar based on the standard values used to define halophiles: 0, 5, 10, 15, 20 and 25 % (w/v). Plates were incubated at 37 °C for 7 days. The presence of a yellow zone around the colony indicated a positive response to the glutaminase assay. The width of the clear zone was expected to be directly related to the amount of extracellular glutaminase that was generated. On SGC-glutamine agar plates, the ratio of enzyme production was used to select the isolates for further study. For the subsequent research, only the isolates with the maximum glutaminase activity on SGC-glutamine agar with high salt concentrations (15 - 25 %, w/v) were examined. In preparation for further research, the cultures were frozen in glycerol containing 20 % (v/v) of glycerol.

As part of the secondary screening, growth and enzyme experiments were performed in 250 mL Erlenmeyer flasks containing 50 mL SGC liquid media supplemented with 1 % (v/v) glutamine. After transferring a loop of the selected strain into 9 mL of SGC germination media with a pH of 7.2 and an appropriate concentration of NaCl, the inoculum was formed. The inoculum was then incubated for 7 days at 37 °C on a rotary shaker (200 rpm). Inoculation was carried out using a seed culture with a concentration of 1 % (v/v) that had been cultivated in the medium described above for 3 days. The inoculated medium was incubated at 37 °C on a rotary shaker (200 rpm) for 7 days. Culture samples (5 mL) were collected aseptically every 24 h and centrifuged for 20 min at 8,000 g and 4 °C. For glutaminase activity experiments, cell-free supernatants were employed as crude enzyme solutions. The harvested cells were washed twice with NaCl solution before being measured for growth. A spectrophotometer was used to measure growth turbidly at 600 nm (UV-1,800 Shimadzu, Japan). The strain that produced the most promising results was chosen for further testing.

Bacterial strain identification

The selected isolate was characterized morphologically and physiologically using the standard approach [30]. According to the approach outlined by Tanasupawat [31], acid formation from carbohydrates was quantified. For 16s rRNA analysis, bacterial genomic DNA was extracted and amplified in a thermal cycler under the following conditions: 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. BF1(5-GAGTTTGATCATGGCTCAG-3′) and BR1(5′-AAGTCGTAACAAGGTAACCG-3′) were employed as universal primers [32]. For 16S rRNA sequence analysis, the selected isolate was analyzed using an automated DNA sequencer. The results were compared to other bacterial 16S rRNA sequences in the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLAST program (blastn). All sequences were concatenated using the program Clustal W [33]. The software tool MEGA, version 10.0, created a phylogenetic tree and a UPGMA phylogeny. The bootstrapping method was used to assess the reliability of the phylogenetic reconstructions (1,000 replications).

Microorganism and inoculum preparation

This study uses the strains of *Tetragenococcus muriaticus* FF5302 chosen based on a previous screening to produce halophilic glutaminase. It was derived from Thai samples of fermented fish sauce and was isolated from those (Nam-pla). The inoculum was generated by putting 15 mL of the chosen strain into a 500 mL Erlenmeyer flask that contained 150 mL of SGC seeding media with a pH of 7.2. The flask was then incubated at 37 °C on a rotary shaker (200 rpm) for 7 days before being inoculated into the bioreactor.

Halophilic glutaminase production in bioreactor

Fermentation was conducted in SGC medium. The growth and enzymes were examined in a 3 L stirred bioreactor containing 2 L liquid SGC media. Also incubated for 7 days at 37 °C, 200 rpm, and 0.05 VVM was the medium of origin. At 24-h intervals, 10 mL of culture samples were collected aseptically and centrifuged at 8,000 g and 4 °C for 20 min. The cell-free supernatants were used for glutaminase activity experiments as crude enzyme solutions. The cells were harvested, washed twice with NaCl solution, and then utilized to measure their growth. A spectrophotometer was used to quantify turbidity at 600 nm to determine the growth. Under various experimental conditions, fermentation was performed.

Optimization for halophilic glutaminase production

Firstly, various physicochemical parameters needed for the maximum synthesis of halophilic glutaminase by strain FF5302 were tuned to achieve optimal results. In the first step of optimization, the critical variables for halophilic glutaminase production and their ranges of variation were estimated using the traditional 'one-variable-one-time (OFAT)' approach at the bioreactor scale. This approach was used to determine the ranges of variation in those variables. The effects of different NaCl concentrations (ranging from 0 to 25 %), incubation times (ranging from 0 to 168 h), pH levels (ranging from 4.0 to 8.0), incubation temperatures (ranging from 25, 30, 35, 40, 45 and 50 °C), various carbon sources (glucose, sucrose, maltose, mannitol, and fructose) at a concentration of 1 % (w/v), nitrogen sources (peptone, yeast extract). All experiments were carried out at least in triplicate, and the mean values were calculated.

Determination of halophilic glutaminase activity

Imada *et al.* [34], described a modified version of the Nessler's reagent method for measuring glutaminase activity. After adding Nessler's reagent to the sample, the proportional release of ammonium ions was used to calculate the enzyme's activity calorimetrically. A 0.5 mL aliquot of the sample enzyme

preparation was combined with a 0.5 mL aliquot of 0.2 M glutamine solution. After 30 min of incubation, 1.0 mL of 10 % trichloroacetic acid was added to the liquid to stop the process (TCA). 0.1 mL of enzyme mixture preparation, 0.2 mL of Nessler's reagent, and 3.7 mL of distilled water were added, respectively. Enzyme activity was estimated using a spectrophotometer and an absorbance spectrum at 450 nm. Under optimum conditions, 1 unit of glutaminase corresponds to the quantity of enzyme that releases one M of ammonia.

Results and discussion

Screening of halophilic glutaminase producing bacterial isolates

Fifty-nine bacteria isolated from Thai fermented fish sauce (Nam-pla) were evaluated on SGC-glutamine agar for their halophilic glutaminase production. Among these, isolate FF5302 exhibited the largest colony size to yellow zone size ratio, demonstrating that it was the most capable of producing glutaminase. The color change from red to yellow is due to the enzymatic reaction that produces acid (glutamic acid) from the substrate glutamine, thereby decreasing the pH of the agar plate medium. After 7 days of incubation (data not shown), the glutaminase activity ratio of this isolate was 4.38 at 20 % (w/v) NaCl. In the presence of high salt concentrations, the results suggest that this strain may be a possible source of glutaminase-producing bacteria. Tetragenococci was the predominant lactic acid bacteria in salt-fermented fish products with 11 % or more salt. In these instances, *T. muriaticus* and *T. halophilus* coexisted in a traditional Thai and Laos salt-fermented fish paste is known as pla-ra or pa-daek [35]. FF5302 was chosen to optimize the synthesis of halophilic glutaminase under liquid culture conditions.

Identification of the selected isolate

Based on the data presented in **Table 1**, the morphological and biochemical properties of Isolate FF5302 were analyzed using established protocols [30-33]. The isolate was found to be morphologically classified as a gram-positive coccus with a cream-colored pigmentation, as was discovered by inspection using a light microscope. At a temperature of 40 °C and NaCl concentrations ranging from 5 to 25 % (w/v), growth was seen, with 20 % being deemed the ideal value. Isolate FF5302 tested positive for the ability to produce oxidase, catalase, urease, and nitrate reduction but tested negative for the generation of H_2S. This isolate was also capable of hydrolyzing casein and glutamine, but it was unable to generate acid from glycerol, lactose, raffinose, or sucrose. Because of its similarity to *Tetragenococcus muriaticus* JCM10006T [24,25], we could identify that FF5302 is closely related to *Tetragenococcus* sp. by analyzing its morphological and physiological properties and coming to this conclusion. The selected isolate underwent additional identification using comparative molecular analysis of the 16S rRNA gene homology with other bacteria found in the database. As shown in **Figure 1**, the investigation revealed that the 16S rRNA gene of FF5302 shares a 99 % degree of similarity with the *Tetragenococcus muriaticus* strain NBRC 100499 (accession number NR113915.1).

Table 1 Characteristics of the morphological and physiological makeup of isolate FF5302.

Characteristics	1	2	3
Gram stain	Positive	Positive	Positive
Morphology	Cocci	Cocci	Cocci
Pigment	Cream	Cream	Cream
Temperature for growth (40 °C)	+	+	−
NaCl range for growth (%)	5–25	15–25	0-25
Optimum NaCl for growth (%)	20	ND	ND
Hydrolysis of:			
Casein	+	+	−
Arginine	−	−	+
Glutamine	+	ND	−
Acid production from:			

Characteristics	1	2	3
L-Arabinose	−	−	+
D-Galactose	+	+	+
Glycerol	−	−	+
Lactose	−	−	−
D-mannitol	+	+	−
D-melibiose	−	−	W
D-sorbitol	−	−	−
Raffinose	−	−	W
Rhamnose	W	W	W
Ribose	+	+	+
Sucrose	−	−	W
Trehalose	−	−	+
Xylose	+	+	W

*Taxa: 1, *Tetragenococcus muriaticus* FF5302; 2 *Tetragenococcus muriaticus* JCM10006^T 3, *Tetragenococcus halophilus* ATCC33315^T (data from Sitdhipol *et al.* [32]). +, Positive; −, Negative; ND, no data available; W, weakly positive

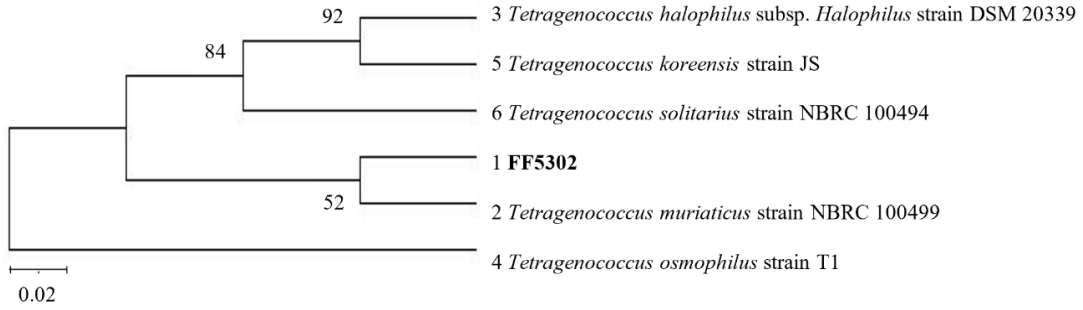

Figure 1 Phylogenetic tree constructed from 16S rRNA gene sequences illustrating the link between strain FF5302 and similar bacterial species. Using the UPGMA algorithm, the branching pattern was constructed.

Optimization of cultural and nutritional conditions for halophilic glutaminase production

Effect of NaCl concentrations

As shown in **Figure 2**, the enzyme activity generated is at its peak when the concentration of NaCl is 20 %. The activity of glutaminase continuously increased with the concentration of sodium chloride up to 5 % but then gradually reduced with the steady increase in the concentration of sodium chloride (up to 25 %). As a result of this finding, the glutaminase enzyme in question has a high salt tolerance. As a result, it has the potential to play a key part in food businesses that demand high salt culture, such as the soybean and fish sauce industries. Research conducted with L-glutaminase from *Bacillus subtilis* [10,21] and *Penicillium brevicompactum* [30], yielded comparable results. Wakayama *et al.* [22], discovered that the L-glutaminase enzyme generated from actinomycetes had a high tolerance to salt. On the other hand, L-glutaminases derived from *Aspergillus sojae* and *A. oryzae* are considerably inhibited by high salt concentrations [3,15].

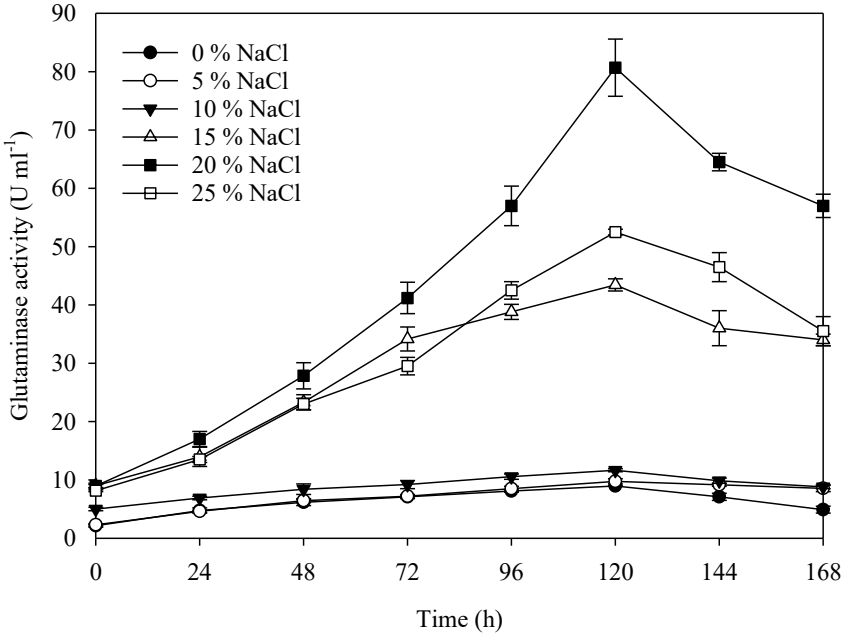

Figure 2 Effect of NaCl concentrations on glutaminase production by *T. muriaticus* FF5302. Data represent mean ± SD ($n = 3$); $p < 0.05$.

Effect of incubation period

Fermentation was performed using various incubation periods ranging from 0 to 168 h in SGC medium (pH 6.8 - 7.0, at 37 °C) at frequent intervals of 24 h to examine the influence of each of these periods on the growth and production of halophilic glutaminase. As the incubation continued, halophilic glutaminase synthesis exhibited a growth-related relationship. As indicated in **Figure 3**, the greatest enzyme production (83.0 U mL^{-1}) was produced from *T. muriaticus* FF5302 culture grown for 120 h. In addition, it was reported that maximum glutaminase productivity (47.12 mL^{-1}) by *Bacillus* sp. DV2-37 was obtained at 96 h of incubation period [36]. However, the marine isolated bacterial strain *Bacillus subtilis* produced the most L-glutaminase after 18 h of incubation [37].

Effect of initial pH on production

To investigate the influence of initial pH, *T. muriaticus* FF5302 produced the most halophilic glutaminase under these conditions. This experiment was determined by adjusting the pH during fermentation from 4.0 to 8.0. As indicated in **Figure 4**, the optimal enzyme activity (75.0 U mL^{-1}) was obtained when the starting pH of the production medium was set to 8.0. At a pH of 4.0, the concentration of halophilic glutaminase dropped to 8.5 U mL^{-1}. Similar effects of pH on the synthesis of L-glutaminase in *Hypocrea jecorina* were reported to be 8.0 [1]. The optimal pH range for L-glutaminase from *Vibrio* sp., and *Aspergillus* sp. was reported to be between 7.0 and 8.5 [15,20].

Effect of temperature

In order to determine how temperature affects the formation of halophilic glutaminase, the flasks were kept at various temperatures ranging from 25 to 50 °C for 120 h (**Figure 4**). The highest enzyme synthesis was seen at a temperature of 45 °C (87.90 U mL^{-1}). The enzyme production decreased below and above a temperature of 45 °C. However, Gomaa [36], confirmed that the maximum glutaminase activity (40.80 mL^{-1}) by *Bacillus* sp. DV2-37 isolated from marine environment was at 37 °C. Maximum L-glutaminase synthesis was encouraged by incubating *Beauria* sp. and *A. fumigatus* at temperatures of 27 and 30 °C, respectively [33].

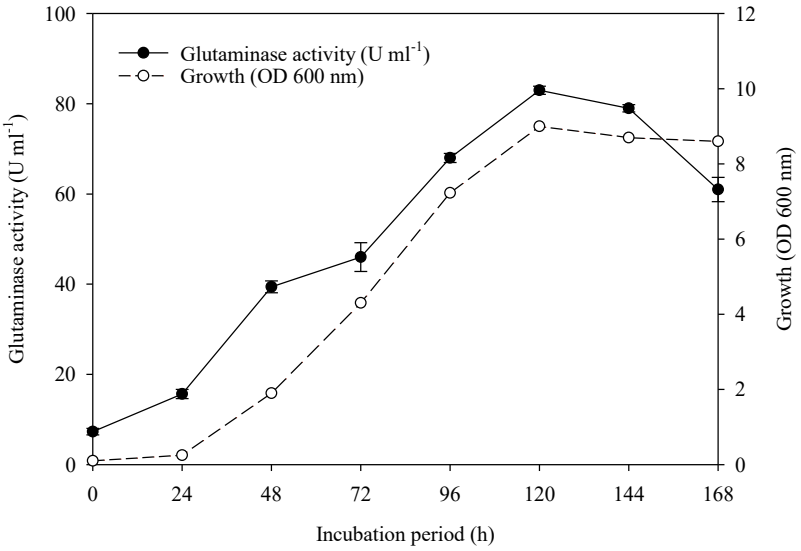

Figure 3 Effect of incubation period on glutaminase production and growth by *T. muriaticus* FF5302. Data represent mean ± SD ($n = 3$); $p < 0.05$.

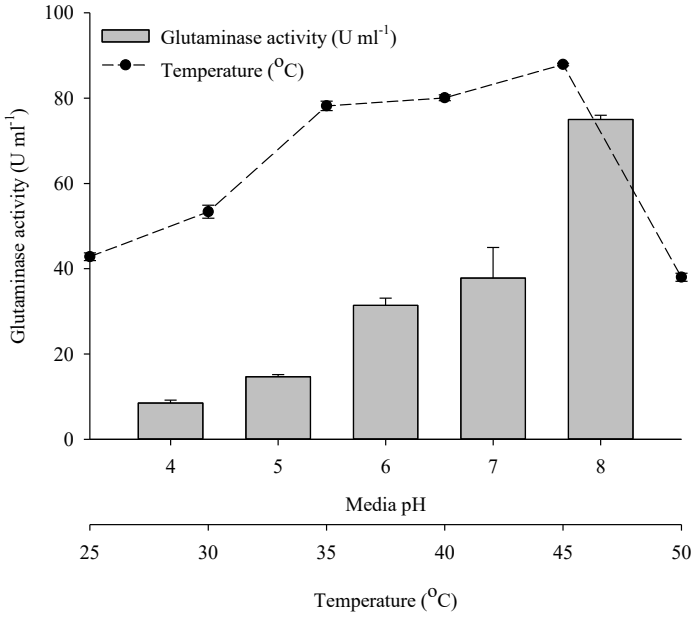

Figure 4 Effect of initial pH and temperature on glutaminase production by *T. muriaticus* FF5302. Data represent mean ± SD ($n = 3$); $p < 0.05$.

Effect of carbon sources

The addition of different carbon sources could boost the production of enzymes. As a result, 1 % (w/v) of various carbon sources was added to the medium. These carbon sources included glucose, sucrose, maltose, mannitol, and fructose. **Figure 5** demonstrates that the rise in halophilic glutaminase synthesis was most noticeably brought about by adding sucrose. This result was followed by adding mannitol, fructose, glucose, and maltose in that order. In general, none of the carbon sources showed much of a substantial increase in the halophilic glutaminase produced as a result of the SGC medium; nevertheless, free carbon produced a comparatively large amount of halophilic glutaminase (88. U mL^{-1}).

Effect of different nitrogen sources

The effects of various organic nitrogen sources, including beef extract, casamino acids, glutamine, malt extract, peptone, and yeast extract, were investigated and analyzed. As seen in **Figure 5**, the influence

of nitrogen sources on glutaminase synthesis revealed that glutamine was a better nitrogen source for halophilic glutaminase (83.45 U mL^{-1}) by *T. muriaticus* FF5302, which was the organism that conducted the study. However, the glutaminase synthesis was significantly lower in the beef extract (32.0 U mL^{-1}). Similar findings were reported by Katikala *et al.* [30], who found that the marine bacterial strain LG24 produced the maximum yield of extracellular L-glutaminase in 120 h, with a value of 22.68 U mL^{-1}, when L-glutamine was provided as the sole carbon and nitrogen source in the media. The reason for this is that the microorganism employs L-glutamine as its nitrogen source, and the only ammonium ions that are engaged in the medium are those created as a result of the enzymatic process that L-glutaminase catalyzes. To accurately determine the enzyme activity, it is necessary to have this circumstance. The microbe was programmed to look for glutamine, and once it found it, it used it as a source of carbon and nitrogen, producing an enzyme called glutaminase.

According to Das and Agsar [38], L-glutamine is the most effective source of nitrogen (30 IU) out of all the sources that were investigated. Previous researchers [5], came to the same conclusions after looking at the data. It demonstrates that the amide nitrogen of glutamine was a source of amino groups in a wide variety of biosynthetic processes. It was also frequently implicated in protein active or binding areas. In addition, it was a source of information. In addition, nitrogen sources are secondary sources of energy for organisms. These sources play a significant part in the growth of organisms and the creation of beneficial enzymes within organisms. Both the kind of substance and the concentration that we utilized to have the potential to alter or inhibit the synthesis of enzymes [39].

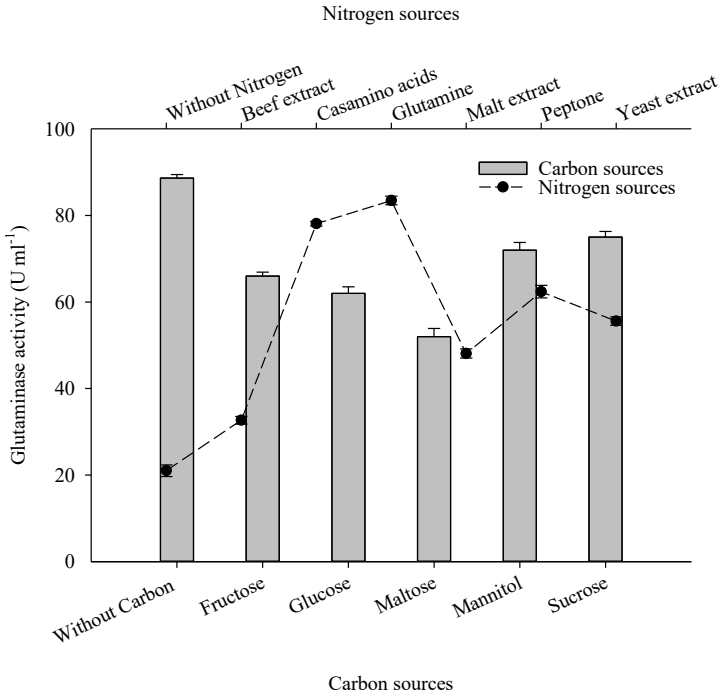

Figure 5 Effect of different carbon and nitrogen sources on glutaminase production by *T. muriaticus* FF5302. Data represent mean ± SD ($n = 3$); $p < 0.05$.

Effect of inoculum size

Figure 6 makes it abundantly evident that the size of inoculum 5 % (v/v) produced the largest amount of halophilic glutaminase activity, which was 58.06 U mL^{-1}. These factors were significantly significant with maximal growth and halophilic glutaminase production (58.06 U mL^{-1}), which indicates that halophilic glutaminase production is substantially related to growth in a bacterial population. Because the bacterial growth was unable to propagate sufficiently at a lower concentration of seed culture, enzyme synthesis was disrupted [40]. This result was because the delayed substrate conversion inhibited the growth of the bacteria. An increase in the total number of cells contained within the inoculums would ensure that the process of rapid proliferation and biomass production goes smoothly.

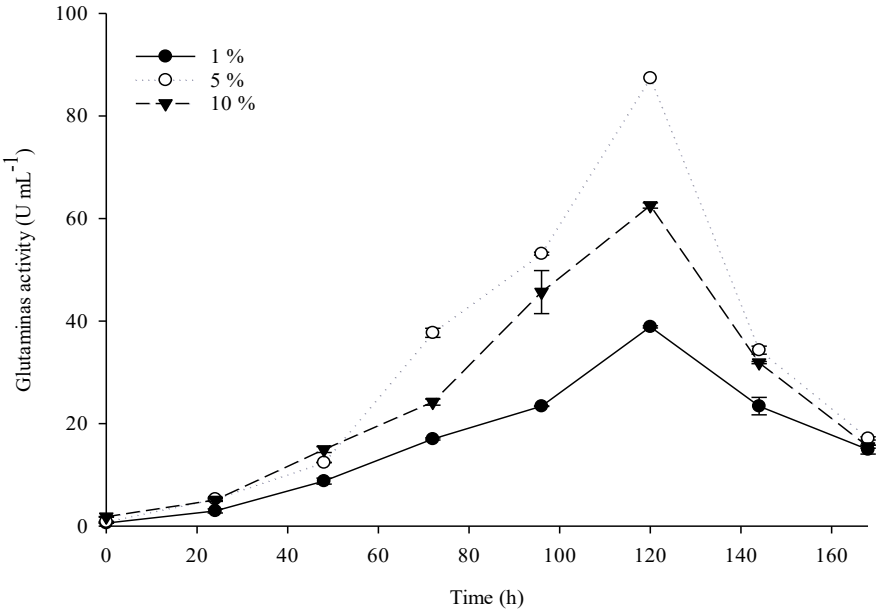

Figure 6 Effect of inoculum size on glutaminase production by T. muriaticus FF5302. Data represent mean ± SD (n = 3); $p < 0.05$.

Conclusions

The finding of this study demonstrates the halophilic glutaminase production in bioreactor scale by *Tetragenococcus muriaticus* FF5302 is principally influenced by various factors such as NaCl concentration, incubation time, initial pH, temperature, carbon source, nitrogen source, and inoculum size. The optimization of these factors has a significant role in enhancing the halophilic glutaminase enzyme yield (87.4 U mL^{-1}). Thus, the isolate *T. muriaticus* FF5302 showed the potential of halophilic glutaminase to tolerate harsh conditions like high concentration of NaCl, which makes it a promising inoculant for enzyme industry as well as in extremely condition.

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References

[1] D Bülbül and E Karakuş. Production and optimization of L-glutaminase enzyme from *Hypocrea jecorina* pure culture. *Prep. Biochem. Biotechnol.* 2013; **43**, 385-97.

[2] A Hegedus, MK Williamson, MB Khan, JD Zeidler, ATD Poian, T El-Bacha, EA Struys and H Huthoff1. Evidence for altered glutamine metabolism in human immunodeficiency virus type 1 infected primary human CD4+ T cells. *AIDS Res. Hum. Retrovir.* 2017; **33**, 1236-47.

[3] K Ito, Y Koyama and Y Hanya. Identification of the glutaminase genes of *Aspergillus sojae* involved in glutamate production during soy sauce fermentation. *Biosci. Biotechnol. Biochem.* 2013; **77**, 1832-40.

[4] C Pallem, S Manipati and SR Somalanka. Process optimization of L-glutaminase production by *Trichoderma koningii* under solid state fermentation (SSF). *Int. J. Appl. Biol. Pharmaceut. Tech.* 2010; **1**, 1168-74.

[5] SAV Jesuraj, MMR Sarker, LC Ming, SMJ Praya, M Ravikumar and MT Wui. Enhancement of the production of L-glutaminase, an anticancer enzyme, from Aeromonas veronii by adaptive and induced mutation techniques. *PLos One* 2017; **12**, 1-17.
[6] SRK Pandian, V Deepak, SD Sivasubramaniam, Nellaiah H and K Sundar. Optimization and purification of anticancer enzyme L-glutaminase from *Alcaligenes faecalis* KLU102. *Biologia* 2014; **69**, 1644-51.
[7] MA Abu-Tahon and GS Isaac. Purification, characterization and anticancer efficiency of L-glutaminase from *Aspergillus flavus*. *J. Gen. Appl. Microbiol.* 2020; **65**, 284-92.
[8] JE Nagamani. Production, partial purification and assay of L-glutaminase enzyme from *Aspergillus niger* by solid state fermentation. *Int. J. Res. Rev.* 2018; **5**, 177-81.
[9] C Thammarongtham, G Turner, AJ Moir, M Tanticharoen and S Cheevadhanarak. A new class of glutaminase from *Aspergillus oryzae. J. Mol. Microbiol. Biotechnol.* 2001; **3**, 611-7.
[10] Y Shimizu, A Ueyama and K Goto. Purification and characterization of glutaminase from *Bacillus subtilis* GT strain. *J. Brewing Soc. Jpn.* 1991; **86**, 441-6.
[11] M Klein, H Kaltwasser and T Jahns. Isolation of a novel, phosphate-activated glutaminase from *Bacillus pasteurii. FEMS Microbiol. Lett.* 2002; **206**, 63-7.
[12] DE Hughes and DH Williamson. Some properties of the glutaminase of *Clostridium welchii. Biochem. J.* 1952; **51**, 45-55.
[13] AF Yakunin. Functional and structural characterization of four glutaminases from *Escherichia coli* and *Bacillus subtilis*. *Biochemistry* 2008; **47**, 5724-35.
[14] YS Mostafa, SA Alamri, MY Alfaifi, SA Alrumman, SE Elbehairi, TH Taha and M Hashem. L-glutaminase synthesis by marine *Halomonas meridiana* isolated from the Red Sea and its efficiency against colorectal cancer cell lines. *Molecules* 2021, DOI: 10.3390/molecules26071963.
[15] N Masuo, K Yoshimune, K Ito, K Mutsushima, Y Koyama and M Moriguchi. *Micrococcus luteus* K-3-type glutaminase from *Aspergillus oryzae* RIB40 is salt-tolerant. *J. Biosci. Bioeng.* 2005; **100**, 576-8.
[16] A Weingand-Ziade, C Gerber-Decombaz and M Affolter. Functional characterization of a salt- and thermotolerant glutaminase from *Lactobacillus rhamnosus. Enzym. Microb. Tech.* 2003; **32**, 862-7.
[17] R Athira, T Elizebeth, T Narendra, T Sheik, S Gupta, M Chaudary, K Siddalingeshwara and T Pramod. Investigation on the production of L-glutaminase from *Pseudomonas stutzeri* strain under solid state fermentation using various agro residues. *J. Drug Deliv. Therapeut.* 2014; **4**, 81-5.
[18] J Calderón, A Huerta-Saquero, GD Pont and S Durán. Sequence and molecular analysis of the *Rhizobium etli* glsa gene, encoding a thermolabile glutaminase. *Biochim. Biophys. Acta* 1999; **1444**, 451-6.
[19] FM Reda. Kinetic properties of *Streptomyces canarius* L-Glutaminase and its anticancer efficiency. *Braz. J. Microbiol.* 2014; **46**, 957-68.
[20] GN Prabhu and M Chandrasekaran. Purification and characterization of an anti-cancer enzyme produced by marine *Vibrio Costicola* under a novel solid state fermentation process. *Braz. Arch. Biol. Tech.* 1999; **43**, 1-5.
[21] T Sathish, D Kezia, P Bramhachari and RS Prakasham. Multi-objective based superimposed optimization method for enhancement of L-glutaminase production by *Bacillus subtilis* RSP-GLU. *Karbala Int. J. Mod. Sci.* 2018; **4**, 50-60.
[22] M Wakayama, T Yamagata, A Kamemura, N Bootim, S Yano, T Tachiki, K Yoshimune and M Moriguchi. Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilla* NYW-81 and its application in Japanese soy sauce fermentation. *J. Ind. Microbiol. Biotechnol.* 2005; **32**, 383-90.
[23] T Kuda, Y Izawa, S Yoshida, T Koyanagi, H Takayashi and B Kimura. Rapid identification of *Tetragenococcus halophilus* and *Tetragenococcus muriaticus*, important species in the production of salted and fermented foods, by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). *Food Contr.* 2014; **35**, 419-25.
[24] X Li and HY Yu. Characterization of a novel extracellular lipase from a halophilic isolate, *Chromohalobacter* sp. LY7-8. *Afr. J. Microbiol. Res.* 2012; **6**, 3516-22.
[25] P Iyer and RS Singhal. Glutaminase production using *Zygosaccharomyces rouxii* NRRL-Y 2547: Effect of aeration, agitation regimes and feeding strategies. *Chem. Eng. Tech*. 2010; **33**, 52-62.
[26] S DasSarma and P DasSarma. Halophiles and their enzymes: Negativity put to good use. *Curr. Opin. Microbiol.* 2015; **25**, 120-6.
[27] MA Cabrera and JM Blamey. Biotechnological applications of archaeal enzymes from extreme environments. *Biol. Res.* 2018; **51**, 37.

[28] S Wu, R Snajdrova, JC Moore, K Baldenius and UT Bornscheuer. Biocatalysis: Enzymatic synthesis for industrial applications. *Angew. Chem. Int. Ed.* 2021; **60**, 88-119.
[29] SN Sehgal and NE Gibbons. Effect of some metal ions on the growth of *Halobacterium cutirubrum*. *Can. J. Microbiol.* 1960; **6**, 165-9.
[30] AM Elshafei, MM Hassan and NH Ali. Purification, kinetic properties and antitumor activity of L-glutaminase from *Penicillium brevicompactum* NRC829. *Br. Microbiol. Res. J.* 2014; **4**, 97-115.
[31] S Tanasupawat, S Okada and K Komagata. Lactic acid bacteria found in fermented fish in Thailand. *J. Gen. Appl. Microbiol.* 1998; **44**, 193-200.
[32] J Sitdhipol, S Tanasupawat, P Tepkasikul, P Yukphan, A Tosukhowong, T Itoh, S Benjakul and W Visessanguan. Identification and histamine formation of *Tetragenococcus* isolated from Thai fermented food products. *Ann. Microbiol.* 2013; **63**, 754-53.
[33] S Dutta, S Ghosh and S Pramanik. L-asparaginase and L-glutaminase from *Aspergillus fumigatus* WL002: Production and some physicochemical properties. *Appl. Biochem. Microbiol.* 2015; **51**, 425-31.
[34] A Imada, S Igarasi, K Nakahama and M Isono. Asparaginase and glutaminase activities of microorganisms. *Microbiology* 1973; **76**, 85-99.
[35] J Marui, S Boulom, W Panthavee, M Momma, K Kusumoto, K Nakahara and M Saito. Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos. *Biosci. Microbiota Food Health* 2015; **34**, 45-52.
[36] EZ Gomaa. Production, characterization, and antitumor efficiency of L-glutaminase from halophilic bacteria. *Bull. Natl. Res. Centre* 2022; **46**, 10.
[37] X Zhang, Z Xu, S Liu, K Qian, M Xu, T Yang, J Xu and Z Rao. Improving the production of salt-tolerant glutaminase by integrating multiple copies of Mglu into the protease and 16S rDNA genes of *Bacillus subtilis* 168. *Molecules* 2019; **24**, 592.
[38] M Das and D Agsar. Production and antioxidant attribute of L-glutaminase from *Streptomyces enissocaesilis* DMQ-24. *Int. J. Sci. Tech. Res.* 2013; **2**, 1-9.
[39] MP Jacob and S Devi. Acid stable α-amylase from *Pseudomonas balearica* VITPS19-production, purification and characterization. *Biotechnol. Rep.* 2021; **30**, e00603.
[40] M Bonnet, JC Lagier, D Raoult and S Khelaifia. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microb. New Infect.* 2020; **34**, 100622.