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Purification and characterization of glutaminase and urease-free L-asparaginase from *Bacillus atrophaeus* with acrylamide reduction potential

Abstract. L-asparaginase (L-asnase) is a versatile enzyme with uses in food industry and medicine. Current study aimed to isolate L-asnase producing microorganism/s without urease and glutaminase and optimize L-asnase production. First, screening and isolation of L-asnase-producing bacterial strains that did not produce glutaminase and urease from chicken gizzards were performed. For this purpose, the enzyme producing bacteria were screened on the agar medium supplied with substrate and phenol red indicator dye. Among the isolated bacteria, 1 isolate showed L-asnase free of glutaminase and urease. The selected strain was identified by biochemical, morphological and 16s rRNA sequencing. The selected strain was identified as *Bacillus atrophaeus* by 16S rRNA sequencing. The effects of incubation temperature (30°C) and time (72 hours), medium pH (8.0) and nutritional sources (glucose and $NaNO_3$) on L-asnase production were determined. L-asnase was purified with acetone, and its molecular weight was determined to be 42 kDa by SDS-Page. Enzyme kinetics were also calculated, and it was determined that V_{max} was 43 μmol/mL/min and Km was 2.7 mM. L-asnase activity was highest at 40 °C and the optimal pH was 8.0. L-asnase activity was stimulated by Mn^{2+}, Mg^{2+}, and Ca^{2+} but inhibited by Co^{2+}, Na^{+}, Zn^{2+}, and Hg^{2+}. L-asnase was utilized to treat potato chips before they were fried in order to assess its capacity to mitigate acrylamide. The result was an 80% reduction in acrylamide concentration when compared to the untreated control. Based on these findings, it appears that L-asnase could have potential use in the food industry.
Key words: L-asparaginase, *Bacillus atrophaeus*, production, purification, acrylamide.

Introduction

L-asparaginase (E.C.3.5.1.1, L-asnase) is an enzyme of the hydrolase group. This enzyme hydrolyzes L-asparagine to ammonia and L-aspartate [1]. L-asnase has many applications in the health and food fields. L-asnase is a chemotherapeutic agent in the treatment of leukemia, Hodgkin's disease, melanosarcoma, lymphosarcoma, and reticulosarcoma [1–3]. L-asnase is eco-friendly, biodegradable, safe, and can reach the desired area directly. L-asnase is important in reducing potential carcinogenic and neurotoxic acrylamide in food technology [4]. Apart from chemical and physical methods, enzymatic methods are also used to prevent acrylamide formation, which occurs when starchy foods are heated to high temperatures by frying or baking [5,6]. Acrylamide is formed by the Maillard reaction of L-asparagine and reducing sugars, which are naturally found in foods (coffee, crackers, and chips). The World Health Organization has declared acrylamide as a carcinogen due to its potential for mutagenicity and toxicity [7]. It is crucial to maintain desirable textural, nutritional and sensory qualities while reducing acrylamide formation. The easiest and most efficient method for preventing or reducing the formation of acrylamide is to utilize L-asnase [3]. Acrylamide formation can be reduced by using L-asnase before heat treatment of foods [8]. It is also known that L-asnase has antioxidant characteristics [9].

Due to its increasing use in the food and health fields, the market for L-asnase is constantly increasing. The market for L-asnase is estimated to be US$413.2 million in 2019 and will increase to US$435.8 million by 2025. Currently, L-asnase, which is used in clinical applications, is produced by *Escherichia coli* and *Erwinia chrysanthemi*. As cancer cases are expected to increase in the coming years, there will be an increase in demand for and use of L-asnase [10].

L-asnase is produced by a wide range of bacteria, fungi, actinomycetes, algae, and plants. Microorganisms are the most suitable sources for the production of L-asnase. However, this enzyme causes undesired side effects (liver, kidney, and

pancreas problems, hepatotoxicity, neurotoxicity, allergic reactions, coagulation anomalies) due to its impurities (glutaminase, urease). Therefore, extra purification steps are required to remove glutaminase and urease [11]. Isolation of glutaminase and urease-free L-asnase producing isolates is unquestionably advantageous in order to reduce purification steps. The aim of this study is to optimize and characterize the glutaminase- and urease-free L-asnase production of newly isolated *B. atrophaeus*. The application potential of the L-asnase in acrylamide reduction in potato chips was also evaluated.

Materials and methods

Collecting samples and isolating bacterial strains. The gizzard samples from the local slaughterhouse were transported directly to the laboratory, after which samples were taken aseptically from the gizzard. Phosphate-buffered saline was added at 5 mL for each gram of sample, and the falcon tube was shaken for 60 s. Serial dilutions were prepared from the resulting homogenate, spread on Nutrient Agar (NA, Merck, Germany) plates, and incubated at 37 °C for 2 days. Individual representative colonies were selected for purification and plated on fresh NA plates. At -86 °C, pure cultures were cryopreserved in Nutrient Broth with 20% glycerol for later use.

Selection of bacterial isolates for L-asnase production. Isolated bacteria were screened using M9 agar plates (% 1.5 agar) containing phenol red and L-asparagine (10 g/L). M9 consisted of (g/L): 2 glucose, 3 KH_2PO_4, 0.5 $MgSO_4.7H_2O$, 0.1 $CaCl_2.2H_2O$, 10 L-asparagine, 0.5 NaCl and 6 Na_2HPO_4. Phenol red 0.009% (w/v) was added as an indicator dye. On M9 agar plates, separated pure cultures were streaked, and the color change formation was observed for two days at 30 °C. Isolates with pink colored regions around bacterial colonies were evaluated as L-asnase producing strains.

Screening of bacterial isolates for glutaminase and urease production. Both quantitative and qualitative techniques were applied to evaluate whether the isolated bacteria produced urease and glutaminase. Glutaminase or urease producing isolates were determined on phenol red indicator plates [12]. Pre-culture of the isolates was grown overnight at 30 °C and 150 rpm in Nutrient Broth. The following day, 1 mL of pre-culture (OD_{600} 1) was added to 50 mL of M9 broth media in 250 mL flasks, and the mixture was incubated for 4 days at 30 °C at 140 rpm. It was determined using M9 media containing L-glutamic acid or urea as the sole nitrogen source, respectively. $NaNO_3$ was used as the only source of nitrogen while preparing control plates. Glutaminase and urease activities were evaluated using the Nesslerization procedure outlined below to assess L-asnase activity. The buffer combination containing L-asparagine-Tris-HCl was substituted with L-glutamine-Tris-HCl and urea-Tris-HCl, respectively, and the enzyme activity was determined using the same approach as stated below.

Identification of L-asnase producer bacterium. Abis Online Software was used for both morphological and biochemical tests to identify the bacterium [13]. The bacterium producing L-asnase was then identified based on 16S rDNA sequencing using universal primer sets 27F and 1102R. The obtained sequence was aligned and compared with the sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and submitted to GenBank.

Analytical methods. The L-Asnase activity was determined by quantifying ammonia formation in the culture supernatant using Nessler's reagent. The enzymatic reaction mixture contains 900 µl of 0.04 M L-asparagine in 50 mM M Tris-HCl buffer (pH 8.6) and 100 µL of crude enzyme. For 30 min, enzyme substrate mixtures were incubated at 37°C. At the end of the incubation, 100 µL of 1.5 M trichloroacetic acid (TCA) was added to stop the activity, and the mixture was incubated for 15 min. at 20 °C for color development. To separate precipitates from the reaction mixture, it was centrifuged at 8,000 rpm for 6 min. Then, 200 µL of Nessler reagent was added to the sample containing 200 µL of supernatant and 1.6 mL of distilled water, and the amount of ammonia released was determined using the UV visible spectrophotometer at 425 nm. At 37 °C, one unit of L-Asnase activity was stated the quantity of enzyme that produced 1 µmole ammonia per minute [11,12].

Enhancement of L-Asnase production using one factor at a time approach. The influence of carbon and nitrogen sources on L-Asnase production was investigated. For this purpose, six different carbon sources (2 g/L) such as glucose, fructose, mannitol, lactose, glycerol and sucrose and six different nitrogen sources (1 g/L) such as ammonium nitrate, ammonium sulfate, ammonium chloride, bacto peptone, tyrptone, and yeast extract were added to M9 broth. The effects of different temperatures (25-40°C), pH (5-10), and incubation times (24-96 hours) on L-asnase production were tested.

Purification of L-asnase. B. atrophaeus AspK1 was grown for 48 h at 180 rpm and 30 °C in optimized M9 medium containing L-asparagine. At the end of the period, the culture medium was centrifuged at 10,000 rpm for 15 min., the supernatant was taken, and the bacterial cells were discarded. Chilled

acetone (-18 °C) was added to the supernatant (crude enzyme) at a rate of 60% to precipitate the proteins and mixed with a constant stirrer at 4 °C for 5 h. Then, centrifugation was done at 15,000 rpm and 4 °C for 20 min. Precipitated proteins were dissolved in 50 mM Tris-HCl buffer (pH 8.0) and washed by ultrafiltration (Amicon® Ultra-15 centrifugal filter units, 10 kDa cutoff) at 5000 rpm at 4 °C [14]. Utilizing Biological LP Chromatography Systems (Biorad, USA), anion exchange chromatography was employed to carry out the remaining purification process. After washing out, concentrated enzyme solution was filtered through a 0.45 µm syringe filters and loaded onto HiTrap Q HP column (GE Healthcare) pre-equilibrated with 20 mM Tris-HCl, pH 8.0. During elution, 2 mL fractions were collected at a flow rate of 0.5 mL/min with a rising linear gradient of NaCl (50-500 mM) in a buffer of 20 mM Tris-HCl pH 8.0. Elution fractions were collected, and protein concentration was measured according to the Lowry Method [15].

Determination of molecular weight and kinetic constants (Km and Vmax). Using 12% (v/v) polyacrylamide gel stained with Coomassie Brilliant Blue R-250 following electrophoresis, crude and purified L-asnase fractions were subjected to SDS-PAGE analysis. By comparing the relative mobilities with the Precision Plus Protein Unstained Standard (250-10 kDa), the molecular weights of the samples were determined.

Using L-asparagine as the substrate at dosages ranging from 0.2-4 mM, the Michaelis-Menten constant (*Km*) and maximum velocity (*Vmax*) of pure L-asnase from *B. atrophaeus* AspK1 were assessed.

Enzyme characterization. The activity of purified L-asnase was measured at various pH, temperatures, incubation times, and metal ion concentrations. The purified enzyme was studied in buffers of 50 mM pH between 3-10. Potassium phosphate (pH 3-7), Tris-HCl (pH 8-9), and glycine-NaOH (pH 10) were selected as buffers and used for residual activity determination. The L-asnase activity was determined at a temperature ranging from 20 to 80 °C. The thermal stability of L-asnase was tested by pre-incubating at different temperatures for 1-5 hours. Metal ions of salt solutions at 1 mM and 10 mM concentrations, such as $MgSO_4$, $CoCl_2$, $FeCl_2$, $CuCl_2$, $ZnCl_2$, and $CaCl_2$ were used for the determination of enzyme activity.

Application of L-asnase in potato chips. After being cleaned, the potatoes were peeled and chopped into 2 mm slices. In addition, distilled water was used to rinse the starch particles that had adhered to the potato's surface. For forty minutes, the potato chips were submerged in a crude enzyme solution containing 40 U/mL at 40 °C, and dried on blotting paper. The potato chips were cooked for 5 min at 180 °C, dried for 20 min. at 60 °C, and then chilled to room temperature. n-hexane was used three times to de-oil potato chips that were pureed and homogenized in a centrifuge tube. Acetonitrile was used to extract acrylamide. Finally, a 0.22 mm microporous membrane filter was used to remove resuspended liquids for additional examination [16]. Acrylamide analysis was performed using an LC-MS/MS (Agilent 6460 Triple Quadrupole) with a mass-selective detector (MSD, Agilent 7000). The injection volume was 10 µL, and the solvent system consisted of 0.1% formic acid in water and 0.1% formic acid in methanol at a flow rate of 0.3 mL/min at 30 °C.

Results and discussion

Isolation of bacterial species. Nine bacteria isolated from chicken gizzards. The digestive systems of animals provide very favorable environments for the life of microorganisms [17]. Bacteria in the digestive system can produce many enzymes that aid digestion. The chicken gizzard, which is one of these environments, constitutes a very complex environment. In the proximal intestine, such as the gizzard and small intestine, microorganisms compete with the host for energy. *Lactobacillus* and *Clostrodium* species are dominant in the gizzard [18,19]. It was also identified in genera such as *Bacillus*, *Enterococcus*, *Corynebacterium*, *Weissella*, *Geobacillus* and *Planococcus* [20]. Spore-forming *Bacillus* species are widely found in ecosystems due to their high resistance to environmental stresses.

Isolated bacteria were screened for L-asnase, L-glutaminase and urease activity on L-aspararagine-, L-glutamine- and urea-agar plates containing phenol red as an indicator. When these substrates break down, the released ammonia reacts with water to form NH_4OH, which increases the pH of the medium. In these environments containing phenol red as a pH indicator, the pink color formation with an increase in pH is an indication of the production of the relevant enzyme [12]. L-asnase, used in chemotherapy, has possible side effects due to its impurities. Therefore, there is a need to discover new high-purity enzyme production sources for therapeutic uses [21]. Since the AspK1 isolate did not produce L-glutaminase or urease, there was no color change in the solid medium containing L-glutamine and urea, and it was determined that it showed a pink-colored region on the L-asparagine plate. This showed that

it produced L-asnase without producing glutaminase or urease. In addition, isolates were screened by spectrophotometry using the Nesslerization method and the AspK1 isolate was identified as the L-asnase producer without glutaminase and urease activity.

Identification of the glutaminase and urease-free L-asnase producing bacterium. Biochemical tests and physical properties showed that the AspK1 strain is a Gram positive, catalase positive, oxidase negative, aerobic, rod, pale brown pigmented, motile, and endospore forming organism (Table 1).

A 1463 bp 16S rRNA sequence was screened by BLAST (GenBank, NCBI) based on the result of the genetic analysis of the AspK1 strain. The nucleotide sequence is recorded in GenBank with the accession number MW866485.1, and Figure 1 shows species closely related to *B. atrophaeus* in the phylogenetic tree.

Table 1 – Morphological and biochemical characteristics of strain AspK1

Characteristic	Result
Gram	+
Shape	Rods
Motility	+
Endospore	Ellipsoidal
Pigment	Brown
Oxidase	-
Catalase	+
Urease	-
Starch hydrolysis	+
Nitrate reduction	+
Anaerobic growth	-

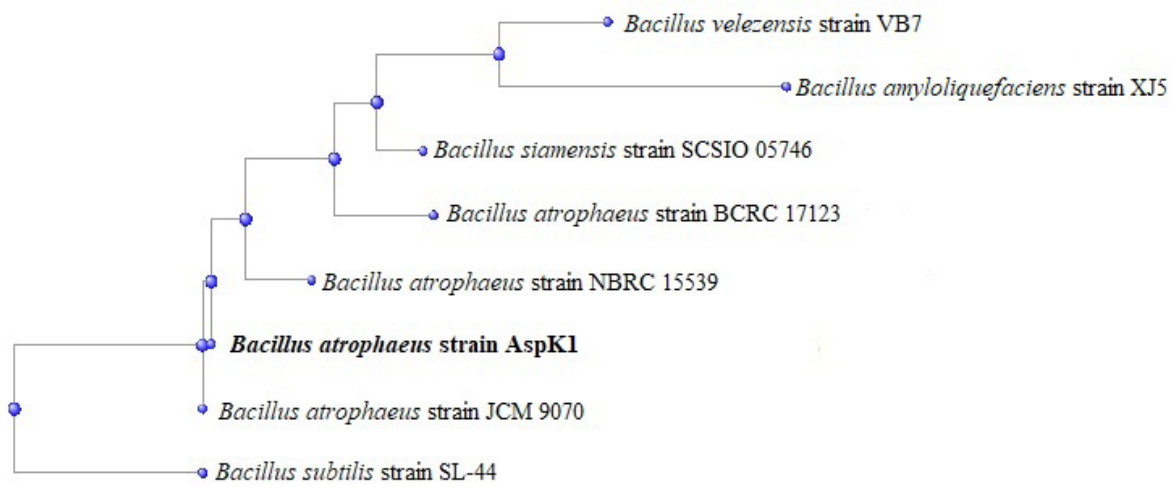

Figure 1 – Phylogenetic tree based on the 16s rDNA sequences of strain AspK1

Optimization of culture conditions for the production of L-asnase. Nutritional and environmental conditions highly affect enzyme production by microorganisms. These properties differ from one organism to another. The optimum temperature for L-asnase production by *B. atrophaeus* AspK1 was determined to be 30ºC (Figure 2a). *Bacillus* species have been reported to produce L-asnase within the temperature range of 25–40 [22].

In addition to temperature, the pH of the culture medium is important in enzyme production as it affects metabolism. Maximum L-asnase production was noted at pH 8.0, followed by a gradual decrease (Figure 2b). Similar to this study, El-Fakharany et al. [23] reported an optimum pH of 8.2 for *B. halotolerans*.

The effect of incubation time on the production of L-asnase is presented on Figure 2c. Maximum L-asnase was achieved in M9 at 48 hours and then gradually decreased. This result is consistent with the result of L-asnase production by *B. velezensis* [21].

The effect of various carbon sources on L-asnase production by *B. atrophaeus* AspK1 was evaluated. It was determined that the synthesis of L-asnase was highest when glucose was used and lowest when sucrose was present (Figure 2d). Similar results have been reported for *B. subtilis* [24], *B. velezensis* [21], *B.*

altitudinis [25], *B. licheniformis* [26], *B. halotolerans* [23] and *Brevibacillus borstelensis* [27].

For L-asnase production from *B. atrophaeus* AspK1, both organic and inorganic nitrogen sources were investigated. Inorganic nitrogen sources have generally resulted in high enzyme production. In this study, the highest L-asnase production with *B. atrophaeus* AspK1 was obtained when NH_4NO_3 was used among the nitrogen sources. In the presence of yeast extract, the least amount of L-asnase was produced (Figure 2e). In L-asnase production, NH_4Cl for *B. velezensis* [26] and $(NH_4)_2SO_4$ for *B. licheniformis* [26] were determined as suitable nitrogen sources. As a nitrogen source, $NaNO_3$ was found suitable for *Paenibacillus validus* and *Streptococcus* sp. [28].

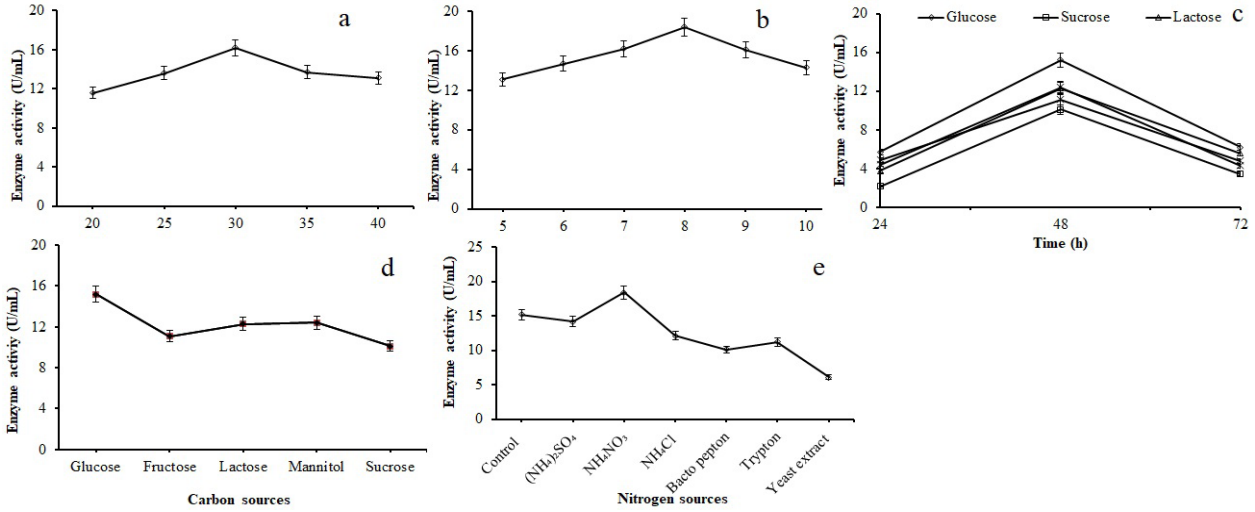

Figure 2 – The impact of a) temperature, b) pH, c) time, d) carbon sources, e) nitrogen sources on L-Asp production by *B. atrophaeus* strain AspK1

Purification and characterization of L-asnase. Extracellular L-asnase found in the cell-free supernatant was used as the source of the crude enzyme. Cells in the broth culture medium were removed by centrifugation at 10,000 rpm for 15 min after 48 h of incubation. The L-asnase was purified using 60% (v/v) chilled cold acetone precipitation followed by Amicon® Ultra-15 centrifugal filter and anion exchange chromatography with the HiTrap Q HP column. The purification steps, protein concentration, specific activity and yield of L-asnase are shown in Table 2.

Table 2 – Purification steps of the L-Asp from *B. atrophaeus* AspK1

Steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Fold purification	Yield (%)
Crude extract	810	105	7.71	1.0	100
Acetone precipation	578	31.5	18.34	1.5	67
Ion Exchange	288	11	26.18	4.8	34

The amount of protein in the L-asnase production medium was determined to be 105 mg. In acetone precipitation, the enzyme yield of L-asnase was measured at 67%, and its protein content was measured at 31.5 mg. It was observed that the specific activity increased in the purification of L-asnase with acetone. A specific activity of 26.18 U/mg protein with a 34% yield and 4.8-fold purification was obtained from the final purified enzyme. In many studies, as the purification coefficient increased, the yield decreased, however, the specific enzyme activity increased [29,30].

After L-asnase was purified, its molecular mass was determined by SDS-PAGE analysis. Molecular mass in the range of 25-47 kDa is common in *Bacillus* L-asnase such as 37 kDa for *B. licheniformis* [26], 39.7 kDa for *B. velezensis* [26], 38.2 kDa for *B. amyloliquefaciens* [31], 41.5 kDa for *B. halotolerans* [23], 42 kDa for *B. australimaris* [32] and 47 kDa for *B. megaterium* [33]. The molecular weight of *B. atrophaeus* L-Asnase was determined to be (42 kDa) (Figure 3) and varied from other *Bacillus* species.

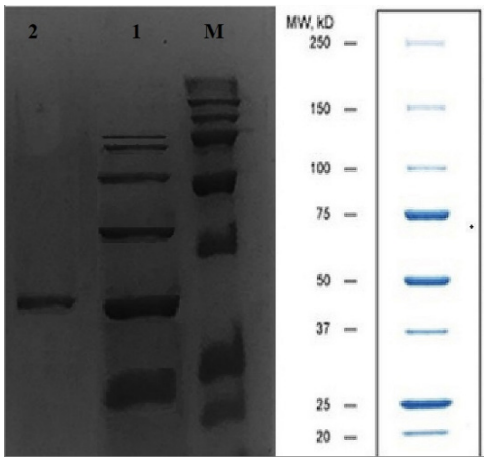

Figure 3 – SDS-PAGE of the purified L-Asp. Note: M – standard protein markers;
Lane 1 – crude enzyme; Lane 2 – purified enzyme

Determination of kinetic constants (Km and Vmax). The Km indicates the enzyme's affinity for its substrate, whereas the Vmax, which is measured in units of product generated per unit of time, indicates substrate turnover [26]. Enzyme kinetic parameters showed *Vmax* at 43 µmol/ml/min and *Km* at 2.7 mM, which indicates the high affinity of this enzyme to its substrate. Many factors (origin, structure, and form of an enzyme, enzyme conditions and assay procedures) influence the kinetic features of enzymes. *Km* and *Vmax* values for an L-asnase from *B. velezensis* were 3.6 mM and 41.49 µmol/ml/min, respectively [26]. The Km was found to be 4.56 mM for the *B. australimaris* NJB19 [32]. The *Km* of purified L-asnase from *B. licheniformis* was 1.4 mM [29].

Effect of temperature and pH on L-asnase activity. Temperature effects on L-Asnase stability were investigated at temperatures ranging from 20 to 90°C (Figure 4).

Results in Figure 4 determined that it showed maximum activity at 40°C. L-asnase activity lost approximately 60% of the total activity at 90°C. Purified L-asnase showed maximum stability after 1h incubation at 40°C, retaining more than 96% of its total activity. In addition, L-asnase lost about 40% of its activity after 5h of incubation at 80°C. L-asnase was found to be quite stable at 30-50°C and retained more than 85-70% of its activity after 5h of incubation. This reasonable thermostability of L-asnase is important for its use in biotechnological fields [31].

L-asnase purified from *B. atrophaeus* AspK1 was studied at pH 3.0-10.0 and was determined to be most active at pH 7.0. Enzyme activity decreased at lower pH values (Figure 5).

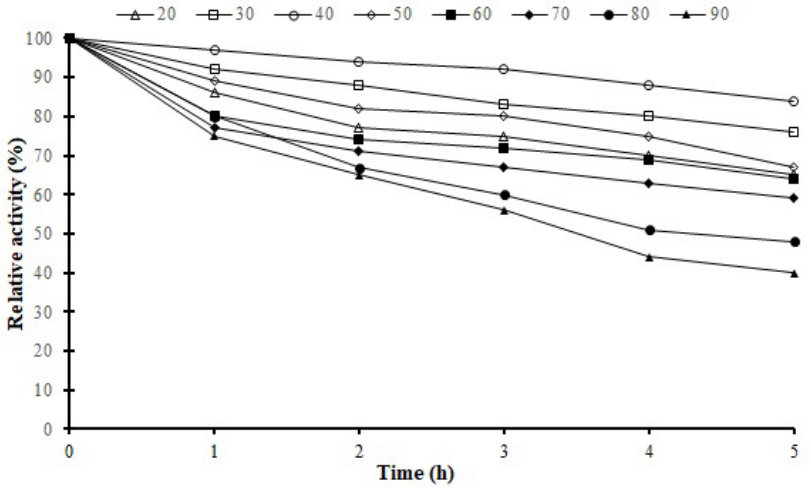

Figure 4 – Thermostability of *B. atrophaeus* AspK1 L-Asp at different temperatures for 5 h

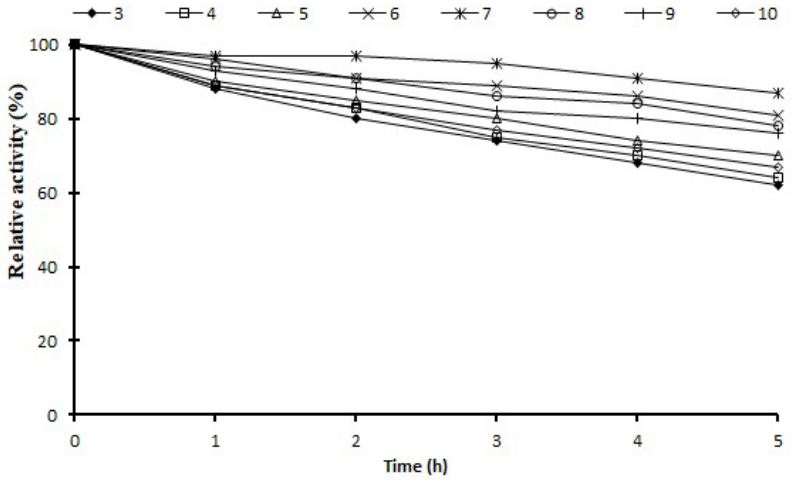

Figure 5 – pH stability of *B. atrophaeus* AspK1 L-asnase incubated in various pH for 5 h

AspK1 L-asnase retained 85% of its activity when incubated for 5h at pH 7. In a study with L-asnase obtained from *B. megaterium*, more than 80% residual activity was determined after 2h of incubation at pH 5.0-8.0 [34].

In addition, it was determined that L-asnase obtained from *B. amyloliquefaciens* showed more than 80% residual activity after 4h of incubation at pH 3.0-11.0 [31]. This is because L-asparagine, when broken down into L-aspartic acid, causes the pH to decrease [31, 34].

Effects of metal ions on the activity of L-asnase. Due to their ability to attach to specific areas of the enzyme, metal ions are important for preserving both its structure and activity [14]. It was determined that the purified enzyme was sensitive to different metal ions. In this study, Mn^{2+} ions (1 mM and 10 mM) increased AspK1 L-Asnase activity by approximately 44-33% (Figure 6).

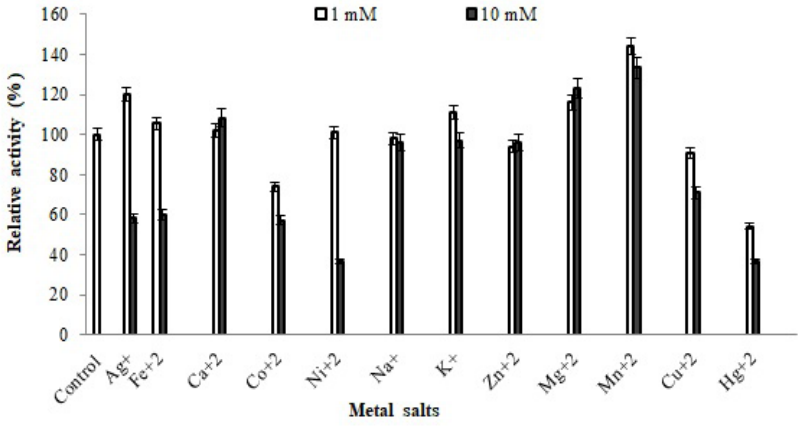

Figure 6 – Effects of different metal ions on L-Asnase from *B. atrophaeus* AspK1

L-asnase activity increased by 105-120% in the presence of 1mM Ag^{2+}, K^{+} and Fe^{+2}. In addition of 10mM of Fe^{2+}, Ni^{2+}, Ag^{+}, Fe^{2+} and Cu^{2+} decreased the activity by more than 40%. The present study determined that some metal ions (Ca^{2+}, Mn^{2+}, K^{+} and Mg^{2+}) can be evaluated as activator for the L-asnase produced by *B. atrophaeus*. Stimulation of L-asnase with Mn^{2+} and Mg^{2+} ions is common [31,35,36]. Also, similar to other studies, the enzyme activity was inhibited by Hg^{2+}, Cu^{2+} and Co^{2+} [37].

Acrylamide mitigation potential of B. atropheus L-asnase. The effect of L-asnase on

the reduction of acrylamide in potato chips was examined by treating the potato slices with 40 U/mL of L-asnase. Approximately 80% less acrylamide was formed during the frying process of potato strips treated with L-asnase than when potato strips were left untreated (Figure 7).

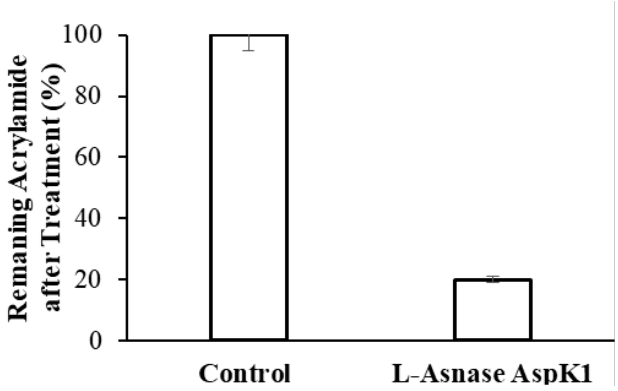

Figure 7 – Content of acrylamide in potato chips after L-asnase treatment

Significantly, *B. atrophaeus* L-asnase's capacity to reduce acrylamide is similar to that of other microbial L-asnase that have been previously documented. In fried potatoes and baked mooncakes, for example, *Paenibacillus barengoltzii*'s L-asnase lowered acrylamide by up to 86% and 52%, respectively [37]. In parallel research, two distinct strains of *B. subtilis* were shown to have L-asnase that could reduce acrylamide by up to 80% [38] and 82% [39].

Similarly, L-asnases from *Acinetobacter soli* [8], *Cobetia amphilecti* AMI6 [40], *Aquabacterium* sp. A7-Y [41], and recombinant *Palaeococcus ferrophilus* [42] have also been shown to reduce acrylamide concentrations by up to 80%, 88.2%, 55.9%, and 79%. However, *Streptomyces koyangensis* SK4 reduced acrylamide levels in potato chips by up to 50% [43], and L-ASNase from *Aspergillus terreus* BV-C observed 93% acrylamide reduction [44]. Recently, the amount of acrylamide in bread and potato chips was decreased by 51.7% and 66.9%, respectively, thanks to an engineered *E. coli* L-asnase [45].

Furthermore, using L-asnase produced by *Fusarium culmorum* reduced the amount of acrylamide in bread and potato chips to 86% and 94%, respectively[46]. Bhagat et al. (2016) [47] reported that the acrylamide concentration in fried potatoes treated with L-asnase from *Pseudomonas oryzihabitans* reduced to 90%.

Conclusion

A newly-isolated *B. atropheus* AspK1 was isolated for L-asnase production. The production of L-asnase has been determined to be influenced by pH, temperature, incubation period, and carbon and nitrogen sources. Glutaminase and urease-free L-asnase from *B. atropheus* AspK1 was found to have a molecular size of 42 kDa. The high pH and thermostability of L-asnase obtained from *B. atrophaeus* is important for its use in biotechnological fields. Glutaminase and urease-free AspK1 L-asnase could possibly be used in the pharmaceutical or food industries. Our isolated *B. atropheus* AspK1 enzyme significantly reduced the creation of acrylamide in potato chips. Therefore, the study is very valuable considering the commercial and vital importance of L-asnase.

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