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Research Article

OPTIMIZATION OF AMYLASE ACTIVITY BY *BACILLUS THURINGIENSIS* ISOLATED FROM FOOD INDUSTRY WASTEWATER

Tuğba UNAL[1a], Kubra OZTAT [2*], Cansu FILIK ISCEN [1b]

[1a] Faculty of Education, Department of Mathematics and Science Education, Eskisehir Osmangazi University, 26480 Eskisehir/Turkey, Tel. +90 222 2393750 email: tuba.unal2002@gmail.com ORCID: 0009-0003-7973-8330

[1b] Faculty of Education, Department of Mathematics and Science Education, Eskisehir Osmangazi University, 26480 Eskisehir/Turkey, Tel. +90 222 2393750; email:cfilik@ogu.edu.tr ORCID: 0000-0001-5463-8825

[2] Graduate School of Natural and Applied Sciences, Eskisehir Osmangazi University, 26480 Eskisehir/Turkey, Tel. +90 222 2393750; email: kocakkubra1995@gmail.com ORCID: 0000-0002-9212-3125

* Corresponding Author

Abstract

This study aimed to isolate and optimize the amylase activity of bacteria isolated from wastewater and treatment sludge in the food industry. Initially, bacterial isolates were obtained and identified, with *Bacillus thuringiensis* emerging as a particularly promising strain due to its significant amylase activity. Following identification, the amylase activity of *Bacillus thuringiensis* was assessed, revealing a baseline activity of 54%. The study then focused on optimizing enzyme production through a systematic evaluation of various culture conditions, including carbon sources, nitrogen sources, pH levels, and incubation times. The results indicated that starch was the most effective carbon source; bacterial peptone was the optimal nitrogen source; a pH of 9 provided the best environment and an incubation period of 48 hours maximized enzyme production. Under these optimized conditions, *Bacillus thuringiensis* demonstrated a remarkable 97.7% amylase activity. This significant enhancement underscores the potential of *Bacillus thuringiensis* for industrial applications, particularly in amylase production. The study offers valuable insights into optimizing enzyme production and establishes a foundation for future research and development in industrial enzyme applications.

Keywords: Amylase enzyme, bacterial amylase, optimization, bacteria isolation

INTRODUCTION

Amylases are crucial enzymes that play a significant role in various industrial applications, including the food, textile, pharmaceutical, and biofuel industries [1]. They catalyze the hydrolysis of starch into simpler sugars such as glucose and maltose, making them indispensable in processes like brewing, baking, detergent formulation, and bioethanol production. The demand for efficient and stable amylase enzymes has led to extensive research into the isolation of amylase-producing microorganisms from diverse environments [2].

Microbial amylases, particularly those produced by bacteria, are favored in industrial applications due to their cost-effective production, broad substrate specificity, and ability to function under a wide range

of environmental conditions [3,4]. Among the different bacterial species, the genus *Bacillus* is well-known for its ability to produce a variety of enzymes, including amylases. *Bacillus* species are widely studied because of their rapid growth, ability to secrete large amounts of enzymes extracellularly, and their resilience to industrial process conditions [5,6].

Wastewater and treatment sludge from the food industry are rich in organic matter and nutrients, making them ideal environments for the growth of diverse microbial communities. These environments often harbor microorganisms capable of producing enzymes that degrade complex organic compounds. Thus, they represent a promising source for isolating novel and potent amylase-producing bacteria [7,8].

In this study, we aimed to isolate amylase-producing bacteria from wastewater and treatment sludge obtained from a food processing industry. The goal was to screen these isolates for amylase activity, identify the most promising strain, and optimize its culture conditions to enhance amylase production. The isolate exhibiting the highest amylase activity was identified as *Bacillus thuringiensis*, a bacterium known for its diverse metabolic capabilities and industrial applications [10,11].

Subsequent optimization of the culture conditions for *Bacillus thuringiensis* was conducted to maximize amylase production. Key parameters such as carbon and nitrogen sources, pH, and incubation time were systematically varied to determine their effect on enzyme activity. The optimization process is crucial, as even slight modifications in culture conditions can lead to significant improvements in enzyme yield and efficiency, thereby making the process more cost-effective and suitable for industrial applications.

MATERIAL AND METHODS

2.1. Culture Media and Solutions

The materials and solutions used in this study included Nutrient Agar (Merck-105450), Tryptic Soy Agar (Fluka-22091), Starch Agar (M107S), Vidyalakshmi's fermentation medium (6 g/L bacterial peptone, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L KCl, 1 g/L starch), iodine solution (0.127 g I_2, 0.083 g NaCl in 100 ml water), 0.06% soluble starch solution, iodine solution (1 g/300 mL iodine crystal, 2 g/300 mL potassium iodide (KI) in distilled water), pH buffers (pH 5- Citrate buffer, pH 7- Phosphate buffer, pH 9- Carbonate-Bicarbonate buffer), and physiological saline (9 g/L NaCl in distilled water).

2.2. Bacterial Isolation from Different Environmental Samples

Samples were collected in sterile bottles from the biological treatment units of food industry factories located in Ankara and Eskisehir (Türkiye), then they were transported to the laboratory under cold chain conditions, where the isolation procedures were initiated. The samples were serially diluted to concentrations of up to 10^{-6}. To obtain a higher yield of bacteria, 100 µl of each dilution was spread

onto two different petri dishes containing Nutrient Agar (NA) and Tryptic Soy Agar (TSA) media, followed by incubation at 37ºC for 24 hours.

2.3. Detection and Comparison of Amylase Enzyme Activity

To detect and compare the amylase activity of the isolated bacteria, Starch Agar was used. Initially, 29 bacterial isolates were inoculated onto Starch Agar plates and incubated at 37ºC for 60 hours. After incubation, Lugol's iodine solution was applied to the surface of the plates to observe any color changes, as Lugol's solution interacts with starch to produce a blue-violet color. The absence of this color around the bacterial colonies was taken as a positive indication of amylase activity [4,12].

To further compare the enzyme activity among the positive isolates, the zone of clearance around the colonies was measured. Fresh 24-hour cultures of each bacterium were prepared, and equal amounts of each culture were inoculated onto separate Starch Agar plates. These plates were then incubated at 37ºC for 24 hours. After incubation, the plates were treated with iodine solution, and the diameter of the clear zones (indicative of amylase activity) was measured to assess and compare the enzymatic activity of each isolate.

2.4. Morphological and Molecular Characterization of Isolate

2.4.1 Morphological Tests

To determine the Gram reaction and morphology of the amylase-positive isolates Gram Staining has been done; bacterial cultures were spread onto microscope slides and allowed to air dry. The samples were then stained with crystal violet for 1 minute, followed by rinsing with a gentle stream of water. Lugol's iodine solution was added and left for 1 minute, after which the slides were washed with 96% ethanol. Finally, the slides were rinsed with distilled water and counterstained with safranin for 20 seconds. After drying, the specimens were examined under the immersion objective of a microscope. Bacteria displaying a pink-red color were classified as Gram-negative, while those exhibiting a purple color were classified as Gram-positive [13].

2.4.2. Molecular Tests

Molecular tests were conducted at Eskisehir MST Lab Biotechnology Company. For the DNA isolation; 200 µl of PBS was added to 2 ml Eppendorf tubes along with zirconium beads, followed by the addition of samples and 600 µl Extraction Buffer, homogenization for 3 minutes, incubation at 56°C for 15 minutes, centrifugation at 8,000 rpm for 1 minute, and transfer of the supernatant to new Eppendorf tubes, then addition of 600 µl Binding Buffer, vortexing for 10-15 seconds, and centrifugation at 8,000 rpm for 1 minute through a spin column, followed by washing with 650 µl Wash Buffer 1 and 500 µl Wash Buffer 2 at 8,000 rpm for 1 minute each, a final wash with 250 µl Wash Buffer 1 at 10,000 rpm

for 3 minutes, elution into 1.5 ml Eppendorf tubes with 50 µl Elution Buffer, and centrifugation at 10,000 rpm for 1 minute.

For PCR amplification, DNA samples that were isolated and quantified were amplified using the A.B.T.™ 2X HS-PCR Master Mix (with Blue Dye) (P02-02-01, Turkey) with the 27F primer (5'-AGAGTTTGATCCTGGCTCA-3') on a SimpliAmp™ thermal cycler (Applied Biosystems™, 15224438). The amplification conditions were set as follows: an initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 60 seconds, and extension at 72°C for 90 seconds; with a final extension at 72°C for 10 minutes and a hold at 4°C indefinitely (Table 2.1). The PCR products were analyzed using 2% agarose gel electrophoresis (Figure 2.1).

Primer: 27F: 5'-AGAGTTTGATCCTGGCTCA-3'

For Sanger Sequencing; the samples were initially subjected to chemical cleanup. This was achieved by mixing 5 µl of amplicon with 2 µl of ExoSAP-IT™ (Applied Biosystems™, 78201.1.ML, UK) in a strip tube and incubating in a SimpliAmp™ thermal cycler (Applied Biosystems™, 15224438) at 37°C for 30 minutes, followed by 80°C for 15 minutes.

For the sequencing PCR, the reaction mixture was prepared by combining 3 µl of ultra-pure water, 2 µl of BigDye™ Terminator 5X Sequencing Buffer (Applied Biosystems™, 4336697, UK), 1 µl of BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™, 4337456, UK), and 2 µl of primer mix, resulting in a total volume of 8 µl per strip tube. To this mixture, 2 µl of the chemically cleaned DNA sample was added and mixed. The samples were then subjected to the following PCR conditions in a SimpliAmp™ thermal cycler: 96°C for 1 minute; 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes; 25 cycles; and a final hold at 4°C indefinitely.

Upon completion of the sequencing PCR, the samples were subjected to physical cleanup. This involved dissolving 1 gram of Sephadex G-50 (Sigma Aldrich, G5080, USA) in 14 ml of double-distilled water, adding 800 µl of this solution to a purification column, and centrifuging the DNA spin column at 3,500 rpm for 3 minutes. The collection tube was discarded and replaced with a new one. Subsequently, the DNA spin column containing Sephadex gel was loaded with the cleaned sequencing PCR samples and centrifuged at 3,500 rpm for 3 minutes. The resulting samples were distributed into plate wells and analyzed using a 3500 Genetic Analyzer (Applied Biosystems®, USA).

Table 2.1 PCR protocol steps

Temperature	**Time**	**Cycle**
95℃	5 min	1 x
95℃	30 sec	35 x
58℃	60 sec	
72℃	90 sec	
72℃	5 min	1x
4℃	∞	1x

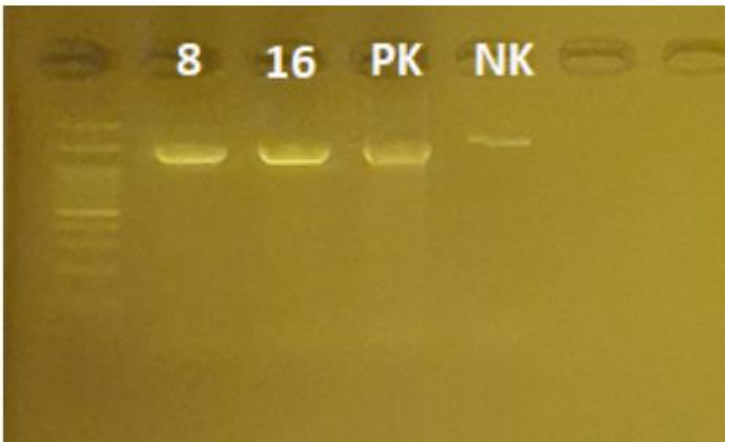

Figure 2.1. Images of PCR results using the 27F primers

2.5. Quantitative Measurement of Amylase Enzyme Activity

The isolate with the code 16 which is *Bacillus thuringiensis* has been selected for further studies. For the measurement of amylase enzyme activity, Vidyalakshmi's fermentation medium was prepared, and an inoculum from isolate was added to the medium. The culture was incubated at 37°C with shaking at 150 rpm. After 24 hours, the culture was adjusted to an optical density of 0.3 at 600 nm using a spectrophotometer to standardize the bacterial density. Subsequently, 150 ml of the medium was inoculated with 1% of the pre-cultured sample and incubated for 48 hours at 37°C with shaking at 150 rpm.

After 48 hours, 10 ml aliquots of the culture were collected into sterile tubes for centrifugation. The samples were centrifuged at 6,000 rpm for 20 minutes. The supernatant was collected and retained as the enzyme source [14].

To measure amylase activity, three types of tubes were prepared: sample, control, and blank. Sample Tube: 2 ml of starch solution and 2 ml of the enzyme source were added to a sterile tube and incubated for 10 minutes, followed by the addition of 4 ml of iodine-acid mixture. Control Tube: 2 ml of starch solution and 2 ml of fermentation medium were mixed in a sterile tube and incubated for 10 minutes, after which 4 ml of iodine-acid mixture was added. Blank Tube: 1 ml of fermentation medium and 3 ml of distilled water were mixed in a sterile tube to calibrate the spectrophotometer.

The spectrophotometer was zeroed with the blank tube. Then, 3 ml from the sample and control tubes were transferred to separate cuvettes, and the absorbance was measured using the Shimadzu UV-2550 Visible spectrophotometer, at 620 nm [14].

The enzyme activity was calculated using the following formula [14].

Activity = $[(R_0-R)/R_0] \times 100$

Where: R_o = Control (reference measurement) and R = Sample (experimental measurement)

2.6. Optimization Studies

Optimization studies were conducted to determine the optimal conditions for carbon source (starch, glucose, sucrose, fructose), nitrogen source (yeast extract, ammonium sulfate, bacterial peptone, casein), incubation time (24, 48, 72, 96 hours), and pH (5, 7, 9). Vidyalakshmi's fermentation medium was used. All the experiment sets have been done in 2 replicates.

2.6.1. Carbon source optimization

To identify the optimal carbon sources, 4 experimental set was established with each medium containing 1% of bacterial peptone, 1% of one of the carbon sources (starch, sucrose, glucose, or fructose), 0.5% of KCl, 0.5% of $MgSO_4$ in dH_2O, while keeping other medium components and culture conditions constant. Prepared media were inoculated with 1% of the sample and incubated at 37°C with shaking at 150 rpm for 72 hours. At the end of the incubation period, the samples were centrifuged at 6,000 rpm for 20 minutes. As previously described, sample, control, and blank cuvettes were prepared in parallel and measured at 620 nm using a spectrophotometer.

2.6.2. Nitrogen source optimization

To identify the optimal nitrogen source, 4 experimental set was prepared with each medium containing 1% of one of the nitrogen sources (bacterial peptone, ammonium sulfate, yeast extract, or casein), 1%

of starch, 0.5% of KCl, 0.5% of $MgSO_4$ in dH_2O, while keeping other medium components and culture conditions constant. At the end of the incubation period, the samples were centrifuged at 6,000 rpm for 20 minutes. As previously described, sample, control, and blank cuvettes were prepared in parallel and measured at 620 nm using a spectrophotometer.

2.6.3. pH and incubation time optimization

The medium prepared with the maximum activity-inducing carbon and nitrogen sources was adjusted to pH values of 5, 7, and 9 using appropriate buffers. The media were inoculated and incubated at 37°C with 150 rpm for 96 hours. Samples were taken every 24 hours to assess enzyme activity. The samples were then centrifuged, and aliquots were prepared for activity measurement.

RESULTS

3.1. Bacterial Isolation, Screening of Isolates for Amylase Enzyme Activity, and Comparison of Amylase Activity Among Isolates

After performing the serial dilution method, 13 bacterial strains were obtained from a food industry facility in Eskisehir, while 16 strains were isolated from a facility in Ankara. These isolates were subsequently cultured and maintained as stock cultures for the evaluation of their amylase enzyme production. Screening for amylase activity revealed that 5 of the isolates exhibited enzymatic activity in terms of amylase, as evidenced by the formation of clear zones around the colonies after iodine solution treatment. Comparative analysis of the active isolates was performed to identify the most suitable strain for further optimization studies. Based on the results, isolate 16 exhibited a clear zone of 2.3 cm as shown in Figure 3.1. and it was selected for subsequent analysis.

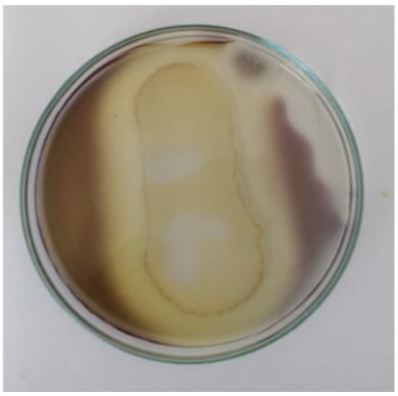

Figure 3.1. The appearance of the isolate coded 16 on petri dishes following exposure to Iodine solution; 2.3 cm of clear zone

3.2. Morphological and Molecular Characterization of Isolate

The isolated bacterium, coded 16, is a Gram-positive rod-shaped organism that exhibits sporulation. A Gram stain microscopy image of this isolate is presented in Figure 3.2. Molecular tests were conducted at Eskişehir MST Lab Biotechnology Company for species identification. The taxonomic tree of the bacterium, as determined by these tests, is presented in Figure 3.3. The DNA of the submitted sample underwent isolation, PCR amplification, and Sanger sequencing, which revealed that the bacterium identified as isolate 16 is *Bacillus thuringiensis*.

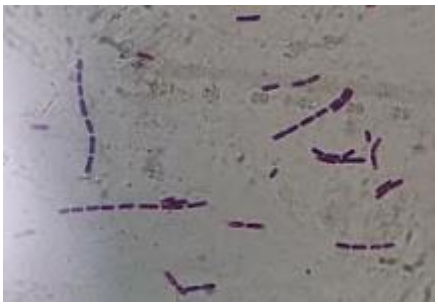

Figure 3.2. Microscope picture of Gram staining of the isolate

The sequencing results yielded the following nucleotide sequence:

5'-
GGATAACTCCGGGAAACCGGGGCTAATACCGGATAATATTTTGAACTGCATGGTTCGAAA
TTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACAC
TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAAT
GGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAAC
TCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
ATCCGGAATTATTGGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGC
CCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAA
GTGGAAATTCCATGTGTAGCGG-3'.

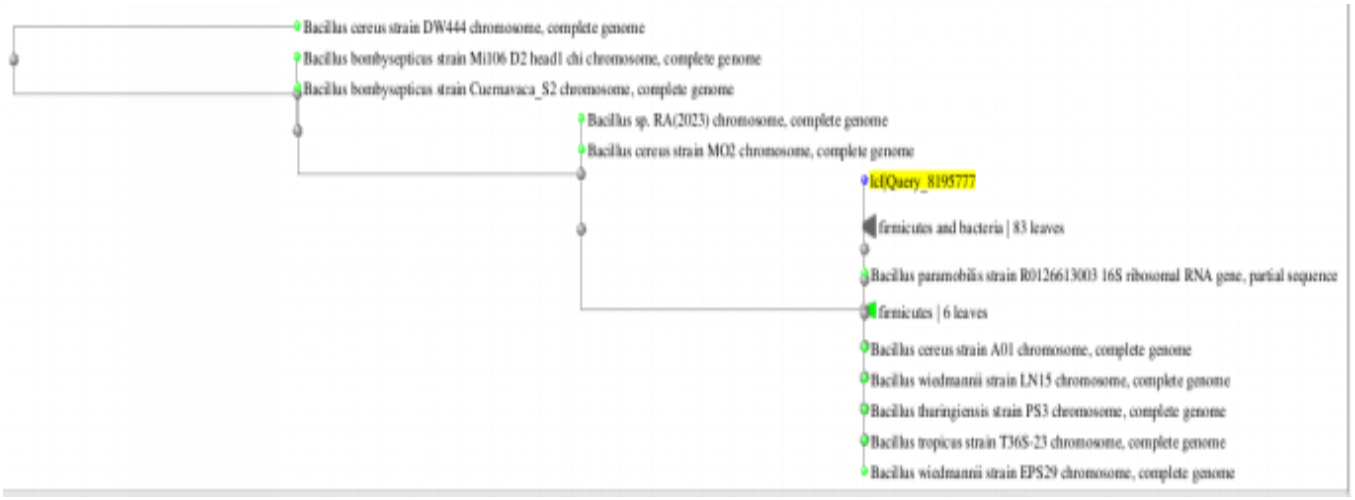

Figure 3.3. Taxonomic Tree of isolate 16

3.3. Optimization Studies

Activity percentages obtained from experimental setups designed to determine optimum conditions were calculated and shown in Figure 3.4. Starch as the carbon source resulted in the highest enzyme activity, achieving a maximum of 84 %, which led to its selection as the fixed carbon source for subsequent experiments. For the nitrogen source, bacterial peptone was chosen as it yielded the highest enzyme activity at 93 %.

Further optimization involved adjusting the pH to 5, 7, and 9, with enzyme activity monitored every 24 hours over a 96-hour incubation period. No activity was observed at pH 5 throughout the 96 hours. At pH 7, enzyme activity reached 95 % at the 96-hour. However, at pH 9, the enzyme activity peaked at the 48-hour with 97.7 %. Due to the quicker attainment of peak activity at pH 9 compared to pH 7, pH 9 was selected as the optimal pH condition.

Additionally, since maximum activity was observed at 48 hours, the optimal incubation time was determined to be 48 hours.

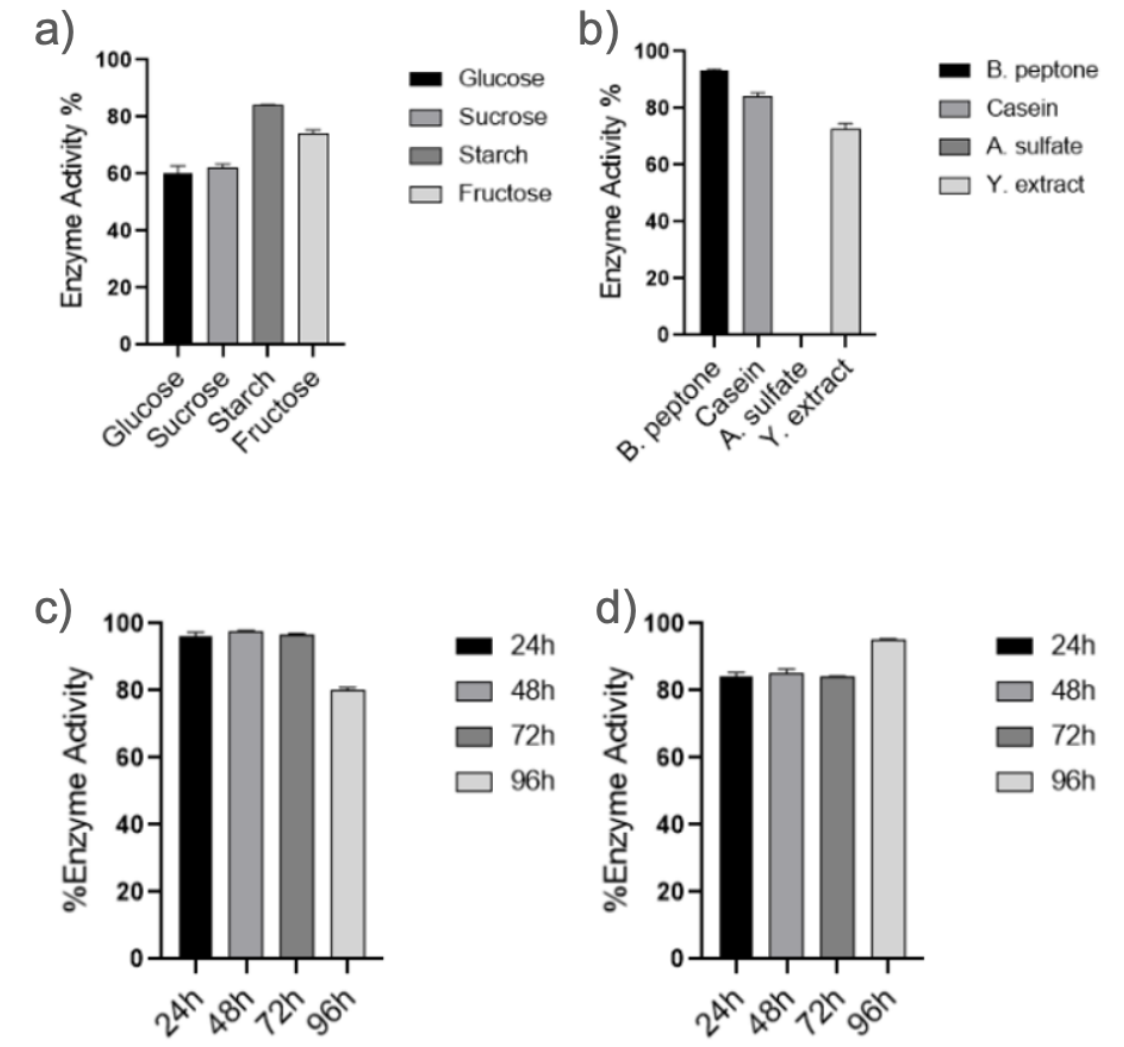

Figure 3.4. Amylase activity percentages of a) carbon sources, b) nitrogen sources, c) pH 9.0 of the media, d) pH 7.0 of the media.

As a result of these optimization studies, the optimal conditions for *Bacillus thuringiensis* were determined to be starch as the carbon source, bacterial peptone as the nitrogen source, a pH of 9, and an incubation time of 48 hours. Under these conditions, enzyme activity was enhanced to 97.7%, a significant improvement from the 54% activity observed prior to optimization.

DISCUSSION

The optimization studies conducted in this project aimed to identify the ideal conditions for maximizing amylase enzyme activity produced by *Bacillus thuringiensis*. This process involved adjusting various parameters, including the carbon source, nitrogen source, pH, and incubation time.

The outcomes of this study provide insights into the potential of *Bacillus thuringiensis* as a robust amylase producer and underscore the importance of optimizing culture conditions to enhance enzyme production. This research not only contributes to the growing body of knowledge on microbial enzyme production but also highlights the potential use of industrial waste materials as a resource for biotechnological applications.

Among the tested carbon sources—starch, glucose, sucrose, and fructose—starch was identified as the most effective in enhancing amylase activity. This result aligns with previous studies that highlight starch as a preferred substrate for amylase production due to its high availability and the enzyme's specific action on polysaccharides. For instance, Akcan (2021) achieved maximum α-amylase production using 1% starch with *Bacillus licheniformis* VO1. Although Akcan's study reported 66.11% activity with yeast extract as the nitrogen source, it did not evaluate bacterial peptone, which yielded 73.13% activity in our study [15].

Additionally, Deb (2013) found that corn flour provided the highest activity among six different carbon sources tested with *Bacillus amyloliquefaciens* P-001, with starch following closely, though the difference was not statistically significant. Regarding nitrogen sources, Deb reported that tryptone and NH_4NO_3 produced the highest amylase activity [16]. Similarly, Rezzukoğlu et al. (2018) investigated the effects of various carbon and nitrogen sources on α-amylase production by Lactococcus sp. PS-2A, finding that 1% starch significantly enhanced enzyme activity compared to other carbon sources. They also identified ammonium chloride as the most effective nitrogen source for enzyme production [17].

Among the nitrogen sources evaluated—yeast extract, ammonium sulfate, bacterial peptone, and casein—bacterial peptone was the most effective, achieving the highest enzyme activity. Bacterial peptone's rich amino acid content likely contributed to its superior performance by promoting microbial growth and enzyme production. This finding is consistent with other studies, which have noted the beneficial impact of amino acid-rich nitrogen sources on enzyme production.

Regarding pH levels, our study found that the highest enzyme activity was achieved at both pH 7.0 and pH 9.0. However, due to maximum activity being reached at 24 hours and 96 hours for pH 7.0 and pH 9.0, respectively, pH 7.0 and an incubation time of 24 hours were considered optimal. This is in contrast to Deb (2013), who reported an optimal pH of 6.5, and Akcan (2021), who identified pH 6.0 and an incubation time of 48 hours as optimal. Rezzukoğlu et al. (2018) observed that maximum activity with Lactococcus sp. PS-2A was achieved at pH 7.0, with an optimal incubation time of 20 hours. These variations underscore the importance of pH in maintaining enzyme activity and stability, with pH 7.0 being effective for our enzyme production.

Incubation times of 24, 48, 72, and 96 hours were tested to determine the optimal duration for enzyme production. The results indicated that the highest enzyme activity was achieved at 48 hours. This duration effectively balances the time required for enzyme synthesis with the risk of activity decline due to degradation or metabolic changes over longer periods. These findings corroborate other research emphasizing the importance of optimizing incubation time to maximize enzyme production.

The final optimized conditions for *B. thuringiensis*—starch as the carbon source, bacterial peptone as the nitrogen source, pH 9, and an incubation time of 48 hours—resulted in a remarkable enzyme activity of 97.7%. These conditions align with the known preferences of *B. thuringiensis* for amylase production and provide a robust framework for scaling up enzyme production in industrial applications.

These results have several practical implications. By optimizing these parameters, the efficiency and yield of amylase production can be significantly improved, which is beneficial for industrial processes that rely on this enzyme, such as in the food and detergent industries. Future research could explore further refinement of these conditions or investigate additional factors that might influence enzyme activity, potentially enhancing the overall productivity and applicability of amylase.

In conclusion, this study successfully determined the optimal conditions for amylase production by *Bacillus thuringiensis*, providing valuable insights for both academic research and industrial applications.

Conflict of Interest

No conflict of interest declared.

Authors Contribution

Unal, Tugba: Formal analysis, investigation, methodology, project administration, resources, software, validation, writing – original draft, writing – review & editing

Oztat, Kübra: Formal analysis, methodology, project administration, software, validation, writing – review & editing

Filik İşçen, Cansu: Methodology, project administration, supervision, writing – review & editing

REFERENCES

[1] Naidu, M. A., & Saranraj, P. (2013). Bacterial amylase: a review. *Int J Pharm Biol Arch*, *4*(2), 274-87.

[2] Karnwal, A., & Nigam, V. (2013). Production of amylase enzyme by isolated microorganisms and its application. *International journal of pharma and bio sciences*, *3*(4), 354-360.

[3] Souza, P. M. D. (2010). Application of microbial α-amylase in industry-A review. *Brazilian journal of microbiology*, *41*, 850-861.

[4] Ekka, A., & Namdeo, N. (2018). Screening, isolation and characterization of amylase producing bacteria and optimization for production of amylase. *IOSR Journal of Biotechnology and Biochemistry*, *4*, 50-56.

[5] Singh, S. U. R. E. N. D. R. A., Sharma, V. I. N. N. I., Soni, M. L., & Das, S. H. I. P. R. A. (2011). Biotechnological applications of industrially important amylase enzyme. *International Journal of Pharma and Bio Sciences*, *2*(1), 486-496.

[6] Nimisha, P., Moksha, S., & Gangawane, A. K. (2019). Amylase activity of starch degrading bacteria isolated from soil. *International Journal of Current Microbiology and Applied Sciences*, *8*(4), 659-671.

[7] Liu, Z., & Smith, S. R. (2021). Enzyme recovery from biological wastewater treatment. *Waste and Biomass Valorization*, *12*, 4185-4211.

[8] Møretrø, T., & Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1022-1041.

[9] Bravo, A., Likitvivatanavong, S., Gill, S. S., & Soberón, M. (2011). Bacillus thuringiensis: a story of a successful bioinsecticide. *Insect biochemistry and molecular biology*, *41*(7), 423-431.

[10] Sanahuja, G., Banakar, R., Twyman, R. M., Capell, T., & Christou, P. (2011). Bacillus thuringiensis: a century of research, development and commercial applications. *Plant biotechnology journal*, *9*(3), 283-300.

[11] Smitha, R. B., Jisha, V. N., Sajith, S., & Benjamin, S. (2013). Dual production of amylase and δ-endotoxin by Bacillus thuringiensis subsp. kurstaki during biphasic fermentation. *Microbiology*, *82*, 794-800.

[12] Sharif, S., Shah, A. H., Fariq, A., Jannat, S., Rasheed, S., & Yasmin, A. (2023). Optimization of amylase production using response surface methodology from newly isolated thermophilic bacteria. *Heliyon*, *9*(1).

[13] Coico, R. (Ed.). (2006). *Current protocols in microbiology*. Wiley.

[14] Dinçbaş, S. (2009). *Alginat Kapsüllerinde Tutuklanan Bacillus Amyloliquefaciens A-Amilaz Enziminin Farkli Nişasta Kaynaklarini Hidrolizleme Yeteneğinin Araştirilmasi* (Master's thesis, Bursa Uludag University (Turkey)).

[15] Akcan, N. (2021). Bacillus licheniformis VO1'den α-Amilaz Üretimi için Tarımsal Endüstriyel Atıkların ve Uluslararası Faktörlerin İncelenmesi. Fen Bilimleri Enstitüsü Dergisi, 11(1), 58-67. https://doi.org/10.21597/jist.780601

[16] Deb, P., Talukdar, S. A., Mohsina, K., Sarker, P. K., & Sayem, S. A. (2013). Production and partial characterization of extracellular amylase enzyme from Bacillus amyloliquefaciens P-001. SpringerPlus, 2, 1-12.

[17] Rezzukoğlu, İ., Fincan, S. A., & Barış, E. N. E. Z. (2018). Peynir altı suyundan izole edilen laktik asit bakterisinden α-amilaz üretimi ve karakterizasyonu. Biyolojik Çeşitlilik ve Koruma, 11(2), 115-122.