Research Article

From Waste to Wealth: Efficient α-Amylase Production Using Agro-Waste Materials: A Study on *Brevibacillus* Isolates

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Abstract | Soil microbiota serves as a rich source of different microbial enzymes including α-amylase. In this study we aimed to isolate, optimize amylase-producing bacterial strains, and evaluate the effectiveness of agro-waste in replacing conventional starch-based fermentation substrates. Soil samples were collected from different areas of Pakistan. Bacteria that produce α-amylase were isolated and characterized. Initial screening *in vitro* was performed by starch hydrolysis assay. This process resulted in the identification of two promising bacterial isolates, AU1 and AU2, which were further characterized through biochemical testing and 16S rRNA sequencing. They were identified as *Brevibacillus reuszeri* and *Brevibacillus brevis*, respectively. Optimization of the fermentation conditions revealed optimal pH 7, temperature of 38 °C, incubation period of 72 h, and yeast extract as an organic nitrogen source. Additionally, using different agro-waste, general waste, and several sources of starch including corn comb, wheat bran, rice bran, potato peel, rice starch, potato starch, and corn starch, *Brevibacillus reuszeri,* and *Brevibacillus brevis* were tested for the production of α-amylase. The maximum amylase production was observed by *Brevibacillus reuszeri* with wheat bran (381 µmol/ min.) and *Brevibacillus brevis* with potato peel (395 µmol/ min.). Our research provides significant insights into the optimization of the fermentation processes for α-amylase production, highlighting the economic and sustainable potential of utilizing agro-waste materials in enzyme production industries.

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Introduction

Over time, the soil microbiota has attracted more attention as the soil is the primary sink of various organisms. These microorganisms carry different biochemical reactions and are rich sources of microbial enzymes including α-amylase (Liu *et al.*, 2022). Amylase is a biological catalyst that acts at random locations of starch molecules and cleaves α-1,4-glycosidic bonds resulting in branched oligosaccharides (Bertoft, 2018). Whereas α-amylase neither can split terminal glucose residues nor cleaves at α-1,6 linkages (Visvanathan *et al.*, 2020). Amylase is classified into three different categories

de-branching, exo-acting, and endo-acting (de Castro et al., 2018). In the world enzyme market, amylase accounts for approximately 25–30 % and is widely used in different industries, including food, paper textile, biopharmaceutical engineering, and bioethanol production (Naik et al., 2023). Although amylase is found in all living organisms, its functionality, specificity, and requirements vary among and within species, and even among different tissues of the same organism. For industrial applications, microbial sources are preferred due to their advantages, mainly cost-effectiveness, reliability, reduced production time and space requirements, and ability to customize according to specific needs (Sidhu *et al.*,1997; Li *et al.*, 2007). Although many microbial species produce amylase, bacteria are the most economical and rapid option. These bacteria inhabit various environments, with soil being their primary habitat. Potent bacterial strains have been isolated using specialized techniques from different sources; particularly soil. Moreover, environments characterized by geothermal activity, such as thermal springs and humus, have also been explored for bacterial amylase producers recognized as significant bacterial ecosystems, known for synthesizing thermally stable enzymes (Prakash *et al.*, 2010; Wang *et al.*, 2015; Mohammad *et al.*, 2017). Bacterial amylase producers are cost-effective and genetically modifiable, making them ideal for enzyme production (Far *et al.*, 2020). The *Bacillus* genus is frequently cited due to its amylase-producing capabilities (Liu *et al.*, 2022). Moreover, this genus offers numerous industrial advantages, including rapid fermentation, protein secretion, ease of handling, and ability to maintain mass manufacturing in cost-effective settings with resistance to elevated temperatures and alkaline environments (Nigam *et al.*, 1995). In the last few years, utilization of agro-wastes and general wastes as fermentation media substrates for α-amylase production has gained attention. While starch has traditionally been the primary substrate for α-amylase biosynthesis, its high cost limits its use in large-scale industrial applications. To overcome this challenge, researchers have turned their attention to more economical and sustainable alternatives, such as agro-wastes and general waste materials, which are abundant in starch and other fermentable components (Gangadharan *et al.*, 2008). These waste materials are increasingly employed in solid-state fermentation (SSF) and submerged fermentation (SmF). Agro-wastes, including lignocellulosic biomass, fruit peels, agricultural residues, and food processing by-products, offer several benefits, such as being inexpensive, readily accessible, rich in starch, and help address solid waste disposal issues, making them commercially viable substrates (Singhania et al., 2009). General waste materials, such as recycled products and non-conventional starch sources, also contribute to reducing overall production costs in enzyme industries (Kumar and Singh, 2018). However, the use of these waste materials presents certain challenges. The presence of complex or indigestible carbohydrates in lignocellulosic biomass may hinder the efficiency of α amylase production. These intricate structures are difficult for microorganisms to metabolize, resulting in reduced enzyme yield. To improve substrate utilization and enzyme production, researchers employed various pretreatment techniques, including biological, physical, and chemical methods, to break down complex carbohydrates into simpler forms that are more easily fermented by microorganisms (Salim *et al.*, 2017). The use of agro-wastes and general waste substrates in fermentation processes showed promise for α-amylase production. When combined efficiency of SmF and potential of SSF, they offer economic advantages, sustainability, and solutions to industrial production and waste management challenges.

The objectives of the present study were to explore the soil microbiota to produce the α-amylase enzyme; in light of the necessity for discovering novel bacterial strains with the capability of producing this enzyme, and optimizing the nutritional and cultural requirements. We seek to optimize the different parameters and potential of the different agro-wastes to enhance the production of α-amylase *via* promising bacterial strains. However, existing literatures lack comprehensive insights into the potential of different agro-wastes as alternative substrates for α-amylase production.

Materials and Methods

Identification and evaluation of microorganisms that produce α-amylase

Eight soil samples were collected from different cities of Pakistan (Lahore, Multan, Faisalabad, and Karachi). The collection sites spanned various environments, including agricultural fields (*i.e.*, rice, potato, and corn), rose root zones, kitchen waste areas, grounds, roadside margins, and leaf litter areas. The samples were collected using sterile containers and transported promptly to the laboratory. Bacterial

isolation was carried out using the serial dilution method followed by the spread plate technique. Each soil sample underwent 10-fold serial dilutions up to 10^{-1}-10^{-10} using sterile dist. water (Kanimozhi *et al.*, 2014). Subsequently, 0.1 ml of the final dilution was aseptically spotted to Petri plates containing starch-agar medium (composition in g/ l: 13 g nutrient broth, 20 g agar, and 10 g starch). The plates were subsequently incubated at 37 ˚C for 24 h to encourage bacterial growth. Initial screening for amylase-producing bacteria was conducted using the starch hydrolysis assay (Luang *et al.*, 2019). After incubation, Gram's iodine solution (10%) was applied to the plates to visualize starch breakdown. The presence of clear zone surrounding the bacterial streak indicated starch hydrolysis, suggesting amylase production by the tested isolate. In contrast, areas that remained dark blue indicated presence of unhydrolyzed starch (Kumar *et al.*, 2014).

Molecular characterization of bacterial isolates

For basic bacterial identification Gram staining and several biochemical assays were carried out. During molecular analysis for DNA extraction (according to the Qiagen (2016) protocol), the DNA easy mini-DNA extraction kit was utilized, adhering to the standard Qiagen DNA extraction Spin protocol. The polymerase chain reaction (PCR) process was executed in four distinct phases. The first phase commenced with an initial denaturation at 94 ˚C for 5 min. The second phase comprised 35 cycles, each involving denaturation at 94 ˚C for 30 sec, followed by annealing at 52.7 ˚C for 35 sec, and then an extension at 72 ˚C for 2 min. In the third phase, a final extension at 72 ˚C for 5 min. was performed to ensure complete elongation of the amplified DNA fragments. The fourth and final phase was a holding stage, where the reaction was kept at 4 ˚C indefinitely to preserve the amplified products for subsequent analysis. The 16sRNA sequencing employed primers with the following sequences: forward 5' (GGA TTA GAT ACC CTG GTA) 3' and reverse 5' (CCG TCA ATT CMT TTR AGT TT) 3'. Using bioinformatics tools such as nucleotide BLAST and Mega-X, the phylogenetic analysis of the amplified product was conducted using the sequencing data.

Preparation of starter culture and growth media

An individual colony of amylase-producing bacterium, isolated from a starch-agar plate, was introduced into Erlenmeyer flasks (250 ml) containing 50 ml of sterile nutrient broth (NB). The flask was then placed in a rotatory shaking incubator at 37 ˚C and 140 rpm for 16-18 h to create a uniform inoculum. Amylase production was conducted through submerged fermentation (Gadhave *et al.*, 2022). The fermentation medium was prepared with the following composition (g/l): soluble starch 10, potassium chloride 0.5, $MgSO_4$ 0.5, and peptone 6. 1 ml of the bacterial inoculum (4.3×10^8 cfu/ml) was inoculated to the fermentation medium and incubated for 48 h at 37±2 ˚C. The enzyme production process involved centrifuging the fermentation media at 4000 g and 4 ˚C for 10 min. The crude enzyme was extracted from the supernatant. This crude enzyme extract was subsequently employed to determine the enzyme activity unit (U).

Amylase enzyme assay

An enzyme assay was carried out using 3,5-dinitrosalicylic acid (DNS) reagent and phosphate buffer (0.05 M) at pH 7.2 to prepare the substrate solution. The enzyme assay was conducted by combining 250 µl of crude enzyme with an equal volume of substrate (prepared in buffer) in sample tubes. Control tubes were prepared by substituting the enzyme with buffer. These mixtures were then incubated in a water bath for 10 min at 50 ˚C. Following incubation, 500 µl of DNS reagent was added to each tube, which was subsequently placed in boiling water on a hot plate for 5 min. After this period, 2 ml of dist. water were introduced to each tube. The entire assay was performed in duplicate (Li *et al.*, 2007). The absorbance (OD value) was then measured at 546 nm using a Spectrophotometer (WE721, Germany) and using the control as a reference. Kumar *et al.* (2014) described the standard assay for amylase activity based on the amount of enzyme needed to produce 1 µmol of a reducing sugar measured as maltose equivalents. The enzyme unit was calculated as:

$$\text{Enzyme unit (µmol/ min.)} = \frac{X}{\textit{Molecular weight of maltose}} \times \frac{1000}{\textit{Incubation time}} \times Df$$

Where, X= maltose released, Df= dilution factor, Time of incubation = 10 min., and the molecular weight of maltose is 342.

Optimization of nutritional and culture conditions for amylase production

Optimization studies weres conducted sequentially. Various physical parameters were optimized, including pH, temperature, incubation time, and nitrogen

sources. To optimize and achieve maximum yield of the amylase enzyme, fermentation conditions ranging from temperature (28-48 °C), pH (5-8), incubation period (24-96 h), and various nitrogen sources were utilized. These included organic nitrogen sources such as beef extract, peptone, yeast extract, and soybean, as well as inorganic sources like ammonium nitrate, ammonium chloride, ammonium sulfate, and urea. Additionally, different agro-wastes (*i.e.*, corncob, rice bran, and wheat bran) and potato peel were employed as general nutrient sources. Furthermore, three distinct types of starch, including corn starch, potato starch, and rice starch were also used for optimization to obtain maximum enzyme yield. The crude enzyme was obtained by centrifuging the fermentation broth at 4000 g and 4 °C for 15 min and then collecting the supernatant.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics, and differences among the substrates (starch versus agro-wastes) were assessed using an independent t-test. A *p*-value of less than 0.05 was considered indicative of statistical significance.

Results

Selection of a bacterial isolate

A total of 50 bacterial isolates were obtained from the different soil samples. Screening on starch-agar plates revealed that 15 isolates demonstrated effective starch hydrolysis. Among these, isolates AU1 and AU2 displayed the largest starch hydrolytic zone, and were selected for further investigation.

Phylogenetic analysis through 16S rRNA sequencing

The phylogenetic analysis of bacterial isolates AU1 and AU2 was performed using the Neighbor-Joining (NJ) method, concentrating on 16S rRNA gene sequences. This evolutionary study utilized MEGA12 with up to three parallel computing threads. Evolutionary distances were determined through the Maximum Composite Likelihood method and are represented as the number of base substitutions per site. The percentage of replicate trees where the associated taxa clustered together in the bootstrap test (1,000 replicates) is indicated above the branches. The phylogenetic trees of AU1 and AU2 illustrate their evolutionary connections within the *Brevibacillus* genus, with bootstrap values offering statistical assurance for each node. AU1 showed 90% similarity with *Brevibacillus reuszeri* (Figure 1) and *Brevibacillus brevis* strains and was assigned an accession no. of OR361798.1 (Figure 2), while the 16S rRNA sequence of AU2 displayed 99.2 % identity with *Brevibacillus brevis* (accession no. MH938812.1). The bootstrap values provided confidence in the clustering patterns, reflecting the level of genetic similarity or divergence among the analyzed *Brevibacillus* isolates.

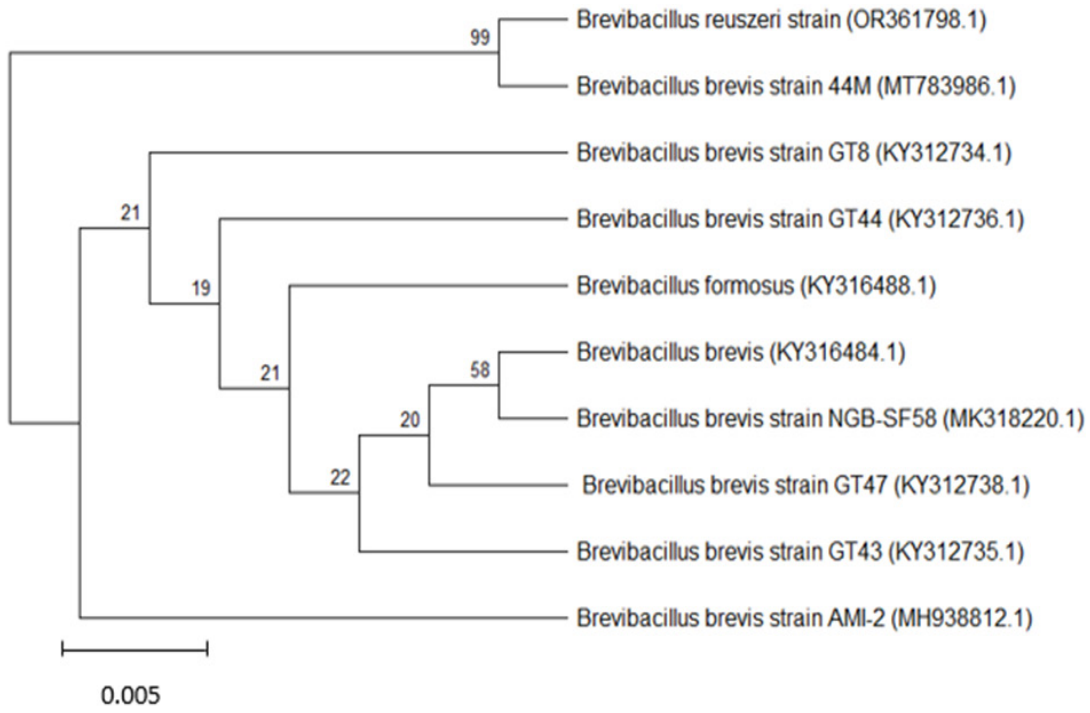

Figure 1: *Phyolgenetic tree of Brevibacillus reuszeri (OR361798.1).*

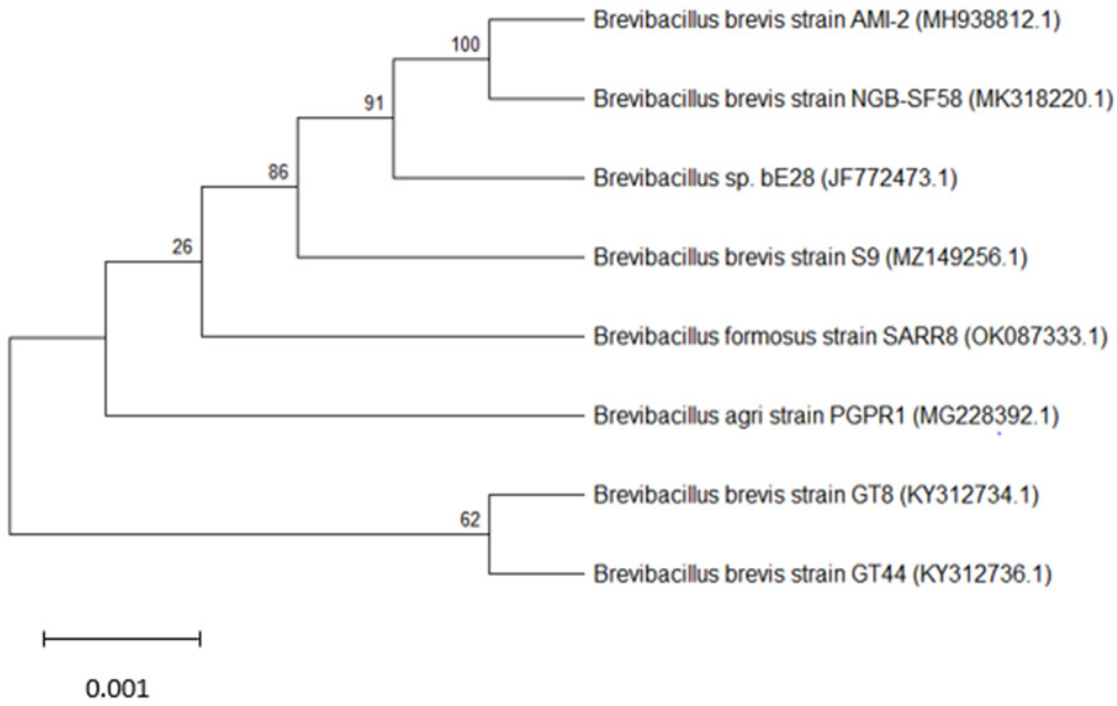

Figure 2: *Phyolgenetic tree of Brevibacillus brevis (MH938812.1).*

Optimization of the fermentation conditions

Optimum temperature: Temperature during fermentation is a crucial factor in submerged fermentation processes. To investigate the α-amylase enzyme's thermal stability across varying incubation temperatures, ranging from 28 ˚C to 53 ˚C and their effect on α-amylase production was depicted in (Figure 3). Experimental results indicated a steady increase in enzyme activity as the temperature rose from 28 ˚C, peaking at 38 ˚C. Beyond this point, activity gradually decreased up to 53 ˚C. Therefore, raising the fermentation temperature above 38 ˚C was not conducive to enzyme secretion for both AU1 and AU2 strains. At 38 ˚C, AU1 and AU2 exhibited peak amylase activity, with C1 reaching 170 µmol/ min. and C2 recording 165 µmol/ min.

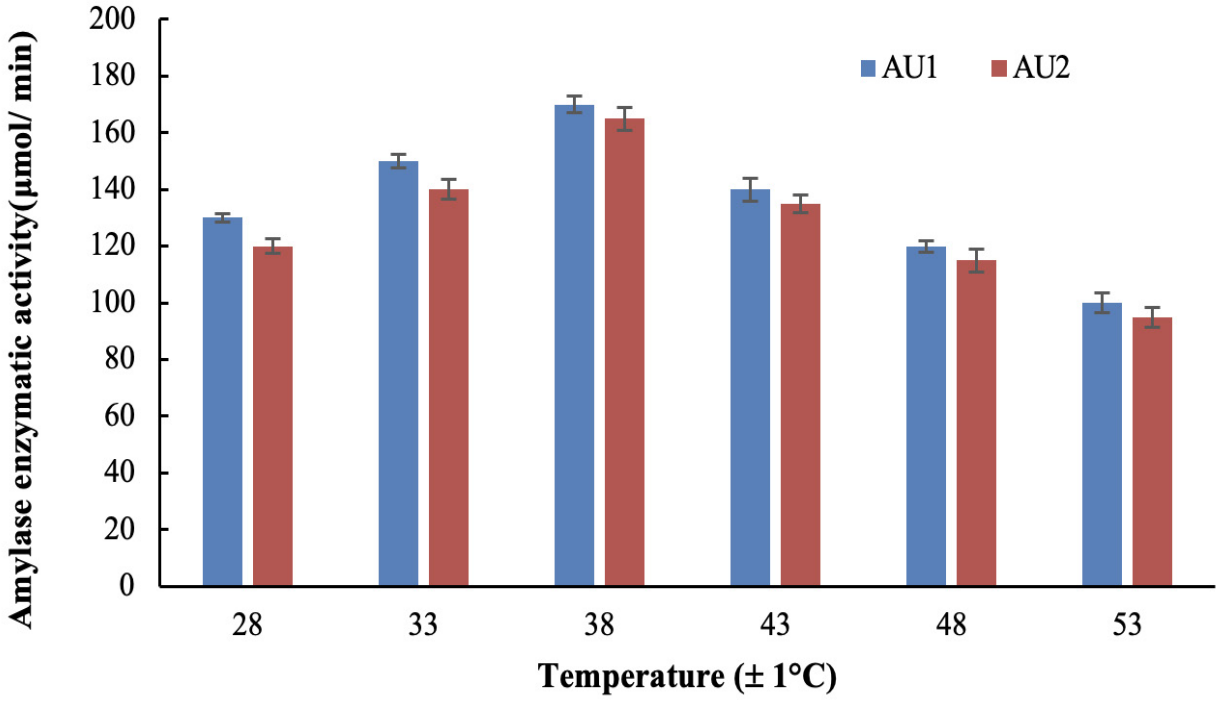

Figure 3: *Temperature influence on α-amylase activity in AU1 and AU2 isolates. Amylase activity (µmol/min.) was evaluated at temperatures ranging from 28 ˚C to 53 ˚C.*

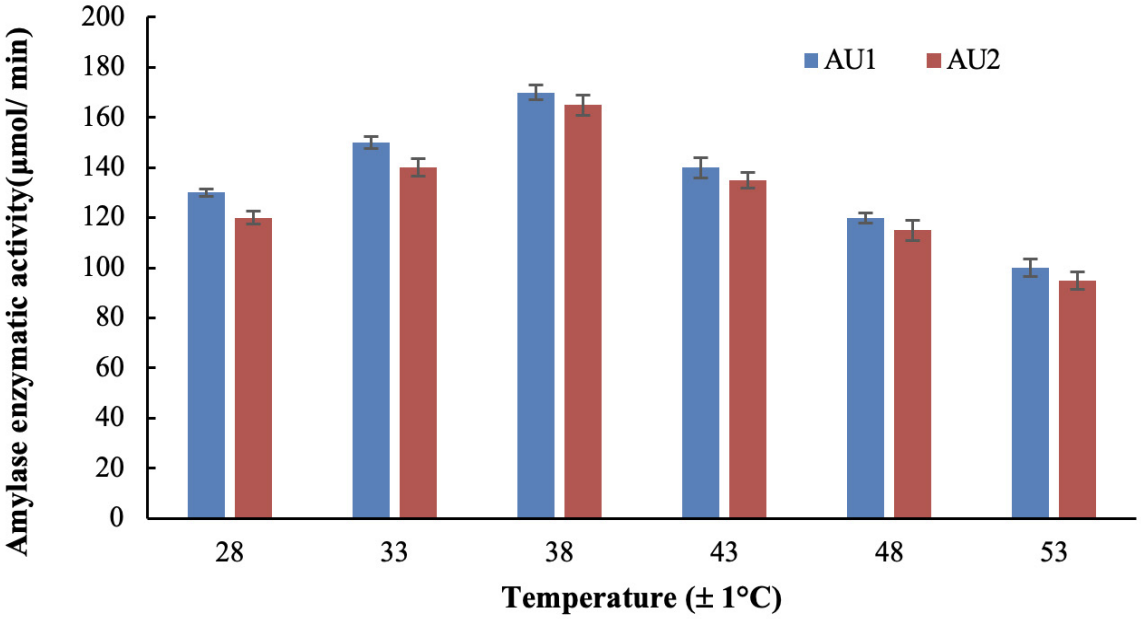

Figure 4: *pH impact on α-amylase activity in AU1 and AU2 isolates. Amylase activity (µmol/ min.) was assessed across a pH range of 5.0 to 8.0. Optimal enzyme activity was recorded at pH 7.5 for both isolates, with a subsequent reduction observed at pH 8.0.*

Optimum pH: The initial pH is another vital parameter influencing microbial growth and subsequent production of enzymes and metabolites. The α-amylase producing activity of strains AU1 and AU2 varied across pH levels from 5 to 8 (Figure 4). At pH 5, AU1 and AU2 showed enzyme activities of 90 µmol/ min and 95 µmol/ min, respectively. The increase in ascending manner continued till pH 75, with AU1 recording 180 µmol/ min and AU2 175 µmol/ min. However, at pH 8, both isolates exhibited markedly reduced activity with AU1 reaching 110 µmol/ min and AU2 recording 115 µmol/min.

Optimum incubation time

The incubation time was affected by both culture's properties and its growth rate. This study focused on determining the ideal fermentation timeline. Each flask containing the fermentation medium was incubated at 38 °C for 24-h intervals, ranging from 24 to 96 h (Figure 5). Maximum α-amylas production by the strains AU1 and AU2 was achieved at 72 h incubation, recording 270 µmol/ min. and 275 µmol/ min, respectively.

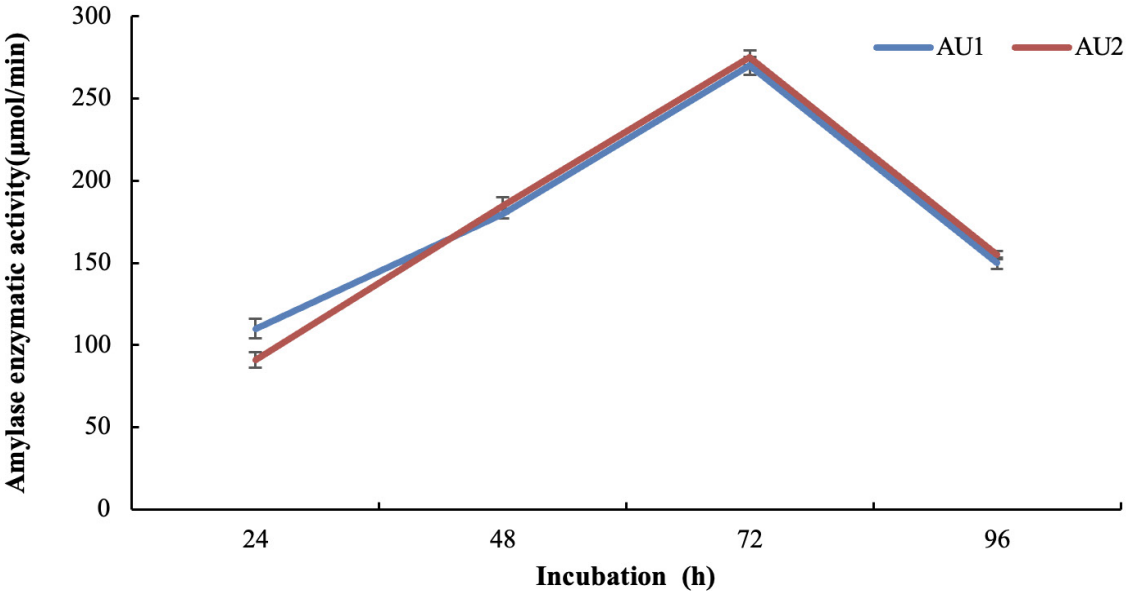

Figure 5: *Effect of incubation time on α-amylase activity in AU1 and AU2 isolates. Amylase activity (µmol/ min.) was assessed across a 24 h interval range.*

Optimum organic and inorganic nitrogen sources

The fermentation medium utilized peptone, beef extract, and soybean as sources of organic nitrogen. Each fermentation flask was maintained at 38 ˚C for duration of 72 h. The highest amylase production was recorded with the inclusion of yeast extract, where AU1 exhibited an activity of 296 µmol/ min., while AU2 achieved 290 µmol/ min (Figure 6). Furthermore, when the fermentation medium was supplemented with inorganic nitrogen sources such as ammonium sulfate, ammonium chloride, urea, and ammonium nitrate, there was a notable decrease in amylase production by both strains (Figure 7), indicating a detrimental effect on amylase production compared to the organic nitrogen sources. Notably, using the inorganic nitrogen sources, both strains showed maximum enzyme activity of 160 µmol/ min. for AU1 and 155 µmol/ min. for AU2.

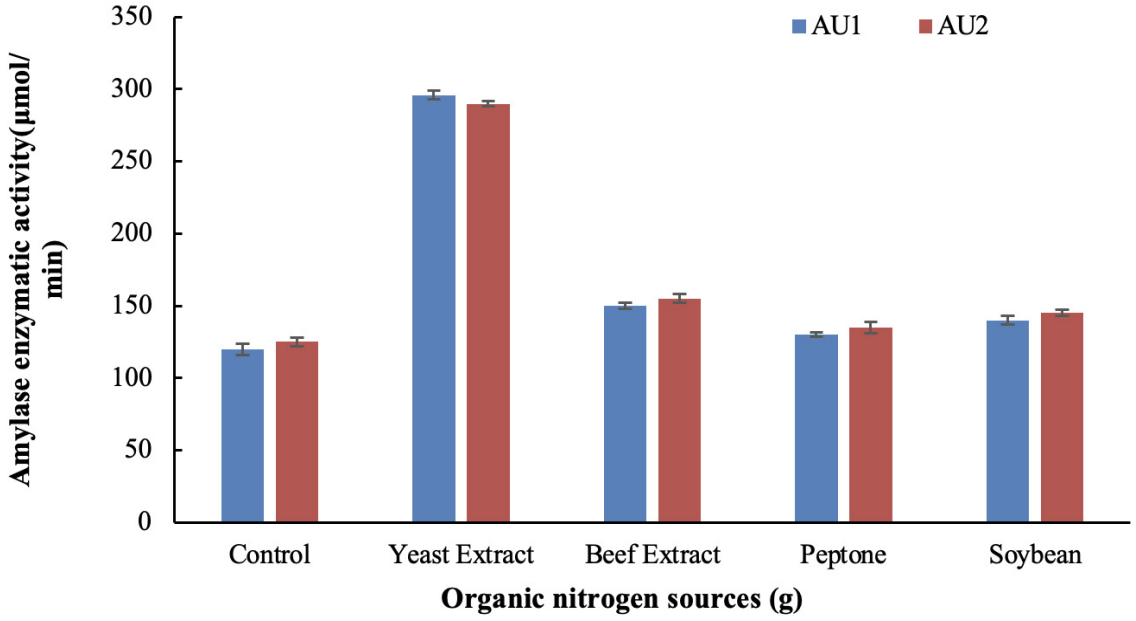

Figure 6: *Influence of various organic nitrogen sources on α-amylase activity in AU1 and AU2 isolates. Amylase activity (µmol/ min.) was evaluated using yeast extract, beef extract, peptone, and soybean as organic nitrogen sources.*

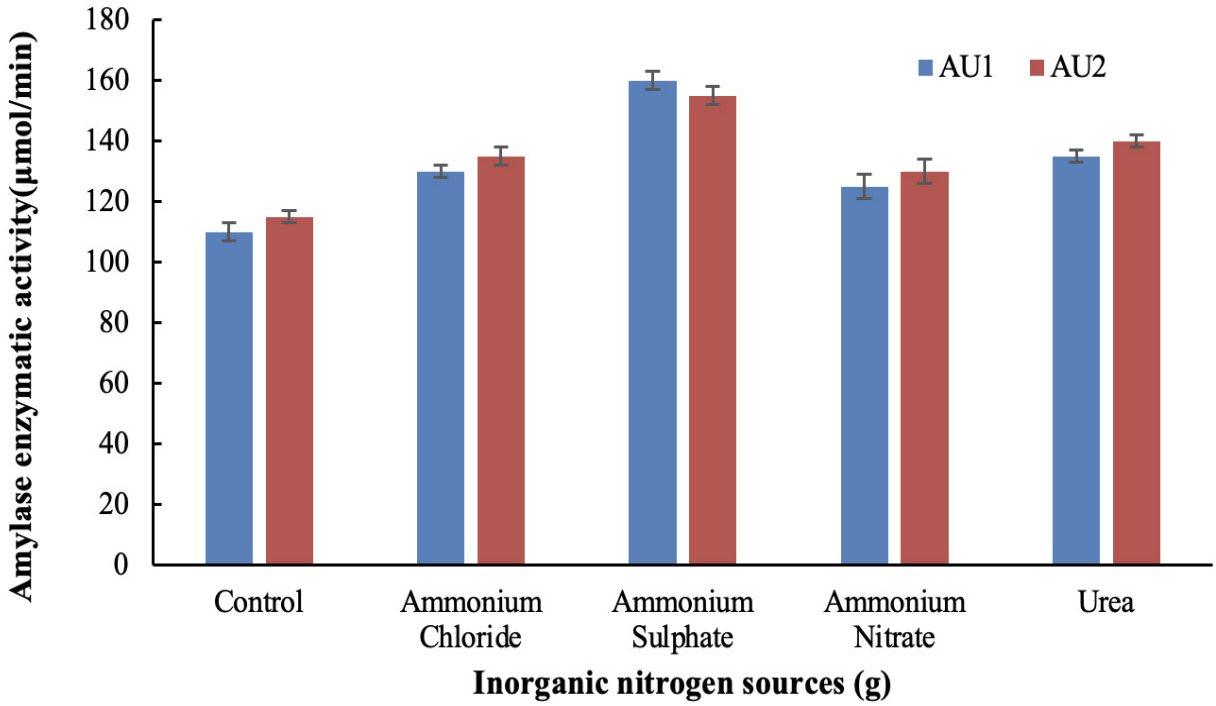

Figure 7: *Impact of various inorganic nitrogen sources on α-amylase activity in AU1 and AU2 isolates. Amylase activity (µmol/ min.) was evaluated using ammonium chloride, ammonium sulfate, ammonium nitrate, and urea as inorganic nitrogen sources.*

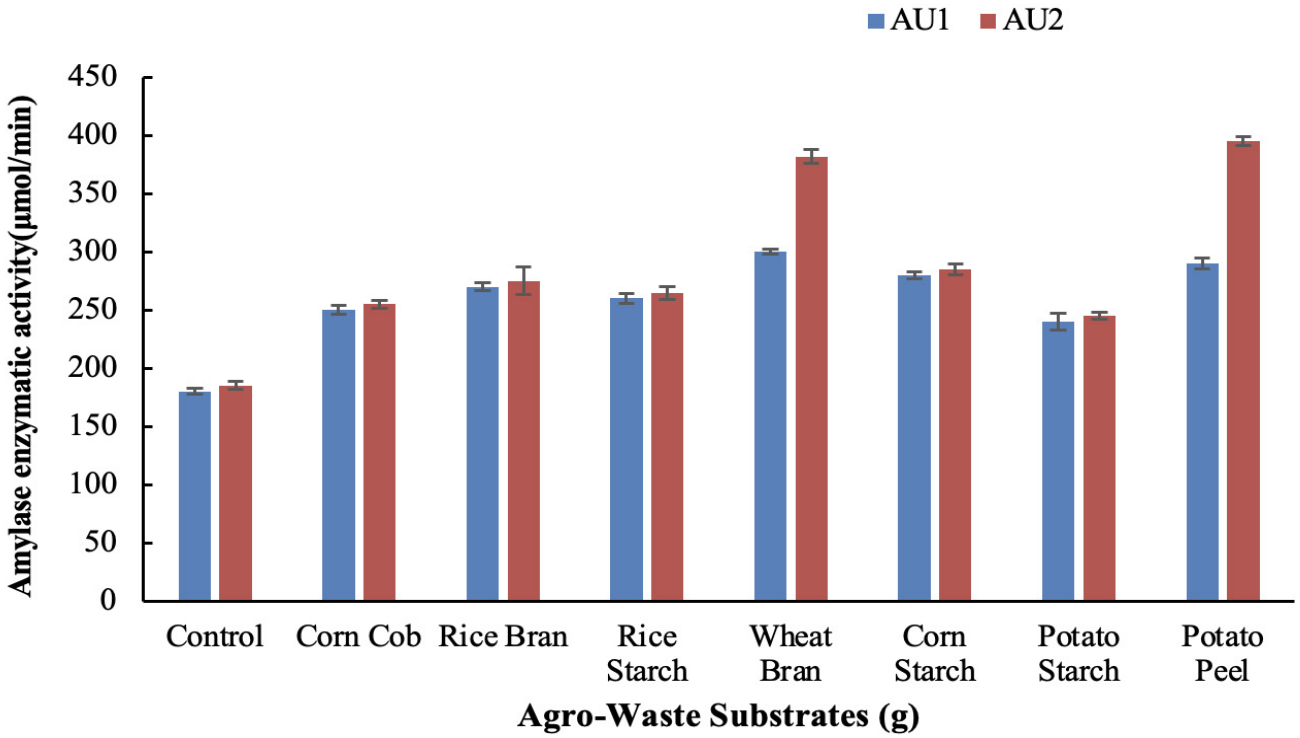

Figure 8: *Amylase activity (µmol/ min.) in bacterial isolates AU1 and AU2 using different agro-waste substrates.*

Impact of the alternative substrates on amylase activity

The production medium was supplemented with different agro-waste materials (*i.e.*, corn cob, rice bran, rice starch, and wheat bran), corn starch, potato starch, and potato peel, as alternatives to conventional starch in the fermentation medium (Figure 8). Strain AU1 achieved its highest enzyme activity with wheat bran recording 381 µmol/min, while AU2 reached its peak activity with potato peel (395 µmol/ min). The results underscore the potential of using agro-waste materials as affordable and eco-friendly substitutes for traditional starch. Notably, wheat bran and potato peel have been identified as the most effective substrates for enhancing α-amylase production in both selected AU1 and AU2 strains, respectively. Statistical analysis results (starch versus agro-wastes) indicated a significant difference (p= 0.006) in amylase production between the two groups, with agro-waste yielding higher enzyme activity compared to starch.

Discussion

Organic matter-rich soil creates an ideal habitat for microorganisms that are sources of microbial enzymes, capable of breaking down complex substrates. These microbial strains have attracted much attention due to maximum amylase production for industrial use. In this study, the thermal stability of amylase was found to be effective up to 38 °C, after which further increase in temperature decreased the enzymatic activity attributable to a catabolic response. This finding aligns with Zaghloul et al. (2021), who reported that *Bacillus amyloliquefaciens* achieved peak amylase production at 37 °C. Suribabu *et al.* (2014) observed a similar trend with *Brevibacillus borstelensis*. However, Shaukat (2021) found that among amylase-producing bacterial isolates, *Bacillus* sp. *G4* exhibited maximum growth at 30 °C, while *Bacillus subtilis* G3 showed optimal growth and enzyme production at 45 °C. Similarly, pH 7 favored maximum amylase production. Our study's findings further align with recent studies on soil-based bacteria. In accordance, Suribabu *et al.* (2014) noted that most starch-degrading bacteria display average growth and enzyme activity at pH 6-7 for *Bacillus sp. MB6* and *Brevibacillus borstelensis*. Additionally, *Bacillus megaterium* RAS103 has shown optimal activity at pH 8 (Rasmey, 2018), while Shaukat (2021) reported peak amylase production by *Bacillus subtilis* G3 at pH 9. Currently, an incubation period of 72 h displayed maximum amylase activity. A previous study conducted by Islam *et al.* (2017) on *Bacillus amyloliquefaciens* and *Bacillus subtilis* (*Bgt5*) in shake culture revealed that enzyme activity increased with fermentation duration, reaching its peak at 48 h of incubation, with no further increase thereafter. Nanganuru (2012) observed comparable findings, noting that *Bacillus subtilis* exhibited maximum amylase production after 72 h of growth, followed

by a decrease. Extended incubation periods resulted in reduced α-amylase production, potentially due to nutrients exhaustion, accumulation of toxic byproducts, α-amylase proteolysis, and other environmental factors related to microorganisms (Abo-Kamer *et al*., 2023). Several studies have shown that supplementing fermentation media with inorganic nitrogen sources may lead to decreased enzyme production, in consistence with this study. Whereas supplementation of organic nitrogen source, mainly yeast extract, expressed maximum amylase production (Gupta et al., 2003). Furthermore, the effect of inorganic nitrogen sources on amylase production corresponds with previous findings observed from studies conducted on *Aspergillus oryzae* (Pedersen and Nielsen, 2000), *Bacillus altitudinis* (Kumar *et al*., 2014), *Bacillus* spp. (Khusro *et al*., 2017), *Bacillus subtilis* (Demirkan, 2011), and *Streptomycetes* spp. (Sobhy *et al*., 2023). However, other studies have reported different results with respect to the organic nitrogen sources. For instance, Sharma *et al*. (2021) found that *Bacillus amyloliquefaciens* produced the most amylase on using tryptophan as an organic nitrogen source. Similarly, Dike *et al*. (2022) noted that *Bacillus circulan* achieved peak amylase production with malt extract. Different agro-wastes are alternative substrates for effective amylase production. Mostafa et al. (2024) reported that potato peel was the most suitable substrate for *Bacillus* spp. NRC1 and *Bacillus subtilis* K-18, respectively. In contrast, Islam et al. (2017) found wheat bran was the best substrate for maximum enzyme production by *Bacillus subtilis*. Our soil-derived bacterial strains exhibited the highest enzymatic activity with wheat bran, which is consistent with previous studies conducted by Ashraf et al. (2003) and Almanaa et al. (2020). These diverse findings suggest that α-amylas production and substrate preferences may vary among different bacterial strains (Almanaa et al., 2020).

Conclusions and Recommendations

The current study conclusively identified the bacterial strains AU1 (*Brevibacillus reuszeri*) and AU2 (*Brevibacillus brevis*) as highly effective producers of α-amylase, with optimal production conditions of; neutral pH, 72 h of incubation, and utilization of yeast extract as an organic nitrogen source. Notably, utilization of different agro-wastes as substrates not only enhanced enzyme yield but also established a cost-effective and environmentally sustainable approach for industrial enzyme production. Additionally, the α-amylase exhibited remarkable thermal stability up to 38 °C, reinforcing its potential for diverse biotechnological applications, including food processing, biofuel production, and pharmaceutical industries. These findings highlight the significance of agro-wastes valorization in enzyme biotechnology and encourage further research into large scale and eco-friendly bioprocessing strategies. Further studies on α-amylase production should be conducted on a range of other agro-wastes, since different substrates could influence the rate of enzyme production. Similarly, pretreatment strategies aiming to enhance substrate digestibility should also be considered, as these may result in increased amount of enzymes. Since genetic engineering can enhance thermal stability and substrate usage, thus amylase production by *Brevibacillus* species is highly recommended for further studies.

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Novelty Statement

This study initiative serves as a pioneering endeavor in the exploration of wheat bran, rice bran, corn cob, and potato peel as economically viable and sustainable fermentation substrates for the biosynthesis of α-amylase, thereby mitigating dependence on costly starch-based media. By converting agricultural by-products into high-value biotechnological assets, this investigation offers a financially feasible alternative while simultaneously addressing the challenges associated with agro-wastes management. Moreover, this study advances the field of green biotechnology by amalgamating wastes valorization with industrial enzyme production, thereby establishing a pathway for environmentally sustainable and economically viable bioprocessing methodologies.

Author's Contribution

FS: Conceptualization, formal analysis and writing original draft.

SSA and HB: Writing original draft and writing-review and editing.
AA: Software and writing-review and editing.

Ethical approval
Not applicable to this paper.

Conflict of interests
The author have declared no conflicts of interest.

References

Abo-Kamer, A.M., Abd-El-salam, I.S., Mostafa, F.A., Mustafa, A.E.R.A. and Al-Madboly, L.A., 2023. A promising microbial α-amylase production, and purification from *Bacillus cereus* and its assessment as antibiofilm agent against *Pseudomonas aeruginosa* pathogen. Microb. Cell Fact., 22(1): 141. https://doi.org/10.1186/s12934-023-02139-6

Almanaa, T.N., Vijayaraghavan, P., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M. and Alyahya, S.A., 2020. Solid state fermentation of amylase production from *Bacillus subtilis* D19 using agro-residues. J. King Saud Uni.-Sc., 32(2): 1555-1561. https://doi.org/10.1016/j.jksus.2019.12.011

Ashraf, H., Iqbal, J. and Qadeer, M.A., 2003. Production of alpha amylase by *Bacillus licheniformis* using an economical medium. Biores. Technol., 87(1): 57-61. https://doi.org/10.1016/S0960-8524(02)00198-0

Bertoft, E., 2018. Analyzing starch molecular structure. Starch in food. Woodhead Publishing. pp. 97-149. https://doi.org/10.1016/B978-0-08-100868-3.00002-0

de Castro, A.M., dos Santos, A.F., Kachrimanidou, V., Koutinas, A.A. and Freire, D.M., 2018. Solid-state fermentation for the production of proteases and amylases and their application in nutrient medium production. Curr. Dev. Biotechnol. Bioeng. Elsevier, pp. 185-210. https://doi.org/10.1016/B978-0-444-63990-5.00010-4

Demirkan, E., 2011. Production, purification, and characterization of alpha-amylase by *Bacillus subtilis* and its mutant derivates. Turk. J. Biol., 35(6): 705-712. https://doi.org/10.3906/biy-1009-113

Dike, P.E.; Mba, E.U. and Ezekiel-Hart, H., 2022. Screening, isolation and characterization of amylase producing bacteria and optimization for production of amylase. J. Adv. Microbiol., pp. 27–51. https://doi.org/10.9734/jamb/2022/v22i330444

Far, B.E., Ahmadi, Y., Khosroshahi, A.Y. and Dilmaghani, A., 2020. Microbial alpha-amylase production: Progress, challenges and perspectives. Adv. Pharma. Bull., 10(3): 350. https://doi.org/10.34172/apb.2020.043

Gadhave, A., Wagh, M.S., Kale, P. and Bhakare, S., 2022. Isolation, screening, characterization and maintenance of amylase producing bacteria from soil. Int. J. Res. Appl. Sci. Eng. Technol., 10(8). https://doi.org/10.22214/ijraset.2022.46121

Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K.M., Sukumaran, R.K. and Pandey, A., 2008. Response surface methodology for the optimization of alpha amylase production by *Bacillus amyloliquefaciens*. Bioresour. Technol., 99(11): 4597-4602. https://doi.org/10.1016/j.biortech.2007.07.028

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K. and Chauhan, B., 2003. Microbial α-amylases: a biotechnological perspective. Process Biochem., 38(11): 1599-1616. https://doi.org/10.1016/S0032-9592(03)00053-0

Islam, M.R., Mondol, O.K., Rahman, M.S., Billah, M.M., Rahman, M.S. and Zohora, U.S., 2017. Screening of α-amylase producing bacteria from tannery wastes of Hazaribag, Bangladesh. Jahangirnagar Univ. J. Biol. Sci., 5(2): 1–10. https://doi.org/10.3329/jujbs.v5i2.32511

Kanimozhi, M., Johny, M., Gayathri, N. and Subashkumar, R., 2014. Optimization and production of α-amylase from halophilic *Bacillus* species isolated from Mangrove soil sources. Appl. Environ. Microbiol. J., 2(3): 70-73.

Khusro, A., Barathikannan, K., Aarti, C. and Agastian, P., 2017. Optimization of thermo-alkali stable amylase production and biomass yield from *Bacillus* sp. under submerged cultivation. Fermentation, 3(1): 7. https://doi.org/10.3390/fermentation3010007

Kumar, V. and Singh, S., 2018. Agro-industrial wastes as substrates for enzyme production: A review. J. Environ. Chem. Engineer., 6(4): 4955–4965. https://doi.org/10.1016/j.jece.2018.07.035

Kumar, Y., Singh, P.K., Singh, A.K., Masih, H., Peter, K., Benjamin, J.C. and Rath, S., 2014.

Production optimization of alpha amylase from *Bacillus altitudinis*. Int. J. Sci. Eng. Technol. Res., 3(4): 654-673.

Li, H., Chi, Z., Wang, X. and Ma, C., 2007. Amylase production by the marine yeast *Aureobasidium pullulans* N13d. J. Ocean Univ. China, 6: 60-65. https://doi.org/10.1007/s11802-007-0060-3

Liu, J.H., Guo, J.N., Lu, H. and Lin, J., 2022. Activity based screening of soil samples from Nyingchi, Tibet, for amylase- producing bacteria and other multifunctional enzyme capacities. Int. J. Microbiol., 2022(1): 2401766. https://doi.org/10.1155/2022/2401766

Luang-In, V., Yotchaisarn, M., Saengha, W., Udomwong, P., Deeseenthum, S. and Maneewan, K., 2019. Isolation and identification of amylase-producing bacteria from soil in Nasinuan Community Forest, Maha Sarakham, Thailand. Biomed. Pharmacol. J., 12(3): 1061-1068. https://doi.org/10.13005/bpj/1735

Mohammad, B.T., Al-Daghistani, H.I., Jaouani, A., Abdel-Latif, S. and Kennes, C., 2017. Isolation and characterization of thermophilic bacteria from Jordanian hot springs: *Bacillus licheniformis* and *Thermomonas hydrothermalis* isolates as potential producers of thermostable enzymes. Int. J. Microbiol., 2017(1): 6943952. https://doi.org/10.1155/2017/6943952

Mostafa, F.A., Wehaidy, H.R., El-Hennawi, H.M., Mahmoud, S.A., Sharaf, S. and Saleh, S.A.A., 2024. Statistical optimization of α-amylase production from novel local isolated *Bacillus* spp. NRC1 and its textile applications. Catalysis Letters. 154(7): 3264-3275. https://doi.org/10.1007/s10562-023-04545-2

Naik, B., Kumar, V., Rizwanuddin, S., Chauhan, M., Gupta, A.K., Rustagi, S., Kumar, V. and Gupta, S. 2023. Agro-industrial waste: A cost-effective and eco-friendly substrate to produce amylase. Food Prod. Proc. Nutr., 5(1): 30. https://doi.org/10.1186/s43014-023-00143-2

Nanganuru, H., 2012. Studies on the production of α- amylase by *Bacillus subtilis*. IOSR J. Eng., 2(5): 1053-1055. https://doi.org/10.9790/3021-020510531055

Nigam, P. and Singh, D., 1995. Enzyme and microbial systems involved in starch processing. Enz. Microb. Technol., 17(9): 770-778. https://doi.org/10.1016/0141-0229(94)00003-A

Pedersen, H. and Nielsen, J., 2000. The influence of nitrogen sources on the α-amylase productivity of *Aspergillus oryzae* in continuous cultures. Appl. Microbiol. Biotechnol., 53: 278-281. https://doi.org/10.1007/s002530050021

Prakash, O. and Jaiswal, N., 2010. α-Amylase: An ideal representative of thermostable enzymes. Appl. Biochem. Biotechnol., 160: 2401-2414. https://doi.org/10.1007/s12010-009-8735-4

Rasmey, A.H., 2018. Kinetic properties of α-amylase produced by *Bacillus megaterium* RAS103 under optimum conditions in submerged fermentation. Egypt. J. Microbiol., 53(1): 219-230. https://doi.org/10.21608/ejm.2018.5628.1075

Salim, A.A., Grbavčić, S., Šekuljica, N., Stefanović, A., Tanasković, S.J., Luković, N., and Knežević-Jugović, Z. 2017. Production of enzymes by a newly isolated *Bacillus* sp. TMF-1 in solid state fermentation on agricultural by-products: The evaluation of substrate pretreatment methods. Bioresour. Technol., 228: 193-200. https://doi.org/10.1016/j.biortech.2016.12.081

Sharma, P. and Chapadgaonkar, S., 2021. Optimization of a-amylase production from *Bacillus Amyloliquefaciens* using Taguchi method. Biosci. Biotechnol. Res. Asia, 18(2): 337–345. https://doi.org/10.13005/bbra/2920

Shaukat, M., 2021. Determination and optimization of crude extracellular amylase extracted from different *Bacillus* species. Pak. J. Sci., 73(1). https://doi.org/10.57041/pjs.v73i1.633

Sidhu, G.S., Sharma, P., Chakrabarti, T. and Gupta, J.K., 1997. Strain improvement for the production of a thermostable α-amylase. Enz. Microb. Technol., 21(7): 525-530. https://doi.org/10.1016/S0141-0229(97)00055-0

Singhania, R.R., Patel, A.K., Soccol, C.R. and Pandey, A., 2009. Recent advances in solid-state fermentation. Biochem. Engineer. J., 44(1): 13–18. https://doi.org/10.1016/j.bej.2008.10.018

Sobhy, H., El-Awady, M., El-Khonezy, M., Hassan, M. and Salam, A.S., 2023. Screening and optimization of the production of alpha-amylase enzyme from *Streptomycetes*. Egypt. Acad. J. Biol. Sci. G. Microbiol., 15(1): 169–178. https://doi.org/10.21608/eajbsg.2023.303300

Suribabu, K., Govardhan, T.L. and Hemalatha, K., 2014. Optimization of physical parameters of α-amylase producing *Brevibacillus borostelensis* R1 in submerged fermentation. Int. J. Res. Eng. Technol., 3(1): 517–525. https://doi.org/10.15623/ijret.2014.0301087

Visvanathan, R., Qader, M., Jayathilake, C., Jayawardana, B.C., Liyanage, R. and Sivakanesan, R., 2020. Critical review on conventional spectroscopic α- amylase activity detection methods: merits, demerits, and future prospects. J. Sci. Food Agric., 100(7): 2836–2847. https://doi.org/10.1002/jsfa.10315

Wang, Q., Cen, Z. and Zhao, J., 2015. The survival mechanisms of thermophiles at high temperatures: An angle of omics. Physiology, 30(2): 97-106. https://doi.org/10.1152/physiol.00066.2013

Zaghloul, W.R., Foda, F.F.A., Saad, S.M.M. and Elmeihy, R.M., 2021. Production and evaluation of alpha-amylase produced from *Bacillus amyloliquefaciens*. Biochem. Microbiol., pp. 361–372.