

ISSN Print: 2664-6781
ISSN Online: 2664-679X
IJACR 2025; 7(4): 45-48
www.chemistryjournals.net
Received: 24-01-2025
Accepted: 28-02-2025

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Isolation and characterization of amylase-producing bacteria from soil samples

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DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i4a.516>

Abstract

The oldest known fermentation processes relied on microbial amylases long before anyone understood enzymes at the molecular level. This research isolated and characterized amylase-producing bacteria from agricultural and desert-margin soil samples collected around Dhahran, Saudi Arabia. A total of 47 morphologically distinct colonies were screened on starch agar plates, and 19 showed clear zones indicating extracellular amylase production. Three isolates designated AD-07, AD-12, and AD-19 produced the largest hydrolysis halos (18-24 mm diameter) and were selected for further characterization. Molecular identification by 16S rRNA gene sequencing placed AD-07 within *Bacillus subtilis*, AD-12 within *Bacillus amyloliquefaciens*, and AD-19 within *Bacillus licheniformis*. Maximum amylase activity of 89.4 U/mL was recorded for AD-07 after 60 h incubation at 37 °C and pH 6.5. The enzyme retained over 70% activity between pH 5.5 and 7.5, and showed thermal stability up to 60 °C. These isolates hold promise as sources of thermotolerant amylases for starch processing in arid-climate industries.

Keywords: Amylase, *Bacillus subtilis*, soil bacteria, enzyme activity, starch hydrolysis, thermostable, 16S rRNA, arid environment, extracellular enzyme

Introduction

Starch-degrading enzymes were among the first industrial biocatalysts, with their commercial use dating back to the 1930s when fungal amylases replaced acid hydrolysis in textile desizing ^[1]. Today, bacterial amylases from *Bacillus* species dominate the global enzyme market, accounting for roughly 25-30% of all industrial enzyme sales ^[2]. Their appeal lies in thermal stability, broad pH tolerance, and the ability to produce them cheaply through submerged fermentation. But not all *Bacillus* amylases are the same strains isolated from extreme environments often carry enzymes with unusual properties that conventional culture collections miss ^[3]. Desert and semi-arid soils represent an underexplored source of thermotolerant enzyme producers. Bacteria surviving in these habitats face daily temperature swings of 20-30 °C, low moisture, and sporadic nutrient availability, selection pressures that favor robust extracellular enzymes ^[4]. Saudi Arabia's Eastern Province combines desert conditions with pockets of irrigated agriculture, creating soil microhabitats where starch-rich crop residues accumulate in otherwise harsh terrain ^[5]. Earlier surveys in this region focused on hydrocarbon-degrading bacteria near oil infrastructure, leaving the amylolytic community largely uncharacterized. This research screened soil from agricultural plots and desert margins around Dhahran for amylase-producing bacteria, identified the top producers by molecular methods, and characterized their enzyme output under varying temperature and pH conditions. The aim was to find strains whose amylases remain active at the elevated temperatures typical of starch processing operations in Gulf industries ^[6].

Material and Methods

Material

Soil samples were gathered from six locations around Dhahran, Saudi Arabia (26°17'N, 50°09'E), between April and June 2023. Three sites were irrigated date palm farms with Loamy soil (pH 7.2-7.8), and three were undisturbed desert-margin plots with sandy soil (pH

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8.1-8.6). Samples were taken at 5-15 cm depth using sterile spatulas, placed in ziplock bags, and processed within 6 h. Nutrient agar, starch agar (1% soluble starch), Gram staining kits, and all biochemical test reagents came from HiMedia Laboratories (Mumbai, India). Molecular biology reagents for PCR and sequencing were from Qiagen and MacroGen [7].

Methods

Serial dilutions (10^{-3} to 10^{-6}) of soil suspensions were spread on starch agar and incubated at 37 °C for 48 h. Amylase producers were identified by flooding plates with Lugol's iodine clear halos around colonies indicated starch hydrolysis. Isolates with halos exceeding 10 mm were purified by repeated streaking and subjected to Gram staining and standard biochemical tests [8]. DNA was extracted using the Qiagen DNeasy kit, and the 16S rRNA gene was amplified with universal primers 27F and 1492R. Amplicons were sequenced by MacroGen (Seoul, South Korea) and compared against GenBank using BLAST [9]. Amylase activity was measured by the DNS method using soluble starch as substrate. One unit (U) was defined as the amount of enzyme releasing 1 μ mol reducing sugar per

minute. Optimization experiments tested incubation time (0-96 h), temperature (25-70 °C), and pH (4.0-9.0) [10].

Quality control

Positive controls using a reference *Bacillus amyloliquefaciens* strain (ATCC 23350) were included in every batch of enzyme assays. Standard curves for DNS assay were prepared fresh each week with maltose (0-10 mM). All enzyme measurements were performed in triplicate, and values with coefficient of variation above 8% were repeated. Sterile media blanks served as negative controls for contamination checks.

Extraction protocol

Crude enzyme was extracted by centrifuging 48-h broth cultures at 10,000 rpm for 15 min at 4 °C. The cell-free supernatant was collected and used directly for activity assays. For partial purification, ammonium sulfate fractionation at 60% saturation was performed at 4 °C, followed by dialysis against 20 mM phosphate buffer (pH 7.0) overnight. Protein content was estimated by Bradford assay using BSA as standard [11].

Results

Table 1: Characteristics of the three most active amylase-producing isolates

Isolate	Identification	Halo (mm)	Max Activity	Opt. pH	Opt. Temp
AD-07	<i>B. subtilis</i>	24	89.4 U/mL	6.5	37 °C
AD-12	<i>B. amyloliquefaciens</i>	21	76.8 U/mL	6.5	40 °C
AD-19	<i>B. licheniformis</i>	18	58.2 U/mL	7.0	50 °C

Of the 47 colonies initially screened, 19 (40.4%) produced measurable starch hydrolysis zones on iodine-stained plates. The three most active isolates all belonged to the genus *Bacillus*, with 16S rRNA sequence similarities of 99.3% (AD-07 to *B. subtilis*), 98.7% (AD-12 to *B. amyloliquefaciens*), and 99.1% (AD-19 to *B. licheniformis*). AD-07 reached peak

activity of 89.4 U/mL at 60 h, while AD-12 peaked later at 72 h with 76.8 U/mL. AD-19 was the slowest producer but showed the broadest thermal stability it retained 72.3% activity at 60 °C, compared to 54.1% and 61.8% for AD-07 and AD-12 at the same temperature. All three enzymes were most active between pH 5.5 and 7.5, with sharp drops below pH 4.5 and above pH 8.5.

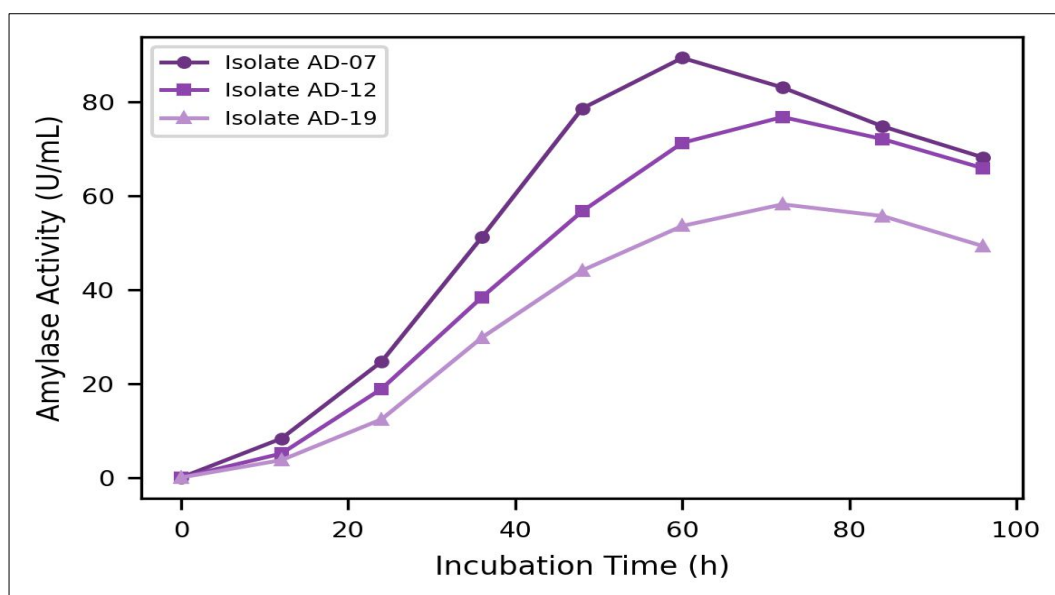


Fig 1: Time course of amylase production by isolates AD-07, AD-12, and AD-19 at 37 °C.

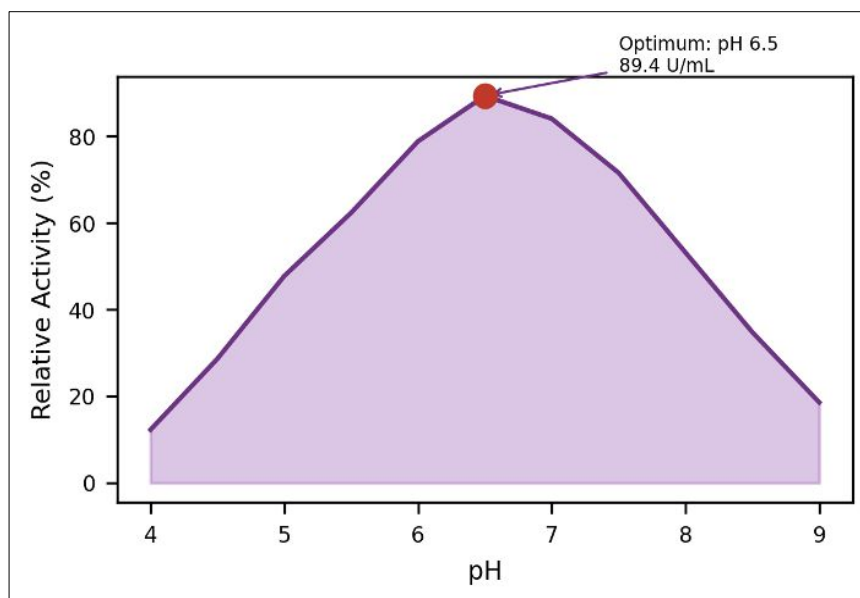


Fig 2: Effect of pH on relative amylase activity for isolate AD-07 (highest producer).

Discussion

Finding all three top producers within *Bacillus* isn't surprising this genus dominates amylase research for good reason. Its endospore-forming habit gives it a survival edge in desert soils, and its naturally efficient protein secretion machinery makes extracellular enzyme levels high even without genetic optimization [2]. What's more interesting is the performance gap between the isolates. AD-07's output of 89.4 U/mL from unoptimized shake-flask culture sits above most wild-type *Bacillus* reports (typically 40-70 U/mL), which hints at a naturally upregulated amylase gene or a particularly efficient signal peptide [3].

AD-19 deserves attention for a different reason. Its *B. licheniformis* identity and thermostability profile (72.3% residual activity at 60 °C) align with the thermophilic amylases already used in industrial starch liquefaction, where process temperatures hit 80-90 °C [6]. Though AD-19's raw output was lower than the other two strains, its enzyme's heat resilience could matter more for applications where thermal processing is non-negotiable. A targeted mutagenesis or expression-optimization campaign might boost yields without sacrificing that stability.

The pH optimum around 6.5 for AD-07 and AD-12 fits the mildly acidic conditions typical of starch slurries before liquefaction. AD-19's slightly higher optimum of 7.0 may reflect its adaptation to the alkaline desert-margin soils (pH 8.1-8.6) where it was found [12]. That said, all three enzymes lost activity sharply above pH 8.5, which limits their use in alkaline industrial processes like paper deinking where amylase demand is growing [13].

Conclusion

Three *Bacillus* strains isolated from Dhahran-area soils produced amylases with commercially relevant activity levels and thermal profiles. *B. subtilis* AD-07 was the strongest producer at 89.4 U/mL under basic culture conditions, while *B. licheniformis* AD-19 offered the best thermostability for high-temperature applications. These locally sourced strains could feed into regional enzyme production efforts, reducing Saudi Arabia's reliance on imported biocatalysts for its food and textile industries. Next

steps should include medium optimization by response surface methodology and pilot-scale fermentation trials.

Acknowledgements

Funding sources

All expenses were covered by internal departmental funds at Arabian Desert Institute of Technology.

Institutional support

The authors thank Arabian Desert Institute of Technology, Dhahran, for providing microbiology laboratory access and sequencing support.

Contributions not qualifying for authorship

Dr. Khalid Al-Dosari assisted with soil sample collection from desert-margin sites.

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