International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 7, 2025
https://www.theaspd.com/ijes.php

Promising Bioactive Streptomyces Sp. Strains E2S7, A6B9 And C2K4 For Amylase Production Isolated From Arid And Semi-Arid Zones Of Algeria

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Abstract
This study aimed to isolate and characterize actinobacteria capable of producing amylase enzymes from soils in arid and semi-arid zones of Algeria, including the Oasis de Djanet, Tassili de Djanet, and Forêt d'Elouricia. From 42 isolates screened on starch agar, three strains–E2S7, A6B9, and C2K4–demonstrated strong amylolytic activity and were identified by 16S rRNA sequencing as Streptomyces hypolithicus, S. indoligenes, and S. candidus, respectively. Amylase production was induced with 1% starch at 37°C. The highest enzymatic activities were recorded at 8.36, 8.90, and 9.32 U/mL under optimal conditions of 30°C and pH 7.0. The enzyme activity showed a pH optimum of 8.0 and a temperature optimum of 45°C. These strains exhibit significant potential for industrial applications, particularly in biotechnology and agriculture.
Keywords: *Streptomyces, amylase, microbial enzymes, arid soils*

1. INTRODUCTION

Arid and semi-arid soil environments are rich reservoirs of microbial biodiversity with considerable potential for biotechnology. These ecosystems, often characterized by extreme conditions such as elevated temperatures and alkaline pH, harbor microorganisms capable of producing robust enzymes suitable for industrial use. Among these enzymes, amylases play a critical role in the hydrolysis of starch into simple sugars. They are extensively used in the food, textile, detergent, and pharmaceutical industries (Ali et al., 2024). Amylases account for 25–33% of the global enzyme market (Ashok et al., 2023), and microbial sources are preferred due to their rapid growth, ease of handling, and cost-effective enzyme production (Ahmad et al., 2020).

Arid and semi-arid ecosystems are characterized by extreme environmental conditions, including high temperatures, low water availability, and nutrient limitations. Despite these challenges, these environments harbore a wide diversity of microorganisms, particularly actinobacteria, which have adapted to thrive under stress. These bacteria are increasingly recognized for their ability to produce stable and potent enzymes with industrial relevance.

Actinobacteria are Gram-positive, filamentous bacteria known for their robust metabolism and secondary metabolite production. In arid ecosystems, actinobacteria have evolved enzymatic systems that remain active under high salinity, alkalinity, or thermal stress—making them particularly attractive for biotechnological applications (Alsharif et al., 2020; Gorrab et al., 2024). Among the enzymes produced are amylases, proteases, cellulases, xylanases, lipases and amylases, many of which function optimally at high temperatures or alkaline pH (Hamdi et al., 2023; El-Sayed et al., 2024, Gorrab et al., 2024; Silaban et al., 2020).

Within this phylum, the genus *Streptomyces* is especially valued due to its prolific enzyme production and biotechnological relevance (Hamdi et al., 2023; Choudhary and Nayak, 2023). This genus has been widely isolated from desert soils, saline areas, and mountainous dry zones. Several Streptomyces species isolated from Algerian, Tunisian, and Middle Eastern deserts have demonstrated remarkable amylolytic and proteolytic activity (Hamdi et al., 2023; Salwan and Sharma, 2018). These strains often produce enzymes with properties that outperform those from mesophilic organisms, particularly in industries that require

thermostable and alkali-tolerant biocatalysts. Several studies have shown that actinobacteria from desert regions can hydrolyze complex substrates such as starch, cellulose, and keratin, even under harsh conditions. For example, thermophilic actinobacteria isolated from compost and saline deserts have produced α-amylases active at 50–70°C and pH 8–10, confirming their value in detergent and bioethanol industries (Syafitri et al., 2019; Prakash et al., 2009). In a global term, actinobacteria from arid and semi-arid regions are a valuable and underexplored resource for novel enzymes with desirable industrial traits. Their adaptation to extreme environments translates into enzymes that are often more stable, active, and resistant to denaturation than their mesophilic counterparts, making them key candidates for future enzyme development and biotechnological innovation.

Despite their promise, few studies have focused on Streptomyces strains isolated from Algerian soils, especially those in extreme environments like Djanet and El Ouricia. These underexplored habitats could yield new strains with enhanced enzymatic profiles. Furthermore, rare actinobacteria from such settings are a rich source of novel bioactive metabolites (Smati et al., 2025).

This study aimed to isolate amylase-producing actinobacteria from arid and semi-arid soils in Algeria, characterize their enzyme production under varying conditions, and identify them through 16S rRNA sequencing. The goal was to evaluate their potential for industrial amylase applications.

2. MATERIALS AND METHODS

2.1. Soil Sampling and processing

Soil samples were collected from three distinct environments in Algeria as described by Pochon and Tardieux, (1962), the Djanet Oasis, the Tassili of Djanet, and the El Ouricia Forest. Soil samples from Djanet oasis were taken around palm trees in the eastern region of Djanet, which has a daily temperature of 48°C and a humidity of 26%. In addition, soil samples from Tassili djanet were taken from western regions of Djanet. It has a temperature of 25°C and 40% of humidity. Furthermore, Elouricia forest soil samples were taken around the forests of the Sétif at el ouricia region (27°C, with a humidity of 45%). Samples were randomly taken from a depth of 15 cm below the surface, transferred to sterile containers, and stored at 4°C until analysis.

2.2. Isolation and Selection of Microorganisms

One gram of each soil sample was added to 9 mL of sterile physiological saline (0.9% NaCl), homogenized by vortexing at 200 rpm for 5 minutes, and used as a stock solution. Serial dilutions (10^{-1} to 10^{-6}) were prepared, and 0.1 mL of each dilution was spread onto starch containing agar plates (10 g soluble starch, 1 g casein, 0.5 g K_2HPO_4, 20 g agar in 1 L distilled water; pH 7.0–7.5). The plates were incubated at 30°C for 7–14 days. Distinct colonies were isolated and purified for further screening.

2.3. Screening for Amylase-Producing Actinobacteria

Purified isolates were cultured on starch-yeast extract agar supplemented with 0.3% soluble starch and incubated at 30°C for 5 days. Amylase activity was detected using Lugol's iodine solution. A clear halo around the colony indicated starch degradation (Fadhil et al.,2020). The amylolytic index (AI) was calculated according to the formula (1)

$$AI=CLZD - CD /CD \quad (1)$$

Where, CLZD: Clear Zone Diameter (mm), and CD: Colony Diameter (mm)

2.4. Molecular Identification via 16S rRNA Sequencing

Genomic DNA was extracted and amplified using universal primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCC-3′). In a 25 µL PCR mixture, the components included 0.5 ng of genomic DNA, a 2X Master Mix (One PCR) containing 100 mM Tris-HCl (pH 9.1), 0.1% TritonTMX-100, 200 mM dNTP, 1.5 mM MgCl2, 0.005 U Taq DNA Polymerase, and 0.2 µM of both forward and reverse primers, with the volume adjusted using nuclease-free water. The PCR thermocycler (Thermo Scientific Hybaid Px2) was set up with the following program: an initial denaturation at 94 °C for 2 minutes for 1 cycle ; 32 cycles of denaturation at 94 °C for 45 seconds, annealing at 54 °C for 45 seconds, and extension at 72 °C for 1 minute ; a final extension at 72 °C for 7 minutes. Samples were maintained at 4 °C until further analysis. The PCR products of 16S rRNAs (~ 1,500 bp) were visualized on a 0.8% agarose gel and purified using the PCR product purification kit, the illustra ExoStar 1-Step (GE Healthcare, Life Sciences, UK Limited). The 16S rRNA gene sequences were then compared with those available in GenBank using the BLAST program (Basic Local Alignment

Search Tools). A phylogenetic tree was constructed using the Muscle method for sequence alignment and the maximum likelihood method with MEGA 12, incorporating 1,000 bootstrap replicates. All 16S rRNA partial sequences of our amylase-producing isolates were deposited on NCBI database (Li et al., 2022).

2.5. Quantitative Colorimetric Amylase Assay

Enzyme production medium

The culture medium for enzyme production comprised (g/l) 5 g Pollard, 5 g soluble starch, 2 g peptone and 2 g yeast extract. One hundred ml of this medium was transferred to a 500 ml conical flask. The flasks were autoclaved at 121°C for 15 minutes. Once cooled, they were inoculated with an overnight culture of actinobacteria. The seeded medium was incubated at 28°C in a shaker incubator for 5 days. At the end of the culture period, the media were centrifuged at 9,000 rpm for 15 minutes to obtain a crude extract, used as an enzyme source. Then, the enzymatic activity of the crude enzyme was verified by amylase assay.

Amylase assay

Amylase activity was assessed by measuring the amount of reducing sugar released from the starch. The amylase assay was performed according to the DNS method (Pinjariand Kotari**,** 2018)**.** One hundred µl of 1% starch solution was added to each tube and incubated for 5 minutes at room temperature to reach thermal equilibrium. An initial addition of 50 µl, 75 µl and 100 µl of amylase solution was made to the respective microtubes. The mixing solution was homogenized by inverting and incubated at room temperature for 5 minutes. The reaction was stopped by adding 100 µl of DNS solution to each tube. The tubes were placed in a heating block and boiled at 100°C for 15 minutes. After boiling, the solutions were cooled on ice for 10 minutes. Once room temperature had been reached, 900 µl of purified water was added to each tube and mixed by inverting. Absorbance was measured at 540 nm using maltose as à standard. One unit (U) of enzyme activity is defined as the amount of enzyme required to release 1 µmol of reducing sugar as glucose per minute under the assay conditions.

2.6. Determination of Optimal pH and Temperature for amylase activity

1% soluble starch solutions were tested at various pH values, ranging from 5 to 10. One milliliter of 1% substrate solution was mixed with 1 mL of the appropriate buffers: 0.05 M citrate buffer (pH 5), 0.05 M sodium phosphate buffer (pH 6 and 7), 0.05 M Tris-HCl (pH 8 and 9) and 0.05 M glycine NaOH (pH 10). One milliliter of crude enzyme was also added to these mixtures. Samples were incubated at 37°C for 30 minutes. Specific amylase activity was measured, and the pH at which maximum activity was observed was noted. In addition, different substrate solutions were prepared by dissolving 1% soluble starch in solutions at pH 7.0. One milliliter of 1% soluble starch was combined with 1 mL of 0.05 M sodium phosphate buffer (pH 7). One milliliter of crude enzyme was also added to these buffers. Samples were incubated at 15, 25, 30, 35, 40, 45, 50 and 55°C for 30 minutes. Specific amylase activity was calculated, and the temperature at which maximum activity was observed was noted. Optimal pH and temperature conditions were used to determine the final specific amylase activity (Li et al., 2018)**.**

2.7. Statistical Analysis

All data are presented as the mean ± SD of triplicate experiments (n = 3). Statistical significance was determined using one-way ANOVA with Design Expert software (v10) (Milanes-Baños., 2024). Significance was accepted at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Isolation and Identification of Amylase-Producing Actinobacteria

A total of 42 actinobacterial isolates were recovered: 11 from the Djanet Oasis, 13 from the Tassili of Djanet, and 18 from the El Ouricia Forest. All isolates were screened on starch agar for amylase activity, and the amylolytic index (AI) was used to evaluate enzyme production. AI values were categorized as follows: Low: $AI < 1$; Moderate: $1 \leq AI \leq 2$; High: $AI > 2$.

As shown in Figure 1, 28% of Djanet Oasis isolates, 20% from Tassili of Djanet, and 11% from El Ouricia Forest displayed high AI values. Three isolates showing the highest activity: E2S7, A6B9, and C2K4 were selected for further study (table 01).

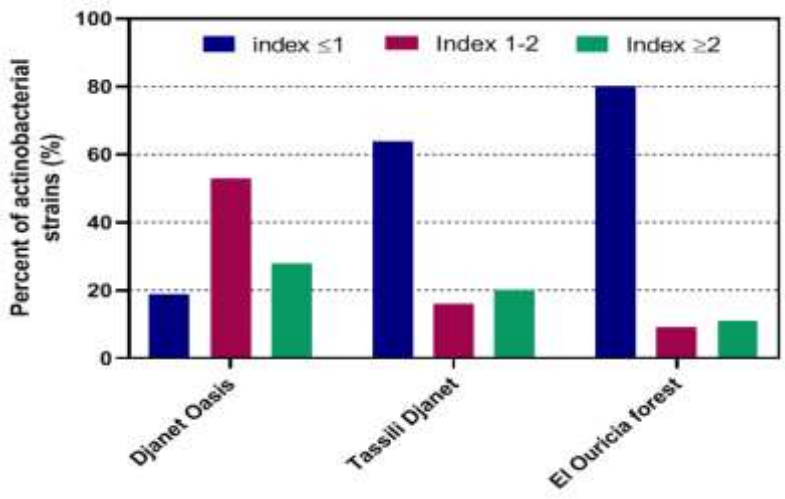


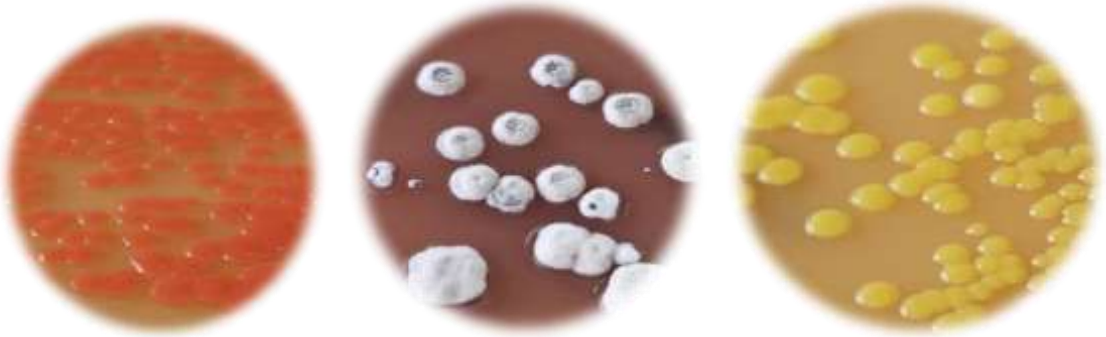
Figure 01. Percentages of amylase activity indices of actinobacterial isolates from different sites (Djanet Oasis, Tassili of Djanet, and El Ouricia Forest).

Table 01. Amylolytic index values and amylolytic activity of selected actinobacterial isolates.

Isolates	Colony Diameter (mm)	Clear zone Diameter (mm)	Index	Amylolytique activity (U/ml^{-1})	Category
E2S7	10.1	39.7	2,93	8,36	High
A6B9	10.2	39.3	2,86	8,90	High
C2K4	10	40.4	3,04	9,32	High

The recorded amylolytic zones were 39 mm or more for the tested Actinobacterial isolates. These results are consistent with the study of Syafitri et al. (2019). Furthermore, they found that the best isolates produced amylolytic activity of 8.36, 8.90, and 9.32 U/ml^{-1}, according to the quantitative DNS assay. This method is based on the degradation of starch into glucose and maltose by amylase. The measurement of amylase activity in cultured Actinobacteria has been reported by several researchers (Zucchi et al., 2011; Raja et al., 2010).

3.2. Phenotypic and genotypic characterization of selected strains



(a) (b) (c)

Figure 02. Morphology of actinobacteries strains : (a) E2S7, (b) A6B9 and (c) C2K4 macroscopic morphology.

Table 02. Morphology characters of strains E2S7, A6B9 and C2K4.

Strains	Colony Forming	Color	Size	Texture	Gram
E2S7	Round	Orange	Large	Mucoid	Positive

A6B9	Irregular	white	Large	Dry	Positive
C2K4	Round	Yellow	Large	Mucoid	Positive

Table 03 : Biochemical characteristics of strains E2S7, A6B9 and C2K4. In the table : +, enzymatic activity présence ; —, enzymatic activity absence. For Caséinase activity, +, low ; ++, medium ; +++, high.

Strains	Oxidase	Amylase	Lacase	Caséinease	Gélatinase	Urease
E2S7	+	+	+	+ + +	+	+
A6B9	+	+	—	+ +	+	+
C2K4	+	+	+	+	+	—

The selected Actinobacterial isolates were subjected to 16S rRNA identification. The phylogenetic analysis grouped the isolates within the *Streptomyces* genus (Figure 2). The results show that the strain A6B9, from the El Ouricia Forest site, was closely related (99.57% similarity) to *Streptomyces hypolithicus*). Also, the strain E2S7 (from the Djanet Oasis site) was related to *Streptomyces indoligenes* with 99.91% of similarity, and the strain C2K4 isolated from the Tassili Djanet site was identified as *Streptomyces candidus* (97.23%). The nucleotide sequences of the three strains where deposited in the Gene Bank database and recorded under the accession numbers PV763888, PV764267, PV764366 respectively (http:// ncbi. nlm. nih. gov). These findings are in agreement with Khadayat et al. (2020), who used similar techniques to identify bioactive *Streptomyces* from soil samples.

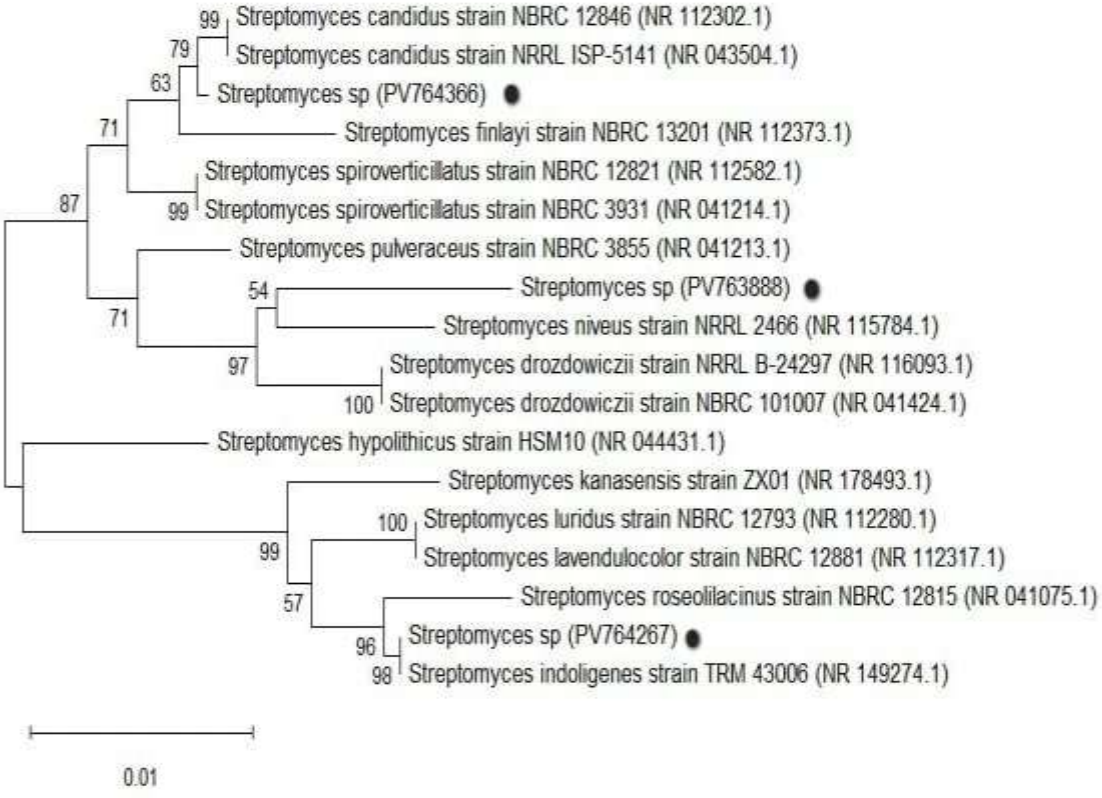


Figure 03. Phylogram derived from 16S rRNA gene sequences of 3 isolates of actinobacteria and their related strain, showing the relationship with type strain in a cluster node of *Streptomyces*. Bootstrap values based on 1000 replications are shown at branch nodes.

3.3. Effect of incubation temperature on amylase activity

The specific amylase activity of all three isolates was evaluated at temperatures ranging from 15°C to 55°C. The results (Figure 4) indicated that the strains E2S7, A6B9, and C2K4 exhibit different behaviors depending on the temperature of incubation. The E2S7 strain reaches a peak amylolytic activity of 12,18 U/ml^{-1} at 50°C, indicating an optimal temperature at this value. The A6B9 strain shows its maximum activity around 45°C, while the C2K4 strain, although it peaks at 50°C, begins to lose activity at 55°C. In summary, E2S7 is optimal at 50°C, while the other strains are optimal around 45°C. This confirms the thermotolerant nature of the isolates. Similar thermal profiles were observed in studies of actinobacterial enzymes for industrial use (Syafitri et al., 2019; El-Sayed et al., 2024).

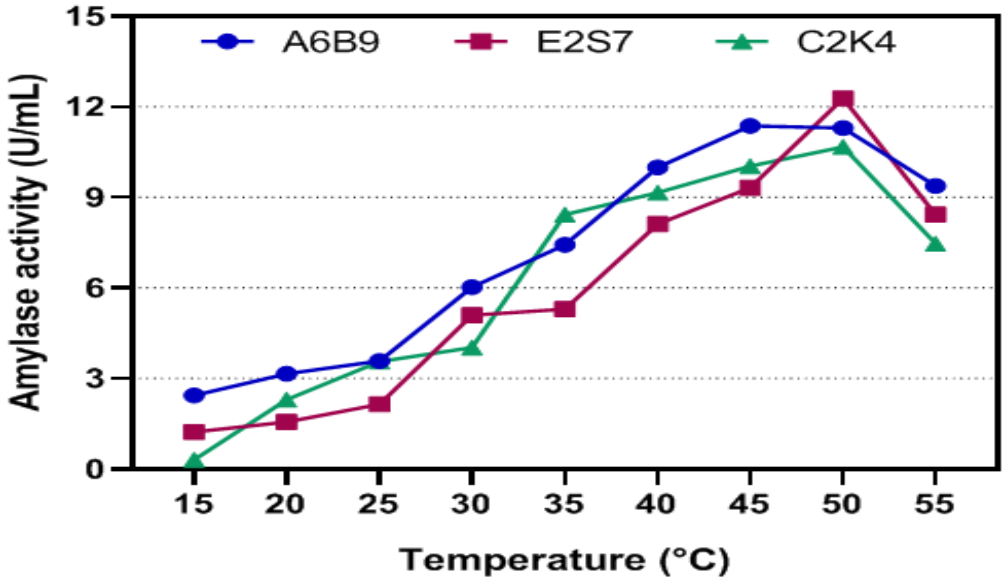


Figure 04. The Influence of Temperature on the Amylolytic Activity of Strains E2S7, A6B9, and C2K4.

Previous studies have shown that amylolytic enzymes produced by Streptomyces strains exhibit a range of optimal temperatures, typically between 40°C and 55°C, with some enzymes maintaining stable activity even at higher temperatures. For instance, a study by Singh et al. (2014) and Hwang et al. (2013) found that the amylase from Streptomyces sp. reached its maximum activity at 50°C, which aligns with the results observed for strain E2S7. This ability to tolerate relatively high temperatures is a significant advantage for industrial applications, such as bioethanol production or starch processing in the food industry. Another study by Kashiwagi et al. (2014) reported that Streptomyces strains produce amylases with optimal temperatures ranging from 45°C to 55°C, further confirming the thermotolerant nature of these enzymes and their potential for use in high-temperature industrial environments. A more recent study by Bukhari et al. (2021) demonstrated that Streptomyces rochei produces a thermostable amylase with optimal activity at 50°C, making it suitable for industrial processes like bioethanol production and starch conversion.

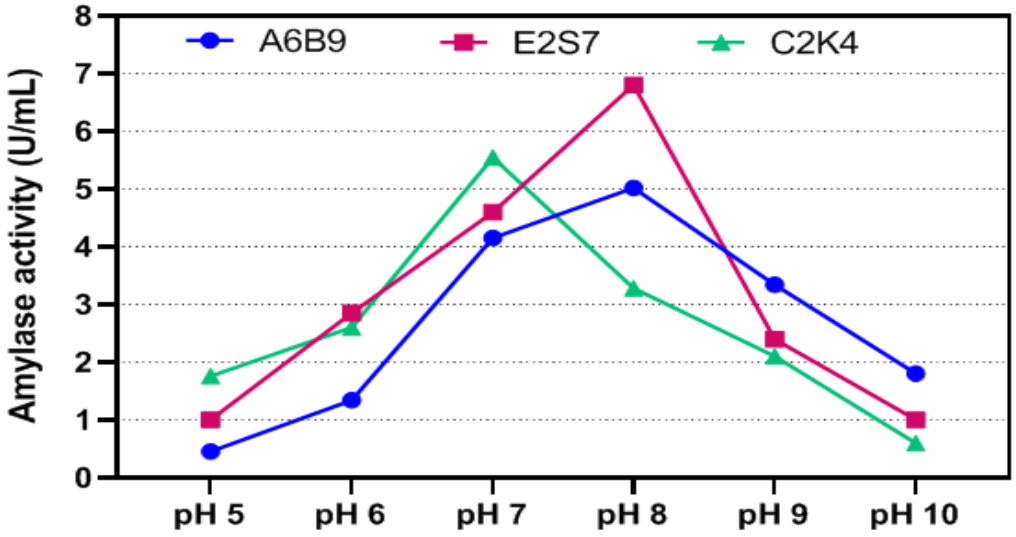
3.4. Effect of pH on Amylase Activity

The amylase activity of all three strains was also assessed across a pH range from 5 to 10 (Figure 5). The three studied strains (E2S7, A6B9, and C2K4) exhibit a similar amylolytic activity profile, with maximum activity observed at pH 8. Strain E2S7 reaches an activity of 6.8 U/ml^{-1} at pH 8 and shows a rapid decline in activity outside this range, indicating a strong preference for slightly alkaline conditions. Strain A6B9 also displays peak activity at pH 8 (5.02 U/ml^{-1}) but demonstrates greater tolerance to slightly acidic conditions, maintaining an activity of 4.60 U/ml^{-1} at pH 7 and showing a significant decrease at pH 10.

As for strain C2K4, it exhibits maximum activity at pH 7 (5.55 U/ml^{-1}), but its activity drops sharply outside this range, with a low activity of 1 U/ml^{-1} at pH 5 and a gradual decrease at pH 9 and 10.

Figure 05. The Influence of pH on the Amylolytic Activity of Strains E2S7, A6B9, and C2K4.

The results indicate the strong alkaliphilic and thermophilic characteristics of the tested actinobacteria, as well as the influence of temperature on amylase enzymatic activity. Among the three isolates, the optimal temperature was found to be 45°C and 50°C. Enzymatic activity was significantly influenced by changes in pH. Therefore, to maximize the efficiency of these enzymes, it would be ideal to use them under conditions close to a pH range of 7.7 to 8 for all three isolates. Actinobacteria are considered prokaryotes of particular interest for the production of a wide variety of enzymes (Tischler et al., 2019). These enzymes play a crucial role as metabolic catalysts in numerous biochemical activities (Salwan & Sharma, 2018; Prakash et al., 2009). Furthermore, another study on screening thermophilic actinobacterial isolates,



obtained by culture methods, showed that these isolates are capable of synthesizing amylases at temperatures ranging from 45°C to 60°C (Syafitri et al., 2019 ;El-Sayed et al., 2024).

4. CONCLUSION

This study highlights the isolation and characterization of amylase-producing actinobacteria from arid and semi-arid soils in Algeria. Out of 42 isolates screened, three strains *Streptomyces indoligenes* (E2S7), *Streptomyces hypolithicus* (A6B9), and *Streptomyces candidus* (C2K4) exhibited the highest amylolytic activity. These strains demonstrated optimal enzyme production at pH 8.0 and temperatures between 45°C and 50°C, confirming their thermotolerant and alkaliphilic nature. The enzymatic activity of these isolates positions them as strong candidates for application in agricultural and industrial processes, especially where high stability under varying conditions is essential.

Further studies should focus on scale-up potential, enzyme purification, and application-specific performance to validate their use in biotechnological industries.

REFERENCES

1. Ahmad, M., Isah, U., Raubilu, I. A., Muhammad, S., & Ibrahim, D. (2020). An overview of the enzyme: Amylase and its industrial potentials. *Bayero Journal of Pure and Applied Sciences, 12*(1), 352–358. https://doi.org/10.4314/bajopas.v12i1.53s
2. Ali, Z., Abdullah, M., Yasin, M. T., Amanat, K., Sultan, M., Rahim, A., & Sarwar, F. (2024). Recent trends in production and potential applications of microbial amylases: A comprehensive review. *Protein Expression and Purification, 106*, 106640. https://doi.org/10.1016/j.pep.2024.106640
3. Alsharif, W., Saad, M. M., & Hirt, H. (2020). Desert microbes for boosting sustainable agriculture in extreme environments. *Frontiers in Microbiology, 11*, 1666. https://doi.org/10.3389/fmicb.2020.01666
4. Ashok, P., Dasgupta, D., Ray, A., & Suman, S. (2023). Challenges and prospects of microbial α-amylases for industrial application : A review. *World Journal of Microbiology & Biotechnology, 40*(2), 44. https://doi.org/10.1007/s11274-023-03821-y

5. Bukhari, S. I., Al-agamy, M., Kelany, M. S., Al Hazani, M. R., & Hamed, M. M. (2021). Production optimization using Plackett-Burman and Box-Behnken designs with partial characterization of amylase from marine actinomycetes. *Environmental Science and Pollution Research*, 10.21203/RS.3.RS-169538/V1. https://doi.org/10.21203/RS.3.RS-169538/V1
6. Choudhary, R., & Nayak, B. (2023). Screening and production of bacterial amylase from different *Streptomyces* species. *International Journal of Engineering Technology and Management Sciences,* 7, 93. https://doi.org/10.46647/ijetms.2023.v07i02.093
7. El-Sayed, M. H., Gomaa, A. E., Atta, O. M., & Hassane, A. (2024). Characteristics and kinetics of thermophilic actinomycetes' amylase production on agro-wastes and its application for ethanol fermentation. *World Journal of Microbiology & Biotechnology*.https://doi.org/10.1007/s11274-024-04009-8
8. Fadhil, M., Oetari, A., & Sjamsuridzal, W. (2020). Starch-degrading ability of *Rhizopus azygosporus* UICC 539 at various temperatures. *Journal of Physics: Conference Series, 2242*, 050014. https://doi.org/10.1063/5.0007874
9. Gorrab, A., Ouertani, R., Souii, A., Kallel, F., Masmoudi, A. S., Cherif, A., & Neifar, M. (2024). A comprehensive review on the properties and applications of extremozymes from extremophilic actinobacteria. *MOJ Applied Bionics and Biomechanics, 8*, 213. https://doi.org/10.15406/mojabb.2024.08.00213
10. Hamdi, C., Arous, F., Boudagga, S., Harrath, N., Nwodo, U., & Jaouani, A. (2023). Actinobacteria isolated from Tunisian forest soils show high diversity and biotechnological potential. *Biologia, 78*, 3653–3665. https://doi.org/10.1007/s11756-023-01515-2
11. Hwang, S. Y., Nakashima, K., Okai, N., Okazaki, F., Miyake, M., Harazono, K., Ogino, C., & Kondo, A. (2013). Thermal stability and starch degradation profile of α-amylase from *Streptomyces avermitilis*. *Bioscience, Biotechnology, and Biochemistry*, 77, 2449-2453. https://doi.org/10.1271/bbb.130556
12. Kashiwagi, N., Miyake, M., Hirose, S., Sota, M., Ogino, C., & Kondo, A. (2014). Cloning and starch degradation profile of maltotriose-producing amylases from Streptomyces species. Biotechnology Letters, 36(11), 2311-2317. https://doi.org/10.1007/s10529-014-1611-5
13. Khadayat, K., Sherpa, D. D., Malla, K. P., Shrestha, S., Rana, N., Marasini, B., Khanal, S., Rayamajhee, B. R., & Bhattarai, N. P. (2020). Molecular identification and antimicrobial potential of *Streptomyces* species from Nepalese soil. *International Journal of Microbiology*.https://doi.org/10.1155/2020/8817467
14. Li, J., Liu, P., Menguy, N., et al. (2022). Identification of sulfate-reducing magnetotactic bacteria using 16S rRNA and primers 27F/1492R. *Environmental Microbiology*.https://doi.org/10.1111/1462-2920.16109
15. Li, S., Yang, Q., Tang, B., & Chen, A. (2018). Improving the enzymatic properties of *Rhizopus oryzae* α-amylase. *Enzyme and Microbial Technology, 117*, 96–102. https://doi.org/10.1016/j.enzmictec.2018.06.012
16. Milanes-Baños, N. A. (2024). A step-by-step guide to performing one-way ANOVA using Jamovi. https://doi.org/10.29057/mjmr.v12i23.10664
17. Mondal, S., Mondal, K., Halder, S., Thakur, N., & Mondal, K. C. (2022). Microbial amylase: Still at the forefront of industrial enzymes. *Biocatalysis and Agricultural Biotechnology*.https://doi.org/10.1016/j.bcab.2022.102509
18. Pinjari, B., & Kotari, V. (2018). Characterization of extracellular amylase from *Bacillus* sp. *Journal of Applied Biology & Biotechnology, 6*(3), 29–34. https://doi.org/10.7324/JABB.2018.60305
19. Prakash, B., Vidyasagar, M., Madhukumar, M. S., Muralikrishna, G., & Sreeramulu, K. (2009). Halotolerant, thermostable, and alkali-stable α-amylases from *Chromohalobacter* sp. *Process Biochemistry, 44*(2), 216–223. https://doi.org/10.1016/j.procbio.2008.10.013
20. Raja, S., Ganesan, S., Sivakumar, K., & Thangaradjou, T. (2010). Screening of marine actinobacteria for amylase enzyme inhibitors. *Indian Journal of Microbiology*.https://doi.org/10.1007/s12088-010-0046-5
21. Salwan, R., & Sharma, A. (2018). Catalytic roles and biotechnological applications of actinobacterial enzymes. *Bioresource Technology*. https://doi.org/10.1016/J.BIORTECH.2007.06.019
22. Silaban, S., Marika, D., & Simorangkir, M. (2020). Isolation and characterization of amylolytic bacteria from rice soil. *Journal of Physics: Conference Series, 1485*, 012006. https://doi.org/10.1088/1742-6596/1485/1/012006
23. Singh, R., Kumar, V., & Kapoor, V. (2014). Partial purification and characterization of a heat stable α-amylase from a thermophilic actinobacteria, Streptomyces sp. MSC702. Enzyme Research, 2014, Article 106363. https://doi.org/10.1155/2014/106363
24. Syafitri, W. A., Ningsih, F., Setyaningsih, P. P., Rachmania, M. K., et al. (2019). Screening of thermophilic actinobacteria for amylase production. *Biodiversitas, 20*(7). https://doi.org/10.13057/BIODIV/D200720
25. Syafitri, W. A., et al. (2019). Thermophilic actinobacteria with high-temperature stability for amylase production. *Biocatalysis and Agricultural Biotechnology*.https://doi.org/10.1016/j.bcab.2021.102068
26. Tischler, D., van Berkel, W. V., & Fraaije, M. (2019). *Actinobacteria*: A source of biocatalytic tools. *Frontiers in Microbiology, 10*, 800. https://doi.org/10.3389/fmicb.2019.00800
27. Zucchi, T., Rossi, G. D., & Cônsoli, F. L. (2011). Characterization of a β-amylase from *Propionicimonas* sp. *Annals of Microbiolo*