Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: www.jacb.journals.ekb.eg

Isolation and Screening of High-Potential Bacterial Isolates for Thermostable Amylase Production

Fatma G. M. Gomaa; Sabrien A. I. Omar; M. A. E. Selim and A. M. M. Elattaapy*

Microbiology Dept., Fac. of Agric., Mansoura Univ., Egypt

Article Information
Received 28 / 10 / 2025
Accepted 3 / 11 / 2025

ABSTRACT

Thermostable amylases produced by thermophilic microorganisms represent a promising solution for efficient industrial processes under harsh conditions. In this study, eighty-one bacterial isolates were isolated and screened for thermostable amylase production under solid-state fermentation (SSF) using wheat bran as the substrate. The residual enzyme activity was measured after an incubation at 80 °C for 1 h. Initial (zero-time) activities values varied widely (≈3.5–60.5 IU/gds), reflecting physiological and genetic diversity in amylase expression among the isolates. Thermal stability also differed substantially: while many isolates lost most of their activity after heat treatment, a subset retained high residual activity (> 70%), indicating exceptional thermostability. The ten most promising isolates were no. 76 (87.3%), 63 (83.6%), 16 (81.9%), 2 (81.2%), 45 (80.1%), 12 (77.1%), 66 (75.2%), 40 (74.8%), 18 (74.5%), and 71 (71.6%). Morphological, biochemical, and physiological characterization revealed that all top-performing isolates belong to the genus *Bacillus*. These findings highlight *Bacillus* spp. as potent producers of thermostable α-amylases.

Keywords: Amylolytic, *Bacillus*, Wheat bran, Starch hydrolysis, solid-state fermentation

INTRODUCTION

Biotechnology has emerged as an efficient and sustainable alternative to conventional industrial processes, offering cleaner production, lower energy consumption, and higher yields compared to chemical catalysis (Saini *et al*., 2017). Microbial enzymes, particularly amylases, are key biocatalysts due to their high catalytic efficiency and selectivity under mild conditions, making them essential in industrial, medical, agricultural, and environmental applications (Far *et al*., 2020; Katsimpouras *et al*., 2021; Tatta *et al*., 2022).

Thermophilic microorganisms are important sources of thermostable enzymes, including amylases, cellulases, proteases, and xylanases, which are widely used in food, pharmaceutical, textile, and environmental applications. These enzymes can retain their activity at high temperatures and under harsh conditions, providing several advantages such as reduced microbial contamination, improved substrate solubility, faster reaction rates, and resistance to chemical or solvent denaturation (Jaiswal *et al*., 2024; Simair *et al*., 2017). Among these microorganisms, Bacteria are especially important due to their rapid growth, high enzyme yield, extracellular secretion, and tolerance to harsh conditions. *Bacillus* species are among the most efficient bacteria for thermostable amylase production, including *Bacillus* subtilis and *B. licheniformis*, widely used for enzyme stability at high temperatures and alkaline pH (Prakash and Jaiswal, 2009; Kholikov *et al*., 2025). In addition, *B. licheniformis* and *B. velezensis* (Esawy *et al*., 2025; Suthar *et al*., 2024), *B. stearothermophilus* and *B. amyloliquefaciens* are important for starch processing, food, textile, and detergent industries (Almanaa *et al*., 2020). These thermophilic and halophilic *Bacillus* strains demonstrate strong thermal stability, highlighting their industrial potential (Yassin *et al*., 2021)

The production of α-amylase by microorganisms can be significantly enhanced by optimizing fermentation conditions. Key factors such as substrate type, moisture content, inoculum size, temperature, pH, and incubation time play a critical role in maximizing enzyme yield (Baysal *et al*., 2003; Saxena and Singh, 2011; Almanaa *et al*., 2020; Abd El Mageed *et al*., 2023). Both solid-state fermentation (SSF) and submerged fermentation (SmF) are commonly employed for enzyme production. SSF, in particular, uses solid substrates, often agro-industrial residues like wheat bran or bagasse, offering cost-effectiveness, higher enzyme concentrations, and simpler equipment. This method is especially beneficial for fungi and thermophilic bacteria, particularly *Bacillus* species, as the solid medium mimics their natural habitat, promoting microbial growth and enhancing enzyme production (Gangadharan *et al*., 2008; Singh *et al*., 2024).

The main aim of this study is to isolate high potential bacterial isolates for production of thermostable amylase using solid-state fermentation.

MATERIALS AND METHODS

Microorganisms and culture conditions

Soil samples were collected from various locations in Dakahlia Governorate, Egypt. Ten grams of each sample were suspended in 90 mL of sterile tap water and shaken at 150 rpm for 30 minutes to obtain a homogeneous suspension. Serial ten-fold dilutions of the suspensions were prepared, and appropriate dilutions were spread onto starch agar plates. The plates were incubated at 55 °C for 24 hours to select for thermostable bacterial isolates. Colonies exhibiting distinct morphology were picked and purified by repeated streaking on nutrient agar. The selected isolates were subsequently subjected to morphological, biochemical, and physiological characterization following standard microbiological

* **Corresponding author.**
E-mail address: a_elattapy@mans.edu.eg
DOI: 10.21608/jacb.2025.436790.1128

procedures according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Screening isolates for α-amylase production

All purified isolates were screened for amylase production by plating them on starch agar medium and incubating at 52 °C for 24 hours. After incubation, the plates were flooded with an iodine solution containing 2% iodine and 0.2% potassium iodide to detect starch hydrolysis (Cappuccino and Sherman, 2014). Colonies producing clear zones around their growth were considered positive for amylolytic activity and were subsequently maintained on starch agar for further experiments (Etesami *et al*., 2023).

Production of α-amylase through solid-state fermentation

For amylase production, solid-state fermentation was carried out using 4 g of dry wheat bran -as the substrate- in a 250-mL Erlenmeyer flask, moistened with 12 mL of tap water. The contents of the flasks were autoclaved at 121 °C for 20 min., then inoculated with 1 mL of a 24-h. bacterial suspension and incubated at 52 °C for 48 h. To extract the crude enzyme, fermented samples were mixed with 40 mL distilled water on a rotary shaker at 150 rpm for 1 h. The mixture was filtered through double-layered gauze, and the filtrate was centrifuged at 5000 rpm for 20 min to remove residual solids. The clear brown supernatant was used as the crude enzyme for subsequent assays (Etesami *et al*., 2023; Sanjaya *et al*., 2024).

Determination of enzyme activity:

Amylase activity was determined by measuring the reducing sugars released from starch hydrolysis using the dinitrosalicylic acid (DNS) method (Miller, 1959). Briefly, 0.9 mL of 0.5% starch solution (prepared in 0.1 mM sodium phosphate buffer, pH 6.5) was mixed with 0.1 mL of crude enzyme and incubated at 70 °C for 10 min. The reaction was terminated by adding 1.5 mL of DNS reagent, followed by boiling for 5 min to develop color. After cooling and appropriate dilution, absorbance was measured at 540 nm against a reagent blank. Glucose was used as the standard. One unit of amylase activity was defined as the amount of enzyme that releases 1 µmol of glucose equivalents per minute under the assay conditions (Isabel *et al*., 2025; Sanjaya *et al*., 2024).

Thermal stability of Amylase:

The thermal stability of the enzyme was evaluated by pre-incubating the crude enzyme at 80°C for 1 h. and then measuring the residual activity at 80 °C (Saxena and Singh, 2011).

Statistical analysis

All experiments were performed in triplicate. Data were expressed as means. Statistical analysis was performed using one-way ANOVA with CoStat software version 6.303 to determine significant differences between means.

RESULTS AND DISCUSSION

Isolation and screening of amylolytic bacterial isolates activity:

One hundred bacterial isolates were obtained and screened for amylase production by plating them on starch agar medium. The plates were incubated at 52 °C for 24 hours and subsequently flooded with iodine solution to visualize starch hydrolysis. Among the tested isolates, 81 exhibited clear zones around their colonies, indicating starch hydrolysis (Tan Gana *et al*., 2014). The diameter of these clear zones reflected high amylolytic activity in the respective isolates. The following images show examples of clear zones formed around bacterial colonies after flooding the starch agar plates with iodine solution. Gunam *et al*. (2021) reported that the ability of a microorganism to produce the enzyme amylase is indicated by the formation of clear zones on a starch-containing medium.

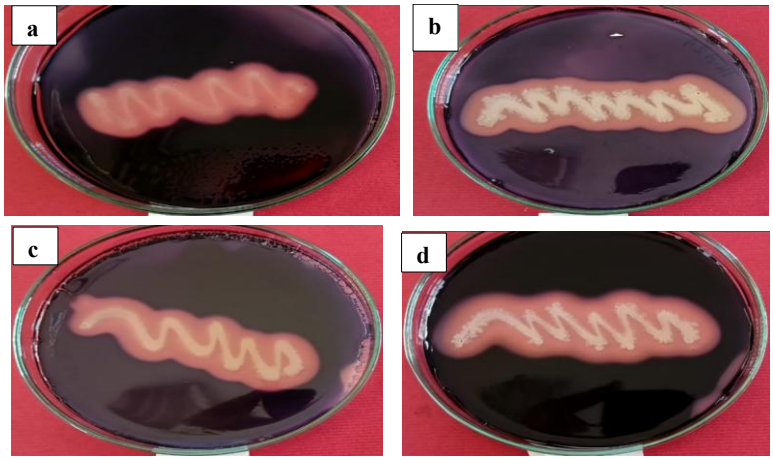

Fig. 1. Hydrolysis zones on 1% starch agar plates produced by different isolates a) isolate no. 1, b) isolate no. 2 c) isolate no. 16 and d) isolate no. 76

Thermostable amylases are highly desirable for industrial processes (starch liquefaction, detergent formulations, textile desizing, paper pulping) because higher operating temperatures increase reaction rates, reduce microbial contamination, and improve substrate solubility. Microbial α-amylases, particularly those from *Bacillus* and thermophilic genera (e.g., *Geobacillus*, *Anoxybacillus*, *Parageobacillus*), are commonly exploited due to ease of production and favorable properties. Solid-state fermentation on agricultural residues such as wheat bran is a cost-effective route to enzyme production and often gives high volumetric yields because the substrate both supports growth and acts as an inducer (El-Shishtawy *et al*., 2014).

Production via SSF on wheat bran is a validated strategy for high α-amylase yields and has been used for both mesophilic and thermophilic amylase producers; published reports demonstrate that wheat bran supports high expression and simplifies downstream handling (lower broth volume, reduced foaming). The present approach is therefore consistent with established efficient production routes (Lim *et*

al., 2020). In this study, a total of 81 bacterial isolates were screened for their ability to produce thermostable amylase under solid-state fermentation (SSF) conditions using wheat bran as the fermentation substrate. Wheat bran serves as an excellent carbon source and inducer for amylase synthesis due to its richness in starch and other polysaccharides, as well as its high porosity, which enhances oxygen transfer and microbial growth during SSF (Paul et al., 2020).

At the initial stage ("zero time"), all isolates exhibited variable levels of amylase activity, ranging from 3.51 to 60.52 IU/gds**,** indicating significant diversity in their enzyme-producing capabilities. This variation likely reflects genetic and metabolic differences among isolates, as well as varying affinities of their amylases for the substrate. Gunam et al. (2021) reported that amylase activity is a key indicator of enzyme production. In this study, isolate R5I4 showed the highest activity (0.897 ± 0.018 U/mL), while T1I1.1 exhibited the lowest (0.284 ± 0.020 U/mL).

Following thermal treatment at 80°C for 1 hour, a considerable reduction in enzyme activity was observed in most isolates, revealing differences in thermal stability. The residual activity ranged from less than 1% (e.g., isolate 23 with 0.78%) to over 87% (isolate 76), demonstrating a broad spectrum of thermostability among the tested strains.

Isolates such as No. 16, 40, 45, 63, 66, 67, 71, and 76 showed remarkably high residual activities (≥70%), suggesting that the amylases produced by these strains are thermostable enzymes capable of retaining catalytic efficiency even after prolonged exposure to elevated temperatures. In particular, isolate 76 exhibited the highest thermostability, maintaining 87.32% of its initial activity after 1 hour at 80°C, followed closely by isolates 63 (83.61%) and 16 (81.94%). Such stability indicates the presence of structurally robust enzymes, possibly stabilized by stronger intramolecular interactions such as disulfide bridges, increased hydrophobicity, or reduced flexible regions that resist thermal denaturation. Conversely, several isolates (e.g., 3, 9, 13, 23, 33, 53) displayed rapid loss of activity (<5% residual activity), indicating production of thermolabile amylases that are susceptible to heat-induced denaturation.

The ten best-performing isolates in this screening were 76 (87.3%), 63 (83.6%), 16 (81.9%), 2 (81.2%), 45 (80.1%), 12 (77.1%), 66 (75.2%), 40 (74.8%), 18 (74.5%), and 71 (71.6%) residual activity relative to zero-time. These results indicate that only a subset of the tested isolates produced enzymes with high thermostability—an essential characteristic for industrial processes such as starch liquefaction, detergent formulation, and food processing, where enzymes must retain activity at elevated temperatures. This screening successfully identified multiple isolates producing highly thermostable amylases after a stringent 1h. of incubation at 80 °C. The top-performing isolates represent promising candidates for both industrial applications and further studies on enzyme thermostability. Fincan *et al.* (2021), Silva-Salinas *et al.* (2021), and Rakaz *et al.* (2021) reported that *Bacillus* species are capable of producing thermostable α-amylase, with enzyme activity remaining largely unchanged even after 90 minutes of incubation at 70 °C.

Table 1. Production of amylase by selected bacterial isolates via solid state fermentation using wheat bran as the substrate, and determination of enzyme activity before and after incubation for 1 h. at 80 ° C.

Isolate no.	Amylase activity IU/gds		Residual activity (%)	Isolate no.	Amylase activity IU/gds		Residual activity (%)	Isolate no.	Amylase activity IU/gds		Residual activity (%)
	At zero time	After 1 h. of incubation at 80°C			At zero time	After 1 h. of incubation at 80°C			At zero time	After 1 h. of incubation at 80°C	
1	60.52	40.25	66.51	28	7.25	1.21	16.69	55	30.40	15.65	51.48
2	52.42	42.54	81.15	29	5.14	0.54	10.51	56	11.95	0.32	2.68
3	18.36	0.24	1.31	30	5.33	0.36	6.75	57	14.48	1.3	8.98
4	31.87	1.24	3.89	31	28.46	18.54	65.14	58	7.13	0.32	4.49
5	38.55	3.65	9.47	32	7.25	1.32	18.21	59	27.04	2.35	8.69
6	27.58	12.54	45.47	33	23.26	0.87	3.74	60	8.11	0.48	5.92
7	9.20	1.12	12.17	34	12.52	2.15	17.17	61	27.43	3.65	13.31
8	12.31	1.15	9.34	35	22.92	5.98	26.09	62	21.13	14.65	69.33
9	17.49	0.33	1.89	36	19.29	5.65	29.29	63	30.32	25.35	83.61
10	16.93	3.65	21.56	37	7.69	1.24	16.12	64	9.65	2.65	27.46
11	36.59	20.47	55.94	38	5.68	2.1	36.97	65	10.18	4.65	45.68
12	41.06	31.65	77.08	39	11.48	3.54	30.84	66	28.63	21.54	75.24
13	11.66	0.24	2.06	40	45.41	33.98	74.83	67	25.35	17.54	69.19
14	32.03	13.65	42.62	41	12.56	1.02	8.12	68	8.11	0.65	8.01
15	29.52	15.62	52.91	42	13.81	2.54	18.39	69	24.87	2.47	9.93
16	40.09	32.85	81.94	43	29.80	18.74	62.89	70	12.95	0.54	4.17
17	14.94	3.54	23.69	44	10.85	3.65	33.64	71	44.18	31.64	71.62
18	33.10	24.65	74.47	45	35.74	28.64	80.13	72	17.62	4.54	25.77
19	21.63	6.65	30.74	46	15.61	4.69	30.04	73	14.04	1.48	10.54
20	3.51	0.21	5.98	47	34.15	13.87	40.61	74	9.09	0.43	4.73
21	14.50	2.54	17.52	48	19.33	4.78	24.73	75	19.41	5.32	27.41
22	24.40	13.54	55.49	49	20.59	11.24	54.59	76	52.28	45.65	87.32
23	18.01	0.14	0.78	50	35.92	23.95	66.68	77	10.03	1.21	12.06
24	11.51	0.36	3.13	51	22.76	12.65	55.58	78	28.23	4.54	16.08
25	38.80	18.98	48.92	52	13.46	1.2	8.92	79	22.32	10.24	45.88
26	10.43	2.21	21.19	53	29.99	2.35	7.84	80	44.84	14.51	32.36
27	16.22	9.87	60.85	54	34.67	13.65	39.37	81	35.54	23.94	67.36
LSD at 0.05	1.96	1.22	3.24	LSD at 0.05	1.96	1.22	3.24	LSD at 0.05	1.96	1.22	3.24

Fermentation media consists of 4 g wheat brane moistened with 12 mL tap water and incubated at 52° c for 48 h.
IU = the amount of enzyme that releases 1 µmol of glucose equivalents per minute under the assay conditions.
gds = gram dry substrate.

After evaluating enzyme activity, ten isolates were identified as potential amylase producers. The morphological and biochemical characteristics of these isolates are summarized in Table 2. The selected isolates (No. 2, 12, 16, 18, 40, 45, 63, 66, 71, and 76) were examined to support their preliminary identification and to correlate phenotypic traits with their amylolytic potential. All isolates were found to be Gram-positive, rod-shaped, and spore-forming, consistent with the general characteristics of the genus *Bacillus*. This genus is well known for its ability to produce extracellular hydrolytic enzymes, including amylases, proteases, and lipases, under a wide range of environmental conditions (Schallmey *et al*., 2004; Morbia *et al*., 2024).

Table 2. Morphological, biochemical, and physiological characteristics of the selected isolates:

Isolate	Gram	Spore	Hydrolysis of			Forming acid from			
no.	stain	forming	Starch	Casein	Gelatin	D-Glucose	D- Mannitol	D- Xylose	L- Arabinose
2	G+ve rods	+	+	+	+	+	+	+	+
12	G+ve rods	+	+	+	+	+	+	+	+
16	G+ve rods	+	+	+	+	+	+	+	+
18	G+ve rods	+	+	+	+	+	+	+	+
40	G+ve rods	+	+	+	+	+	+	+	+
45	G+ve rods	+	+	+	+	+	+	+	+
63	G+ve rods	+	+	+	+	+	+	+	+
66	G+ve rods	+	+	+	+	+	+	+	+
71	G+ve rods	+	+	+	+	+	+	+	+
76	G+ve rods	+	+	+	+	+	+	+	+

CONCLUSION

In this study, 100 bacterial isolates were screened for amylase production. Among them, 81 isolates exhibited clear zones on starch agar plates after iodine flooding, indicating amylolytic activity. Ten isolates (No. 2, 12, 16, 18, 40, 45, 63, 66, 71, and 76) demonstrated the highest enzyme activity, showing strong potential for amylase production. Notably, isolate 76 displayed superior thermal stability, highlighting its promise for industrial applications where high-temperature enzyme performance is essential.

REFERENCES

Abd El Mageed, B. M., Ali, S. G., Hammouda, O., & Raslan, M. (2023). Optimization of α-amylase activity enzyme produced by *Bacillus subtilis* and *Aspergillus niger*. Egyptian Journal of Chemistry, 66(3), 421-426.

Almanaa, T. N., Vijayaraghavan, P., Alharbi, N. S., Kadaikunnan, S., Khaled, J. M., & Alyahya, S. A. (2020). Solid state fermentation of amylase production from *Bacillus subtilis* D19 using agro-residues. Journal of King Saud University-Science, 32(2), 1555-1561.

Baysal, Z., Uyar, F., & Aytekin, Ç. (2003). Solid state fermentation for production of α-amylase by a thermotolerant *Bacillus subtilis* from hot-spring water. Process Biochemistry, 38(12), 1665-1668.

El-Shishtawy, R. M., Mohamed, S. A., Asiri, A. M., Gomaa, A. B. M., Ibrahim, I. H., & Al-Talhi, H. A. (2014). Solid fermentation of wheat bran for hydrolytic enzymes production and saccharification content by a local isolate *Bacillus megatherium*. BMC biotechnology, 14(1), 29.

Etesami, H., Jeong, B. R., & Glick, B. R. (2023). Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops—A review. Current Research in Biotechnology, 5, 100128.

Far, B.E., Y. Ahmadi, A.Y. Khosroshahi and A. Dilmaghani (2020). Microbial alpha-amylase production: progress, challenges and perspectives. AdvancedPharmaceutical Bulletin. Jul;10(3):350.

Fincan, S. A., Özdemir, S., Karakaya, A., Enez, B., Mustafov, S. D., Ulutaş, M. S., & Şen, F. (2021). Purification and characterization of thermostable α-amylase produced from *Bacillus licheniformis* So-B3 and its potential in hydrolyzing raw starch. Life Sciences, 264, 118639.

Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K. M., Sukumaran, R. K., & Pandey, A. (2008). Response surface methodology for the optimization of alpha amylase production by *Bacillus amyloliquefaciens*. Bioresource technology, 99(11), 4597-4602.

Gunam, I. B. W., Sujana, I. G. A., Wijaya, I. M. M., Setiyo, Y., Putra, I. W. W. P., & Suriati, L. (2021, November). Isolation and selection of amylase-producing microbes isolated from ragi tape and cassava tape available on the markets. In IOP Conference Series: Earth and Environmental Science (Vol. 913, No. 1, p. 012041). IOP Publishing.

Holt J.G., Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's Manual of Determinative Bacteriology, 9th edn. Williams and Wilkins, Baltimore

Isabel, J. B., & Premalatha, M. (2025). Harnessing amylase producing novel bacterial strains from industrial waste: A bioprocessing and optimization approach. International Journal of Biological Macromolecules, 146769.

jaiswal, N., and Jaiswal, P. (2024). Thermostable α-amylases and laccases: paving the way for sustainable industrial applications. Processes, 12(7), 1341.

Lim, S. J.; S. N. Hazwani-Oslan and S. N. Oslan (2020). Purification and characterisation of thermostable α-amylases from microbial sources. BioResources. 15(1), 2005-2029.

Morbia, M., Pandey, A., Mahla, P., & Gohil, S. (2024). Isolation of α-Amylase Producing Microorganisms from Soil of Kachchh, Gujarat. J. Pure Appl. Microbiol, 18, 9218.

Prakash, O., & Jaiswal, N. (2010). α-Amylase: an ideal representative of thermostable enzymes. Applied biochemistry and biotechnology, 160(8), 2401-2414.

Qureshi, A. S., Bhutto, M. A., Khushk, I., & Dahot, M. U. (2011). Optimization of cultural conditions for protease production by *Bacillus subtilis* EFRL 01. African Journal of Biotechnology, 10(26), 5173-5181.

Rakaz, M. A., Hussien, M. O., & Ibrahim, H. M. (2021). Isolation, Extraction, Purification, and Molecular Characterization for Thermostable α-Amylase from Locally Isolated *Bacillus* Species in Sudan. Biochemistry research international, 2021(1), 6670380.

Saini, R., Saini, H. S., & Dahiya, A. (2017). Amylases: Characteristics and industrial applications. J. Pharmacogn. Phytochem, 6(4), 1865-1871.

Sanjaya, E. H., Suharti, S., Alvionita, M., Telussa, I., Febriana, S., & Clevanota, H. (2024). Isolation and Characterization of Amylase Enzyme Produced by Indigenous Bacteria from Sugar Factory Waste. The Open Biotechnology Journal, 18(1).

Saxena, R., & Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus* sp. Brazilian Journal of Microbiology, 42, 1334-1342.

Schallmey, M., Singh, A., & Ward, O. P. (2004). Developments in the use of *Bacillus* species for industrial production. Canadian journal of microbiology, 50(1), 1-17.

Silva-Salinas, A., Rodríguez-Delgado, M., Gómez-Treviño, J., López-Chuken, U., Olvera-Carranza, C., & Blanco-Gámez, E. A. (2021). Novel Thermotolerant amylase from *Bacillus licheniformis* strain LB04: purification, characterization and agar-agarose. Microorganisms, 9(9), 1857.

Suthar, S., Joshi, D., Patel, H., Patel, D., & Kikani, B. A. (2024). Optimization and purification of a novel calcium-independent thermostable, α-amylase produced by *Bacillus licheniformis* UDS-5. World Journal of Microbiology and Biotechnology, 40(12), 385.

Tan Gana, N. H., Mendoza, B. C., & Monsalud, R. G. (2014). Isolation, screening and characterization of yeasts with amyloytic, lipolytic, and proteolytic activities from the surface of Philippine bananas (Musa spp.). Philippine Journal of Science, 143(1).

Yassin, S. N., Jiru, T. M., & Indracanti, M. (2021). Screening and Characterization of Thermostable Amylase-Producing Bacteria Isolated from Soil Samples of Afdera, Afar Region, and Molecular Detection of Amylase-Coding Gene. International journal of microbiology, 2021(1), 5592885.

عزل واختيار عزلات بكتيرية عالية الكفاءة في إنتاج الأميليز الثابت حراريا

فاطمة جمعة محمد جمعة، صابرين أحمد إبراهيم عمر، محمد عبدالله العوضي سليم وعبد الرحمن محمد محمود العتابي

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنصورة - مصر

الملخص

تمثل إنزيمات الأميليز الثابتة حرارياً، والتي تُنتَج بواسطة الكائنات الحية الدقيقة المحبة للحرارة، حلاً واعداً لزيادة كفاءة العمليات الصناعية التي تتم في ظروف قاسية. وفي محاولة لعزل بكتيريا عالية الكفاءة في إنتاج إنزيم الأميليز الثابت حرارياً، تم فحص واحدٍ وثمانين عزلة بكتيرية من حيث قدرتها على إنتاج الأميليز الثابت حراريا باستخدام طريقة التخمر في الحالة الصلبة (SSF) على نخالة القمح كركيزة للإنتاج، كما تم قياس النشاط المتبقي بعد تعريض العينات لاختبار حراري لمدة ساعة واحدة عند ٨٠°م. وقد تباينت قيم النشاط الابتدائي (عند الزمن الصفري) بشكلٍ واسع (حوالي ٣,٥–٦٠,٥ وحدة/غرام من المادة الجافة الصلبة)، مما يعكس التنوع الفسيولوجي والجيني في تعبير إنزيم الأميليز. كما أظهرت ثبات الإنزيم حراريا بعد ساعة من التحضين عند ٨٠°م تفاوتًا كبيرًا؛ إذ فقدت معظم العزلات جزءًا كبيرًا من نشاطها، في حين احتفظت مجموعة محددة بنسبة عالية من النشاط المتبقي (>٧٠٪)، مما يشير إلى كونها مرشحة واعدة لإنتاج إنزيم الأميليز الثابت حراريا. وأظهرت أفضل عشر عزلات أداءً في هذا الاختبار النتائج التالية: العزلات رقم ٧٦ (٨٧,٣٪)، ٦٣ (٨٣,٦٪)، ١٦ (٨١,٩٪)، ٢ (٨١,٢٪)، ٤٥ (٨٠,١٪)، ١٢ (٧٧,١٪)، ٦٦ (٧٥,٢٪)، ٤٠ (٧٤,٨٪)، ١٨ (٧٤,٥٪)، و٧١ (٧١,٦٪)، استناداً إلى الخصائص المورفولوجية والكيميائية الحيوية والفسيولوجية، فإن جميعها تنتمي إلى جنس الـ *Bacillus* .