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Screening and Optimizing the Fermentation Conditions for Cold-Active Amylase Production by Some *Penicillium* and *Talaromyces* Species in Submerged Conditions

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ABSTRACT

In the current study, 126 isolates representing 24 distinct fungi species from the genera *Penicillium* and *Talaromyces* were screened to assess their abilities for generating cold-active amylase at 10 and 30 oC. According to preliminary screening results, all investigated isolates (100%) developed cold-active amylase at 10 ºC, with 32 exhibiting a moderate capability and 94 exhibiting high activity. However, 122 isolates (96.7%) produced positive results at 30 ºC, with 35 exhibiting high amylase activity, 50 exhibiting moderate activity, and 37 exhibiting low activity. Fungal isolates that demonstrated high activity at 10 and 30 ºC in the preliminary test (94 and 35, respectively) were further investigated for cold-active amylase production under submerged fermentation (SmF) conditions. At 10 and 30 ºC, respectively, *Penicillium citrinum* AUMC 576 and *Penicillium goetzii* AUMC 498 were the most active strains under SmF. The pH, temperature, nitrogen source, and fermentation time were all changed for *P. goetzii* AUMC 498 and *P. citrinum* AUMC 576 in order to promote the production of cold-active amylase. After 7.0 days, *P. goetzii* demonstrated its maximum amylase production (3.7±0.34 U/mL) at pH 7.0 and 10 ºC using the beef extract as the nitrogen supply. However, *P. citrinum* demonstrated that after 3.0 days of employing the yeast extract as a nitrogen source, amylase activity peaked (9.92±0.84 U/mL) at pH 7.0 and 25 ºC.

1. INTRODUCTION

To biodegrade the starch molecule into glucose, maltose, maltotriose, and dextrin, amylases—more especially, α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2), and γ-amylase (EC 3.2.1.3)—are the biocatalysts that break down the glycosidic bonds in the starch polymer [1–3]. By 2025, the enzyme market is expected to have grown from its 2018 forecast of US$5.5 billion to US$7.0 billion [4]. With between 25 and 33 % of the global enzyme market, amylases are among the most important enzymes [3, 5]. They are widely utilised in a variety of industries, such as the food, beverage, textile, bioethanol, and paper sectors; they are also utilised in a wide range of other domains, such as pharmaceuticals, analytical chemistry, biotechnology, and medicine [3, 6–14].

Cold-adapted enzymes are capable of catalysing at temperatures as low as 30 °C and maintaining catalytic activity at 0 °C [15]. They differ from mesophilic and thermophilic enzymes in that they have a low reaction energy, a higher substrate affinity that can lower the activation energy of enzymatic reactions, and low thermal stability at high temperatures, which causes them to rapidly lose more than half of their activity after 10 minutes at 50-60 °C or several hours at 37 °C [16–18]. Because of their plentiful supply, quick production cycle, high yield, ease of separation, easily regulated reaction conditions, ease of monitoring and control of manufacturing processes, and purification, microbial cold-active enzymes have garnered a lot of attention [19, 20]. Food processing, detergent production, bioremediation, environmental preservation, straw resourcing, and fundamental molecular biology research are just a few of the many uses and applications for these enzymes that have been thoroughly investigated and employed [21].

Because of their advantages for the economy and ecology, cold-adapted amylases are becoming more and more common in industrial processes [15]. Stain removal in detergents, antistaling in bread baking, syrup production in pharmaceuticals, textile desizing, viscosity control in starch slurry in paper and pulp, wastewater treatment, and low-temperature bioremediation are just a few of the commercial uses for cold-active α-amylases of microbial origin [22, 23]. Very little is known about cold-adapted α-amylases and about the microbiological origins of cold-active α-amylases. In food preparation and other industrial applications where operating temperatures are lower than 29 °C, cold-active α-amylases can be required.

Only a small number of microorganisms are allowed in the food industry, and enzyme producers are typically keen to expand their product lines into the food processing sector [24, 25]. Scientists are now concentrating their efforts on finding new enzymes that could replace industrial chemical catalysis since enzymatic processes are more sustainable and environmentally friendly than chemical ones [26, 27]. Therefore, the hunt for novel microbes that can be used to produce amylase is still going on [28]. Given the dramatic rise in the use of microbial enzymes in industrial processes in recent years, the Penicillia appear particularly well-positioned to play a larger role in the future production of these enzymes [24, 25]. This study was therefore tasked with screening many isolates of *Penicillium* and *Talaromyces* for amylase activity on agar medium and submerged culture at 10 and 30 ºC. Additionally, the fermentation conditions were optimized to maximise the amount of amylase produced by the most active strains.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The sources of all the chemicals and reagents utilized in this investigation were LOBA CHEMIE PVT.LTD (Jehangir Villa, 107, Wodehouse Road, Colaba, Mumbai, India) and Sigma-Aldrich Chemical (Sigma-Aldrich, St. Louis, MO).

2.2 Fungal strains

There were 126 different fungal strains in this study. They included 11 isolates from three species of Talaromyces and 115 isolates from 21 species of Penicillium. The fungi were all taken from the culture collection of the Assiut University Mycological Centre in the Assiut Governorate, Egypt.

2.3 Fermentation medium

Sucrose-free Czapek's mineral medium was used as fermentation medium. The medium contained (g/L): sodium nitrate, 2.0; dipotassium hydrogen orthophosphate, 1.0; potassium chloride, 0.5; magnesium sulphate, 0.5; ferrous sulphate, 0.01; zinc sulphate, 0.01; and copper sulphate, 0.005

2.4 Preliminary screening of amylase activity on agar medium

The examined fungi were allowed to colonize for seven days before 50 µL of spore suspension (made in 10 % Tween 80) was collected and inoculated into wells that were 5.0 mm in diameter and created on the agar medium. After that, the plates were incubated for 72 hours at 10 and 30 °C. A 0.25 % aqueous iodine solution (iodine, 1.0 g; potassium iodide, 3.0 g, and 400 mL of distant water) was added to the plates after they had been incubated. The clear zone surrounding fungal colonies against the blue media colour indicates the amylase activity.

2.5 Screening of amylase activity in submerged fermentation (SmF)

A 250 mL Erlenmeyer conical flask with 50 mL of the fermentation medium was used to culture each of the fungal strains with the highest amylase activity. Each flask was inoculated with a 5.0 % (v/v) spore suspension made from cultures that were 7 days old, containing 1.8×10^8 spores/mL. After that, the flasks were shaken at 150 rpm for seven days at 10 and 30 °C.

2.6 Extraction and assay of amylase and estimation of total protein content

Following the incubation time, the cell-free supernatant was extracted using centrifugation (10,000 rpm for 10 minutes at 4 °C) and utilized as a source of amylase. By combining 0.5 mL of filtered crude enzyme with 0.5 mL of 1.0 % starch (made in 50 mM Na-citrate buffer, pH 5.0), amylase activity was measured. The cold-active amylase was incubated for 20 minutes at 10 °C, and the mesophilic amylase was incubated for 20 minutes at 50 °C [29]. After that, 2.0 mL of 3, 5-dinitrosalicylic acid (DNS) was added, and the mixture was boiled for 10 minutes in a water bath to stop the reaction [30]. Following cooling, a UV-Visible spectrophotometer (T80+; UK) was used to detect the colour absorbance at 540 nm. The quantity of amylase needed to release 1.0 µmol glucose per millilitre per minute under standard test conditions was referred to as one unit

of amylase activity. Amylase activity calculations were performed in accordance with AL-Kolaibe, Moharram [32].

$$\textbf{Amylase activity} = \left(\frac{\text{Absorbance x DF}}{(X)(Y)(t)(\text{slope})}\right) \quad \text{U/mL/min}$$

Where: DF = the dilution factor for enzyme; x = the volume of enzyme used; y = the volume of hydrolysate used for assay of reducing sugars; t = the time of hydrolysis; slope was determined by the standard curves of glucose. Folin Lowry's approach [31], which used bovine serum albumin (BSA) as a reference, was used to measure soluble protein.

2.7 Optimization of fermentation parameters

The most powerful strains produced the highest levels of amylase when two factors at a time (TFAT) conditions were met. For the tests, 50 mL of the fermentation medium supplemented with 1.0 % soluble starch served as the sole carbon source in 250 mL Erlenmeyer flasks. Each flask was separately inoculated with 5.0 % (v/v) of spore suspension that contained 1.5×10^8 spores/mL of the potent strains that were 7 days old. The flasks were subsequently incubated at 10, 15, and 20 ºC for the cold-active amylase and at 25, 30, and 35 ºC for the mesophilic amylase production, with varying operating parameters, including pH (4–10) each. The incubation period, which varied from one to ten days, also included several nitrogen sources (peptone, yeast extract, beef extract, sodium nitrate, ammonium sulphate, and ammonium chloride, each at 0.2%). There were three trials carried out.

2.8 Morphological identification of the potent strains

Petri plates containing Cz, MEA, and CYA were inoculated with a fungal spore suspension (made with 30% glycerol, 0.2% agar, and 0.05 % Tween 80 solution) of the powerful strains using an inoculum size of 1.0 µL/spot [33]. Following seven days of incubation at 25 °C, microscopic characteristics were analysed using lacto-phenol cotton blue and a Zeiss microscope (Axio Star, Germany).

3. RESULTS

3.1 Preliminary screening of cold-active amylase activity on agar medium

This test included 126 isolates from two genera, *Penicillium* and *Talaromyces*, representing 24 different fungal species. All examined isolates (100%) were found to be positive producers of cold-active amylase at 10 ºC, with 94 exhibiting high activity and 32 a moderate capability, based on the preliminary screening results. However, 122 isolates, or 96.8% of the screened isolates, showed positive results at 30 ºC; there were 35 isolates with high activity, 50 with moderate activity, and 37 with low amylase activity (Table 1).

Table 1. Screening of cold-active amylase activity of 126 *Penicillium* and *Talaromyces* isolates on agar medium and submerged fermentation at 10 ºC and 30 ºC.

Fungal species	No. of isolates	Screening at 10 °C							Screening at 30 °C						
		Positive	On agar			Submerged fermentation			Positive	On agar			Submerged fermentation		
			H	M	L	H	M	L		H	M	L	H	M	L
Penicillium	115	115	59	17	-	3	6	51	115	24	31	21	4	1	20
P. aurantiogriseum	4	4	3	1	-	-	-	3	4	2	2	-	1	-	1
P. brevicompactum	1	1	1	-	-	-	-	1	2	2	-	-	-	-	2
P. camemberti	1	1	1	-	-	-	-	1	1	-	-	1	-	-	-
P. canescens	1	1	1	-	-	-	-	1	1	-	-	1	-	-	-
P. chrysogenum	18	18	10	8	-	1	2	7	18	3	12	3	1	-	2
P. citrinum	7	7	5	2	-	-	-	5	7	2	4	1	1	-	1
P. corylophilum	3	3	3	-	-	-	1	2	3	1	-	2	-	-	1
P. digitatum	2	2	2	-	-	-	-	2	2	1	1	-	-	-	1
P. expansum	5	5	5	-	-	-	1	4	4	-	2	2	-	-	-
P. goetzii	1	1	1	-	-	1	-	-	1	1	-	-	-	-	1
P. griseofulvum	5	5	3	2	-	-	-	3	5	2	2	1	-	-	2
P. italicum	4	4	2	2	-	-	-	2	4	2	1	1	-	-	2
P. janczewskii	1	1	1	-	-	-	-	1	1	-	-	1	-	-	-
P. oxalicum	5	5	5	-	-	-	-	5	5	-	1	4	-	-	-
P. polonicum	1	1	1	-	-	-	-	1	1	-	1	-	-	-	-
P. scleratiogenum	1	1	1	-	-	-	-	1	1	1	-	-	-	-	1
P. simplicissimum	2	2	2	-	-	-	1	1	2	2	-	-	-	-	2
P. solitum	10	10	9	1	-	-	1	8	10	4	5	1	-	1	3
P. velutinum	1	1	1	-	-	-	-	1	1	1	-	-	-	-	1
P. dierckxii	1	1	1	-	-	-	-	1	1	-	-	1	-	-	-
P. jensenii	2	2	1	1	-	-	-	1	2	-	-	2	-	-	-
Penicillium spp.	39	39	28	11	-	1	4	23	35	10	14	10	1	-	9
Talaromyces	11	11	7	4	-	-	2	5	11	1	5	5	-	-	1
T. duclauxii	7	7	4	3	-	-	1	3	7	1	2	4	-	-	1
T. pinophilus	1	1	1	-	-	-	-	1	1	-	-	1	-	-	-
T. purpureogenus	3	3	2	1	-	-	1	1	3	-	3	-	-	-	-
Total	126	126	94	32	-	3	12	80	122	35	50	37	4	1	30
No. of genera	2	2	2	2	2	1	2	2	2	2	2	2	1	1	2
No. of species	24	24	24	9	-	2	7	23	24	14	12	15	3	1	14

3.2 Determination of cold-active amylase activity in SmF

The purpose of this test was to determine the cold-active amylase activity of 94 and 35 fungal isolates under SmF conditions, respectively, that showed high activity at 10 and 30 ºC in the preliminary test. Only three of the ninety-four isolates that were examined for amylase production at 10 ºC were found to be very active; *Penicillium goetzii* AUMC 498 was the most active strain. Eighty isolates produced modest amounts of amylase at 10 ºC, whilst twelve isolates exhibited considerable amounts. Thirty isolates were found to produce little amylase at 30 ºC, and only one fungus displayed intermediate activity. *Penicillium citrinum* AUMC 576 was the most active isolate among these, with four others exhibiting strong amylase production (Table 1). In order to maximise their

production of the cold-active amylase, *P. citrinum* AUMC 576 and *P. goetzii* AUMC 498 were chosen for their optimal fermentation conditions.

3.3 Optimization of fermentation conditions

3.3.1 Cold-active amylase production by *P. citrinum* AUMC 576

The pH and temperature of the fermentation medium have been adjusted for *P. citrinum* AUMC 576 in order to enhance the synthesis of cold-active amylase. This led to the production of cold-active amylase, which was active throughout a wide pH range (4–10) and temperature range (25–35 ºC). The amylase activity peaked at 7.63±0.68 U/mL at pH 7.0 and 25 ºC (Figure 1). When yeast extract was given as a nitrogen source after three days of incubation, the cold-active amylase activity peaked at 9.92±0.84 U/mL (Figure 2).

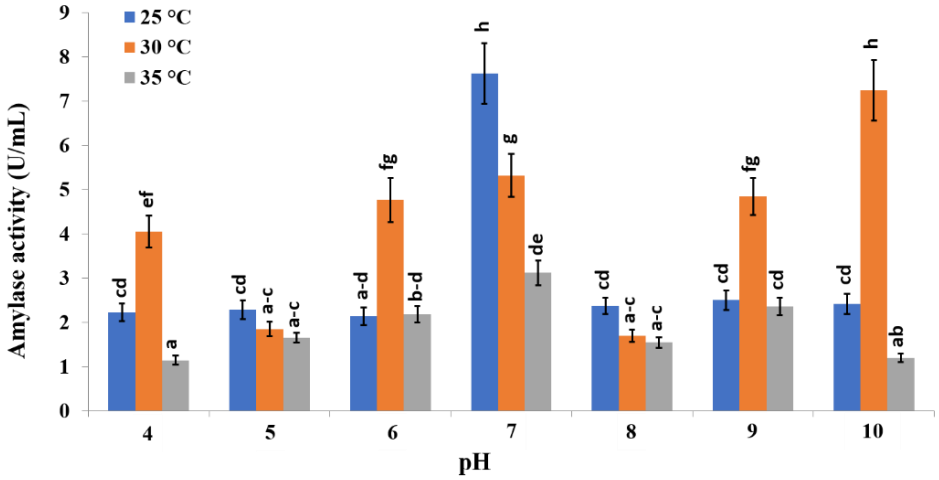

Figure 1. Effect of medium's pH and temperature on the cold-active amylase activity produced by *P. citrinum* AUMC 576 (Mean values±SD with different letters are significantly different; $p < 0.05$; $n = 3$).

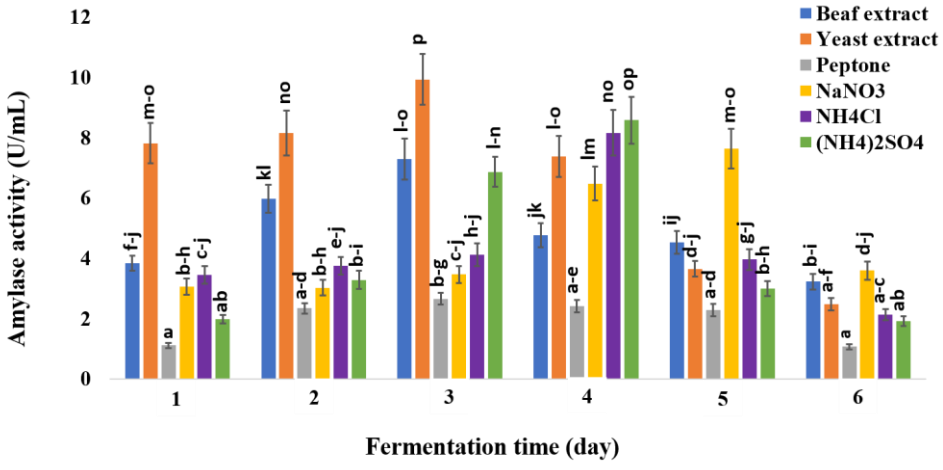

Figure 2. Effect of medium's nitrogen source and fermentation time on the cold-active amylase activity produced by *P. citrinum* AUMC 576 (Mean values±SD with different letters are significantly different; $p < 0.05$; $n = 3$).

3.3.2 Cold-active amylase production by *Penicillium goetzii* AUMC 498

In order to boost the production of cold-active amylase in *P. goetzii* AUMC 498, the pH and temperature of the fermentation medium were changed. Amylase was produced as a result, and it showed activity over a wide pH range (4–10) and temperature range (10–20 ºC). The maximum amylase activity, 2.187±0.2 U/mL, was found at pH 7.0 and 10 ºC (Figure 3). Following seven days of incubation, amylase activity peaked at 3.7±0.34 U/mL when beef extract was introduced as the nitrogen source (Figure 4).

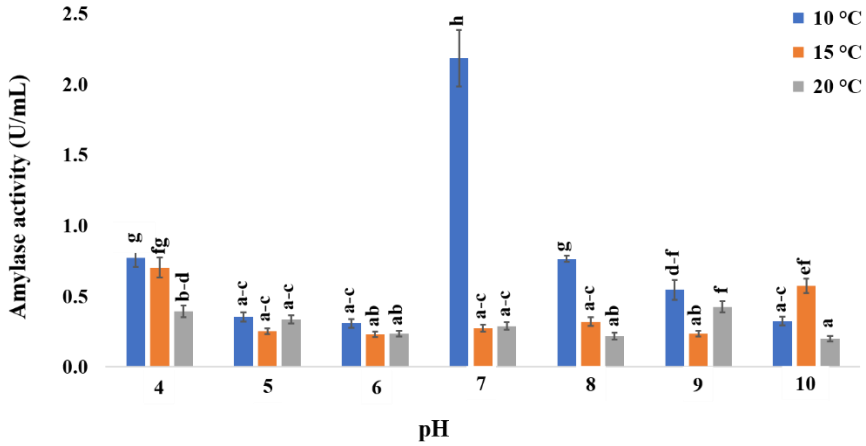

Figure 3. Effect of medium's pH and temperature on the cold-active amylase activity produced by *P. goetzii* AUMC 498 (Mean values±SD with different letters are significantly different; $p < 0.05$; $n = 3$).

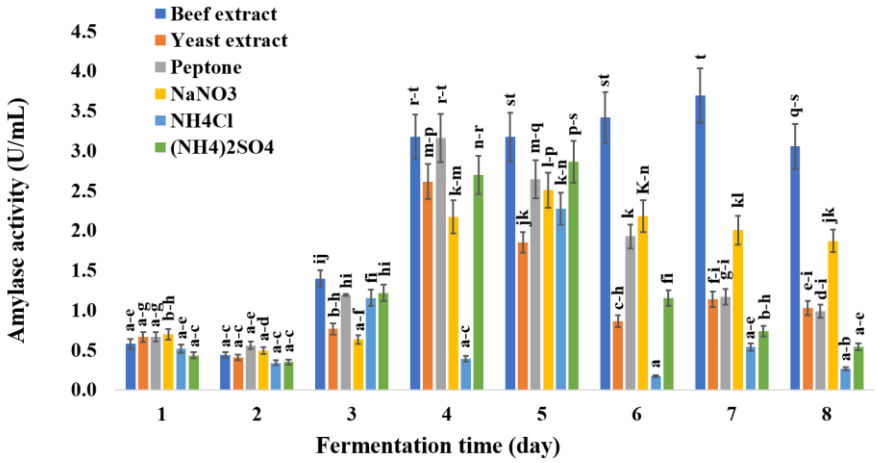

Figure 4. Effect of medium's nitrogen source and fermentation time on the cold-active amylase activity produced by *P. goetzii* AUMC 498 (Mean values±SD with different letters are significantly different; $p < 0.05$; $n = 3$).

3.4 Morphological identification of the potent strains

3.4.1 Penicillium citrinum AUMC 576

The strain *P. citrinum* AUMC 576 in this study showed the morphological characteristics of the type species *P. citrinum*. Conidial color varied ranging from gray green to dark

green, with moderate to good sporulation. Conidiophores biverticillate; stipes smooth, width 2.0–3.0 µm; metulae, 12–16 × 2.0–2.7 µm; phialides ampulliform, 7.5–10 × 2.0–2.5 µm; conidia smooth walled, globose to subglobose, 20–2.5 × 1.8–2.5 µm (Figure 5).

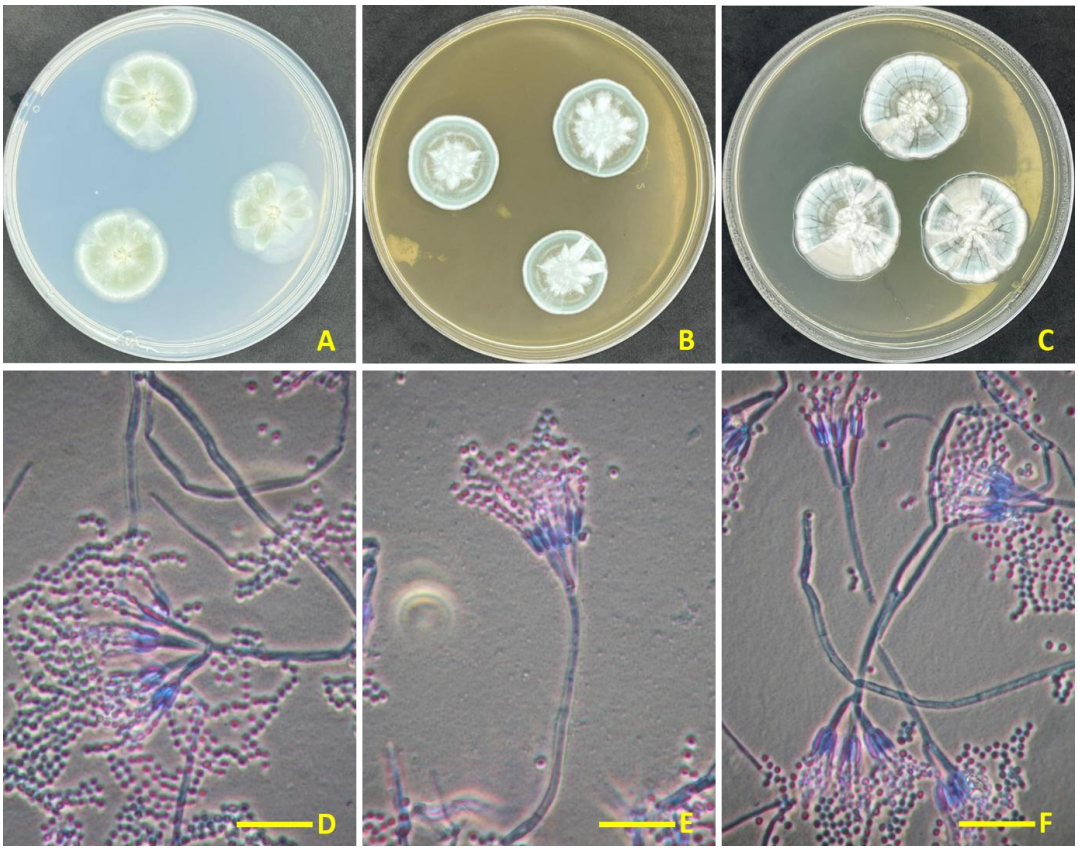

Figure 5. *Penicillium citrinum* AUMC 576. (A–C) Seven-day-old colonies on Cz, MEA, and CYA at 25 C. (D–F) Smooth biverticillate conidiophores bearing metulae and ampulliform phialides and globose to subglobose conidia (Scale bar = 20 µm).

3.4.2 Penicillium goetzii AUMC 498

Mycelium white, conidia grey-green, exudate sparsely produced. Colonies on MEA floccose; sporulation variable, absent to dense, conidia grey-green; mycelium white, exudate droplets absent or produced as clear or light brown droplets. Conidiophores borne from surface and aerial mycelium, terverticillate to quarterverticillate, 200–400 × 2.5–3.5 µm, smooth walled. Branches 12–20 × 2.5–3.5 µm. Metulae, 8–12(−15) × 2.5–3.5 µm. Phialides ampulliform, 7–9(−10) × 2–3 µm. Conidia broadly ellipsoidal, smooth, 2–2.5 × 2–3 µm (Figure 6).

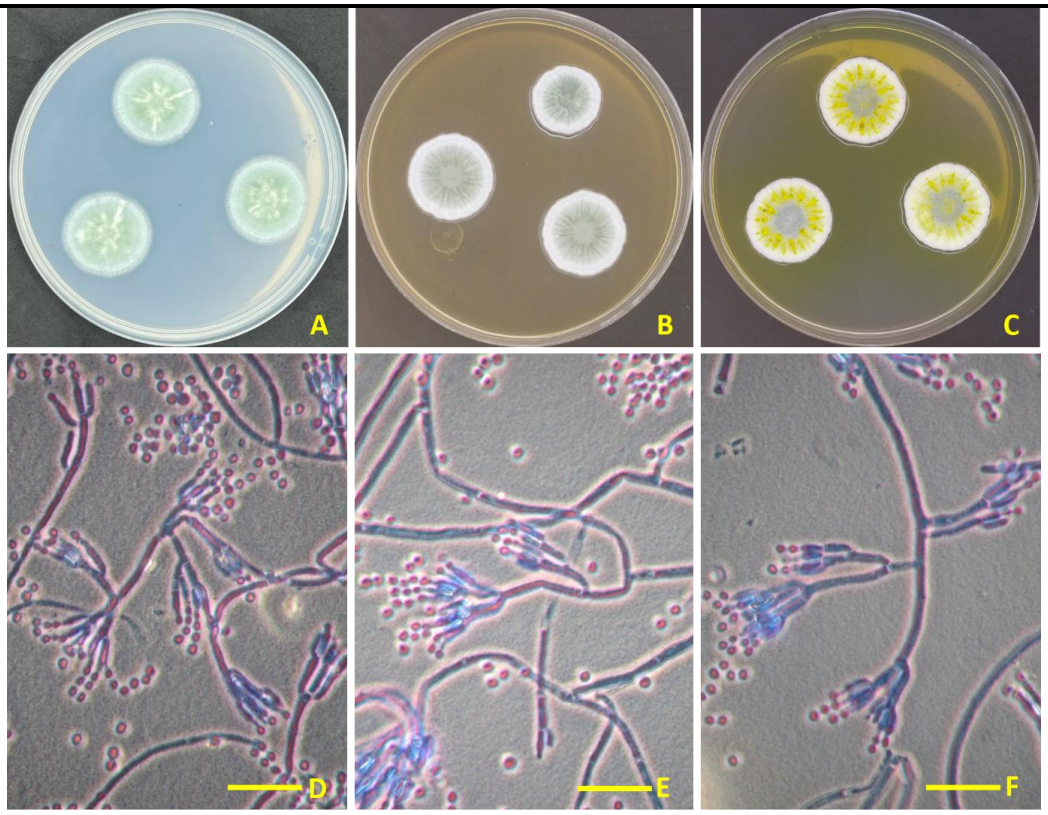

Figure 6. *Penicillium goetzii* AUMC 498. (A–C) Seven-day-old colonies on Cz, MEA, and CYA at 25 C. (D–F) Smooth biverticillate conidiophores bearing metulae and ampulliform phialides and globose to subglobose conidia (Scale bar = 20 µm).

4. DISCUSSION

In recent years, research on the extracellular enzymatic behaviour of several microorganisms has gained attention due to the possibility of using them as biotechnological repositories of industrially required enzymes [10, 34–39]. Amylases are essential enzymes used to hydrolyse polysaccharides, such as starch, into simple sugar components [34, 39–41]. Despite being present in a wide range of materials, such as plants and animals, amylases are usually derived from microbial sources for industrial use [35]. In the field of starch processing, microbial amylases have effectively counteracted chemical starch degradation. They continue to have potential use in a number of production processes, such as the dairy, baking, brewing, detergent, fabric, and paper industries, in addition to their use in starch saccharification. Amylase is now used in a wide range of fields, such as clinical, pharmaceutical, and analytical chemistry, thanks to the development of modern technological frontiers [35].

The synthesis of amylase from a range of fungi has been the subject of numerous studies [1, 39, 42-47]. In order to identify better fungal strains for large-scale production and to specify culture conditions, the majority of research on the use of fungi to create α-amylase has concentrated on mesophilic fungal species [6]. The two most common fungal sources for amylase synthesis are *Aspergillus* and *Penicillium* [48, 49]. Media optimization, particularly for fungal strains that produce amylase, is one of the most

important factors to consider when creating fermentation technology [44, 50]. *P. citrinum* AUMC 576 was able to create a cold-active amylase that was active over a broad pH range (4–10) and temperature range (25–35 ºC) when the amylase production conditions were optimized for this investigation. The highest amylase activity of 7.63±0.68 U/mL was produced at pH 7.0 and 25 ºC. After three days of incubation, the activity peaked at 9.92±0.84 U/mL when yeast extract was used as the nitrogen source. However, at pH 7.0 and 10 ºC, *P. goetzii* AUMC 498 generated the highest amylase activity, 2.187±0.2 U/mL. After seven days of incubation, the amylase activity peaked at 3.7±0.34 U/mL when beef extract was used as the nitrogen source.

Penicillium chrysogenum's α-amylase synthesis peaked at 6–8 days at 30 °C with an initial pH of 4.0–5.0, addressing this worry. The ideal circumstances for different fungus species vary greatly, according to published studies. Accordingly, each species' ideal culture circumstances should be carefully examined. *Aspergillus clavatus* (1.29 U/mL), *P. camemberti* (0.4 U/mL), *P. citrinum* (0.27 U/mL), *P. chrysogenum* (0.48 U/mL), and *Penicillium* sp. (0.27 U/mL) [14] all produced amylase activity that was lower than that of *Penicillium chrysogenum*, which showed 155 U/mL [43], *P. janthinellum* NCIM 4960 yielded 120 U/mL [45], and *P. expansum* MT-1 produced 25.3 U/mL [46].

CONCLUSION

Penicillium citrinum AUMC 576 and *P. goetzii* AUMC 498 were shown to be the most active strains under SmF at 10 and 30 ºC, respectively. After 7.0 days, *P. goetzii* demonstrated its maximum amylase production at pH 7.0 and 10 ºC using the beef extract as the nitrogen source. While using the yeast extract as a nitrogen source, *P. citrinum*, on the other hand, demonstrated that amylase production peaked at pH 7.0 and 25 ºC after 3.0 days.

Supplementary material: Table 1. *Penicillium* and *Talaromyces* species, number of isolates per species, and isolation source of the isolates.

Authors' contributions

All authors participated equally to data analysis, authoring, and revising the article. The final version of the manuscript has been reviewed and approved by all authors.

Competing interests

The authors declare no conflict of interest.

Data availability

All data related to this manuscript is incorporated in the manuscript and supplementary material.

REFERENCES

1. N. Arora, S. Kaur, and S. Kaur, 2017. Use of Agro industrial residue for the production of Amylase by *Penicillium* sp. for application in food industry. Journal of Biotechnological Biomater. **7**(2): p. 1-4.

2. O.A. Al-Bedak, R.S. Sakr, and A.M. Al-Kolaibe, 2022. The microbial amylases: an overview with practical consequences and applications. Journal of Microbiol Exp. **10**(4): p. 130-134.
3. R.K. Bhatia, S. Ullah, M.Z. Hoque, I. Ahmad, Y.H. Yang, A.K. Bhatt, and Bhatia S.K., 2021. Psychrophiles: a source of cold-adapted enzymes for energy efficient biotechnological industrial processes. Journal of Environmental Chemical Engineering. **9**(1): p. 104607.
4. H. Abd El-Latif, G. Heba, E.S. Abd El-Fatah Bahaa, S. Fathy and O.A. Al-Bedak, 2019. Molecular identification of naturally isolated *Candida tropicalis* AUN-H100 and optimization of their extracellular amylase production, International Journal of Agricultural Sciences and Veterinary Medicine, **7**(4): p. 28-34.
5. A.D.S. Pereira, R.I.D.C. Fontan, M.Franco, E.C.D. Souza, C.M. Veloso, V.S. Sampaio and R.C.F Bonomo, 2018. Study of alpha-amylase obtained by solid state fermentation of cassava residue in aqueous two-phase systems. Brazilian Journal of Chemical Engineering, **35**: p. 1141-1152.
6. B.R. Shruthi, R.N.H. Achur, and T.N. Boramuthi, 2020. Optimized solid-state fermentation medium enhances the multienzymes production from *Penicillium citrinum* and *Aspergillus clavatus.* Current Microbiology, **77**(9): p. 2192-2206.
7. Y. Liu, N. Zhang, J. Ma, Y. Zhou, Q. Wei, C. Tian, and S. Zhang, 2023. Advances in cold-adapted enzymes derived from microorganisms. Frontiers in Microbiology, 14: p. 1152847.
8. M. Santiago, C.A. Ramírez-Sarmiento, R.A. Zamora, and L.P. Parra, 2016. Discovery, molecular mechanisms, and industrial applications of cold-active enzymes. Frontiers in microbiology, 7: p. 1408.
9. A. Debroy and N. George, 2023. Psychrophiles and psychrozymes: Structural adaptations and applications. in AIP Conference Proceedings, AIP Publishing.
10. A.M. Moharram, A.N.A. Zohri, A.E.L. Hesham, H.E. Abdel-Raheam, M. Al-Ameen Maher and O.A. Al-Bedak, 2022. Production of cold-active pectinases by three novel *Cladosporium* species isolated from Egypt and application of the most active enzyme. Scientific Reports, 12(1): p. 15599.
11. H.D. Kim, S.M. Kim and J.I. Choi, 2018. Purification, characterization, and cloning of a cold-adapted protease from Antarctic Janthinobacterium lividum.
12. J. Ge, X. Jiang, W. Liu, Y. Wang, H. Huang, Y. Bai and H. Luo, 2020. Characterization, stability improvement, and bread baking applications of a novel cold-adapted glucose oxidase from *Cladosporium neopsychrotolerans* SL16. Food Chemistry, 310: p. 125970.
13. P. Yadav, R.P. Singh, S. Rana, D. Joshi, D. Kumar, N. Bhardwaj and A. Kumar, 2022. Mechanisms of stress tolerance in cyanobacteria under extreme conditions. Stresses, 2(4): p. 531-549.

14. Y. Liu, K. Jia, H. Chen, Z. Wang, W. Zhao and L. Zhu, 2023. Cold-adapted enzymes: mechanisms, engineering and biotechnological application. Bioprocess and Biosystems Engineering, 46(10): p. 1399-1410.
15. M. Kuddus, Roohi, N. Bano, G.B. Sheik, B. Joseph, B. Hamid and A. Madhavan, 2024. Cold-active microbial enzymes and their biotechnological applications. Microbial Biotechnology, 17(4): p. e14467.
16. Y.R. Maghraby, R.M. El-Shabasy, A.H. Ibrahim and H.M.E.S. Azzazy, 2023. Enzyme immobilization technologies and industrial applications. ACS omega, 8(6): p. 5184-5196.
17. M. Kuddus, 2018. Enzymes in food biotechnology: production, applications, and future prospects.
18. S. Bruno, D. Coppola, G. di Prisco, D. Giordano and C. Verde, 2019. Enzymes from marine polar regions and their biotechnological applications. Marine drugs, 17(10) p. 544.
19. F. Sarmiento, R. Peralta, and J.M. Blamey, 2015. Cold and hot extremozymes: industrial relevance and current trends. Frontiers in bioengineering and biotechnology, 3: p. 148.
20. Y.F. Qian, J.Y. Yu, J. Xie and S.P. Yang, 2023. A mini-review on cold-adapted enzymes from psychrotrophic microorganisms in foods: benefits and challenges. Current Research in Biotechnology, p. 100162.
21. D. Smith and A.H. Onions, 1994. The preservation and maintenance of living fungi. (CAB international.
22. M.J. Bailey, P. Biely and K. Poutanen , 1992. Interlaboratory testing of methods for assay of xylanase activity. Journal of biotechnology, 23(3) p. 257-270.
23. G.L. Miller, 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31(3): p. 426-428.
24. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193: p. 265-275.
25. A.M. AL-Kolaibe, A.M. Moharram, and O.A. Al-Bedak, 2021. Worthwhile enzyme production and eco-friendly bioconversion of three agricultural residues by *Aspergillus curvatus* and *Aspergillus gaarensis*, promising enzyme-producers isolated from extreme environment. Journal of Basic and Applied Mycology (Egypt), 12: p. 1-14.
26. R.A. Samson, C.M. Visagie, J. Houbraken, S.B. Hong, V. Hubka, C.H. Klaassen and J. Frisvad, 2014. Phylogeny, identification and nomenclature of the genus *Aspergillus.* Studies in mycology, 78(1): p. 141-173.
27. J.I. Pitt, 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*, .

28. C.M. Visagie, J. Houbraken, J.C. Frisvad, S.B. Hong, C.H.W. Klaassen, G. Perrone and R.A. Samson, 2014. Identification and nomenclature of the genus *Penicillium.* Studies in mycology, 78(1): p. 343-371.
29. K.A. Gomez and A.A. Gomez, 1984. Statistical procedures for agricultural research. : John wiley & sons.
30. A.H. Moubasher, M.A. Ismail, R.A. Mohamed and O.A. Al-Bedak, 2016. Xylanase and cellulase production under extreme conditions in submerged fermentation by some fungi isolated from hypersaline, alkaline lakes of Wadi-El-Natrun, Egypt, Journal of Basic and Applied Mycology, 7: p. 19-32.
31. M.A. Ismail, A.H. Moubasher, R.A. Mohamed and O.A. Al-Beddak, 2018. Agro–industrial residues as alternative sources for cellulases and xylanases production and purification of xylanase produced by *Aspergillus flavus* AUMC 10331 isolated from extreme habitat. Current Research in Environmental and Applied Mycology, 8(3): p. 313-322.
32. A.H. Moubasher, M.A. Ismail, , R. Mohamed and O.A. Al-Bedak, 2019. Production and purification of extreme xylanase from *Aspergillus flavus* AUMC 10331 in sub-merged fermentation, European Journal of Biological Research, 9(1): p. 20-28.
33. O.A. Al-Bedak, E.A. Teama, E.A. Ali, M.T. Said, E.M. Shalaby and Z.A. Moharram, 2020. Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. Journal of Basic and Applied Mycology (Egypt), 11: p. 77-97.
34. A. Akpan and R. Morgan, 2002. Oral candidiasis, Postgraduate medical journal, 78(922): p. 455-459.
35. M.A. Farooq, S. Ali, A. Hassan, H.M. Tahir, S. Mumtaz and S. Mumtaz, 2021. Biosynthesis and industrial applications of α-amylase: a review. Archives of Microbiology, : p. 1-12.
36. S.E. Jujjavarapu and S. Dhagat, 2019. Evolutionary trends in industrial production of α-amylase. Recent patents on biotechnology, 13(1): p. 4-18.
37. A. Pandey, C.R. Soccol, P. Nigam and V.T. Soccol, 2000. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. Bioresource technology, 74(1): p. 69-80.
38. S. Erdal and M. Takin, 2010. Production of alpha-amylase by *Penicillium expansum* MT-1 in solid-state fermentation using waste Loquat (*Eriobotrya japonica* Lindley) kernels as substrate. Romanian Biotechnological Letters, 15(3): p. 5342-5350.
39. B.K. Sethi, B. Dikshit, S.L. Sahoo, C. Pradhan, S. Sena and B.C. Behera, 2017. Extracellular production of amylase and protease by *Penicillium Purpurogenum* BKS9. Biosci Bioeng, 5: p. 1-6.

40. R. Gupta, P. Gigras, H. Mohapatra, V.K. Goswami and B. Chauhan, 2003. Microbial α-amylases: a biotechnological perspective. Process biochemistry, 38(11): p. 1599-1616.
41. S. Mishra and N. Behera, 2008. Amylase activity of a starch degrading bacteria isolated from soil receiving kitchen wastes. African journal of Biotechnology, 7(18).
42. S.P.E. Jakheng, J. Thomas, M. Umar, F.S. Ojeleye, A.K. Albert, J.O. Oko and N.A. Kutman, 2020. Screening of Locally Isolated *Penicillium* Species from the Soil for Amylase Production. Journal of Applied Life Sciences International, 25(12): p. 76-83.
43. Q.D. Nguyen, J.M. Rezessy-Szabó, and A. Hoschke, 2000. Optimisation of composition of media for the production of amylolytic enzymes by *Thermomyces lanuginosus* ATCC 34626. Food Technology and Biotechnology, 38(3): p. 229-234.
44. K. Kathiresan and S. Manivannan, 2006. Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. African journal of Biotechnology, 5(10).
45. B. Balkan and F. Ertan, 2005. Production and properties of α-amylase from *Penicillium chrysogenum* and its application in starch hydrolysis. Preparative Biochemistry and Biotechnology, 35(2): p. 169-178.
46. R. Sindhu, G. Suprabha and S. Shashidhar, 2009. Optimization of process parameters for the production of a-amylase from *Penicillium janthinellum* (NCIM 4960) under solid state fermentation. African Journal of Microbiology Research, 3(9): p. 498-503.
47. M. Ahmad, A. Bilkisu and U. Shamsuddeen, 2024. Production of amylase enzyme by *Aspergillus* and *Fusarium* species using sugar cane bagasse. UMYU Journal of Microbiology Research, 9(1): p. 202-213.
48. S. Arifeen, J. Jamil, A. Sarwar, N. Ullah, R. Nelofer, T. Aziz and T. Albekairi, 2024. Biosynthesis and optimization of amylase from *Bacillus* sp. Isolated from soil samples using agro-industrial waste as substrate. Applied Ecology and Environmental Research, 22(4): p. 2927-2940.
49. A. Samanta and S. Jana, 2024. Optimization of cold active amylase production by mesophilic *Bacillus cereus* RGUJS2023 under submerged fermentation. Journal of Environmental Biology, 45(1): p. 16-24.
50. O. Adeoyo and O. Adewumi, 2024. Characterization of α-amylase and antimicrobial activity of *Penicillium chrysogenum.* Journal of BioScience and Biotechnology, 13(1): p. 23-28.