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Research article

Optimizing conditions for augmented production of amylase by *Talaromyces islandicus* AUMC 11391 ☆

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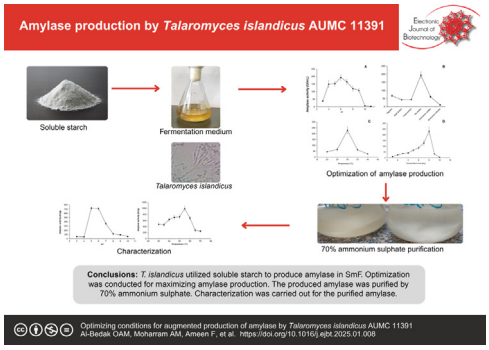
GRAPHICAL ABSTRACT

Optimizing conditions for augmented production of amylase by *Talaromyces islandicus* AUMC 11391.



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ABSTRACT

Background: To satisfy rising biotechnological needs, climate change, and depleting water supplies, amylases that can survive high temperatures, and salt concentrations must be developed. Amylases are the most important enzymes that comprise approximately 25–33% of the international enzyme market and have a great role in many industries.
Results: In this investigation, *Talaromyces islandicus* AUMC 11391 was used to produce amylase in submerged fermentation (SmF) in an augmented concentration of 232 ± 36 U/mL after 8 d of incubation at pH 6.0 and 30°C using sodium nitrate as a nitrogen supply. The obtained enzyme was partly purified employing 70% ammonium sulfate and then dialysis. The activity of the produced amylase exhibited reached the peak (992.14 ± 80 U/mg protein) at pH 5.0 and 55°C. Cu, Co, Fe, Ni, Ca, and Zn had an activating effect on the activity of the amylase enzyme at pH 5.0 and 55°C by 134.57, 123.1, 115.4, 109.76, 105.43, and 103.2%, respectively. The K_m and V_{max} were recorded as 132.1 mM and 60.6 µmol/min, respectively. *T. islandicus* AUMC 11391's-amylase hydrolyzed the raw starch of maize, sorghum, wheat,

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Starch
Talaromyces

rice, and oat at rates of 12.3, 113.7, 32.25, 34.67, and 73.6% in contrast to the soluble starch.
Conclusions: This enhanced α-amylase from *T. islandicus* AUMC 11391 looks to be a potential option to meet the present amylase requirements of a variety of industrial processes because of its improved production and beneficial properties.
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1. Introduction

Amylases (namely α-amylase, E.C.3.2.1.1; β-amylase, EC 3.2.1.2; and γ-amylase, EC 3.2.1.3) are the biocatalysts, which undergo biodegradation of starch molecule into glucose, maltose, maltotriose and dextrin through the breakdown of the glycosidic bonds in the starch polymer [1]. Amylases are the most important enzymes that comprise approximately 25–33% of the international enzyme market and have a great role in many industries such as bioethanol production, starch saccharification, food, textile, brewing and paper industries as well as in many other fields such as medicinal and pharmaceutical, analytical chemistry and biotechnology [2,3,4]. Because of their simplicity of manufacture and low cost, fungal amylases are more dependable and favored over other sources.

Amylases have a variety of uses in a number of different sectors, including the food, fermentation, textile, paper, detergent, and sugar industries. It may be utilized in biotechnology-related fields such as environmental pollution removal, converting starch into required substrate by a variety of microbes, absorbing waste that contains starch, and producing biochemical materials with the aid of starch substrate [3,5,6].

Due to their affordability and simplicity of manufacturing, fungi provide amylases that are more dependable and preferable than those from other sources. In the food, fermentation, paper, and textile sectors, all of these enzymes have several uses [7,8]. Since many years ago, α-amylase has been widely used in starch-based industries. Although there are numerous microbiological sources for the effective synthesis of this enzyme, only a few carefully chosen strains of fungi and bacteria are suitable for industrial production. It is a continual effort to look for novel microbes that can be used to produce amylase. Just a few microorganisms are allowed in the food industry, and enzyme producers are typically keen to expand their product lines into the food processing sector [9,10].

Yet, the use of microbial enzymes in industrial processes has increased dramatically in recent years, and the Penicillia appears to be particularly well-positioned to play a bigger role in the future production of these enzymes [9,10]. The manufacturing of enzymes should be concentrated on using low-value agro-industrial wastes as substrates, since this would lower the cost of production and assist in addressing the disposal and pollution issues associated with these materials. As a result, the current work sought to optimize the amylase production fermentation conditions of *Talaromyces islandicus* AUMC 11391 in submerged fermentation (SmF) in order to create amylase enzyme, which was subsequently partly isolated and characterized.

2. Materials and methods

2.1. Isolation of the fungal isolate

Utilizing the direct plating technique [11] on Czapek's Dox agar [12], *T. islandicus* used in this investigation was isolated from rice grains collected from El-Minia Governorate, Egypt [13]. It was discovered to be the most prolific amylase-producing fungus on sucrose-free Cz agar supplemented with 1% soluble starch, and it was chosen for this study's amylase production. The strain was kept at the Assiut University Mycological Centre's culture collection as frozen cultures in 15% glycerol-water at −70°C, as well as lyophilized specimens with accession number AUMC 11391.

2.2. Morphological and molecular identification of the fungal isolate

Morphological characteristics in the culture and growth rates of the strain were studied on Czapek's gar (CZ), malt extract agar (MEA), and Czapek's yeast Autolysate agar (CYA) [12]. Inoculations were made from spore suspension in a three-point design using a 1.0 μL inoculum. The dim, opposite, cultures were incubated at 25°C for 7 d. DNA of the fungus in this study was isolated [14], and the PCR reaction was performed at SolGent Co. (South Korea), using SolGent EF-Taq [15]. For ITS region amplification, ITS1 and ITS4 universal primers were used [16]. Contiguous sequence of the *Talaromyces* species included in this study was produced using the DNASTAR (version 5.05). The total ITS dataset for phylogenetic analysis contained 25 sequences, sequence of *Aspergillus terreus* ATCC 1012 as outgroup, sequence for *Talaromyces* sp. AUMC 11391 in this work, and 23 sequences retrieved from GenBank related to the genus *Penicillium*. All sequences were aligned together using MAFFT (version 6.861b) with the default options [17]. BMGE was used to optimize the alignment gaps and parsimony uninformative characters [18]. Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analyses were performed by MEGA X (version 10.2.6) [19], and the robustness of the most parsimonious trees was applying 1000 replications [20]. Utilizing Modeltest 3.7′s Akaike Information Criterion (AIC), the optimum nucleotide substitution model for ML analysis was identified [21]. The resulting tree was edited and saved as TIF format [22].

2.3. Optimization of the amylase production in SmF

Under one factor at a time (OFAT) settings [23,24], the strain's respective pH, nitrogen supply, temperature, and fermentation period were changed. The studies used 50 mL of sucrose-free Czapek's broth [25] as the fermentation medium in 250 mL Erlenmeyer flasks. As the only carbon source, 1% soluble starch was supplied. The flasks were each individually inoculated with spore suspension made from the 7 d-old culture of the *T. islandicus* strain AUMC 11391 (2.0%; v/v) grown on CYA at 25°C. The flasks were then incubated under various operating conditions, including pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0), nitrogen source (peptone, yeast extract, beef extract, sodium nitrate, ammonium chloride, and ammonium sulfate; each at 0.2%), temperature (20, 25, 30, 35, and 40°C), and incubation time (1–10) days. Three separate experiments were done.

2.4. Extraction and assay of amylase enzyme and total protein estimation

The cell-free supernatant was recovered after the incubation time by centrifugation (10,000 × g at 4°C for 10 min), and it was employed as an amylase source. By combining 0.5 ml of filtered crude enzyme with 0.5 ml of 1.0% starch (prepared in 50 mM Na-citrate buffer, pH 5.0), amylase activity was measured. The reaction mixture was incubated at 50°C for 20 min [26,27]. The procedure was then halted by adding 2 ml of 3, 5-dinitrosalicylic acid (DNS) and heating it for 10 min in a water bath. After cooling, a UV–Visible spectrophotometer (T80+; UK) was used to detect the absorbance at 540 nm. Soluble protein was determined using Folin Lowry's method [28] and bovine serum albumin (BSA) as standard. One unit of the amylase activity (U) was defined as the amount of amylase needed to release 1.0 μmol glucose per min. Using glucose as the standard, the amylase activity was determined [3]. The total protein determination was carried out following the method of Lowry et al. [28].

2.5. Production and partial purification of amylase in SmF

The same fermentation medium and processing parameters as those previously indicated have been employed in SmF to produce amylase. After incubation, 10,000-rpm centrifugation was employed to separate the cell-free supernatant for 10 min at 4°C. The amylase enzyme was obtained from the supernatant by applying 70% ammonium sulfate precipitation. Using a Freeze dryer (VirTis, Model #6KBTES-55; USA), the precipitated protein was separated and lyophilized. The dried enzyme was dissolved in citrate buffer (pH 5.0) and dialyzed twice against the same buffer for two hours each time, removing the buffer after each dialysis. Then leave out tiny molecules and obtain an enzyme by buffing it overnight at 4°C. The partially purified dialyzed amylase was subsequently lyophilized using a Freeze dryer and utilized in subsequent tests.

2.6. Effect of pH and temperature on amylase activity

The partially purified and soluble starch totaled 0.01 g in the reaction mixture (each was prepared independently in a 50 mM buffer solution of 1.0 mL). The reaction was carried out at a range of pH values from 3.0 to 11.0 in increments of 1.0. At the ideal pH level, amylase activity was tested at a temperature improvement of 5°C between 30°C and 80°C. Each component of the reaction mixture, 0.01 g partial-purified amylase and 0.01 g soluble starch, had been produced separately in 1.0 mL of a 50 mM buffer solution. The enzyme activity was calculated using the glucose standard curve and the estimated absorbance at 540 nm [3,27].

2.7. Effect of some ions and inhibitors on amylase activity

The addition of monovalent or divalent ions (NaCl, KCl, $CaCl_2$, $MgSO_4$, $MnSO_4$, $CoCl_2$, $NiCl_2$, $CuSO_4$, $FeSO_4$, and $ZnSO_4$) to the reaction mixture (5 mM) allowed us to explore the impact of certain ions on the amylase enzyme's activity. Using ethylenediaminetetraacetic acid (EDTA) or sodium dodecyl sulfate (SDS) at a concentration of 5 mM, the impact of an enzyme inhibitor was also assessed. Under controlled circumstances, the amylase enzyme's activity was tested to determine 100% activity in the absence of ions or inhibitors.

2.8. Determination of kinetic constants (K_m and V_{max})

K_m (Michaelis-Menten constant) and V_{max} (maximum reaction velocity) values of the amylase enzyme were determined by measuring enzyme activity at different concentrations of soluble starch (1–20 mg/mL) and were estimated [29].

2.9. Substrate specificity of amylase enzyme

The amylase substrate specificity was investigated using 1.0% (w/v) raw starch from several sources (Corn, sorghum, wheat, rice, and oat). The activity was measured for 20 min at pH 5 and 55°C, and the amount of reducing sugars released was quantified using the DNS technique at 540 nm and compared to that on soluble starch.

3. Results

3.1. Morphological and molecular identification of Talaromyces islandicus

The *Talaromyces* strain in this study shared the identical morphological features of the type species. Colonies on Cz, MEA, and CYA are slow-growing, attaining 18, 25, and 25 mm in diameter, respectively, after 7 d at 25°C. Colonies funiculose to floccose, with Greyed-Orange margin. Conidiophores short up to 100 μm long, smooth. Penicilli typically biverticillate symmetrical. Metulae in verticils of 4–6, 8–14 × 1.5–3.5 μm. Phialides in verticils of 5–8, closely packed, lanceolate, 9–13 × 1.5–2 μm. Conidia elliptical, smooth, 2.5–3.5 × 1.5–2.5 μm (Fig. 1).

Phylogenetic analysis of ITS dataset was employed to determine the taxonomic status of the *Talaromyces* strain in this study to other *Talaromyces* species. The maximum parsimony dataset consisted of 573 characters of which 461 characters could aligned unambiguously, 113 variable characters which were parsimony-uninformative, and 47 characters were counted as parsimony informative. Tamura 3-parameter and using a discrete Gamma distribution with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (T92 + G + I) was the perfect model for nucleotide substitution. Maximum Parsimony analyses resulted in 3 most parsimonious trees with a tree length of 248 steps, a consistency index of 0.497041, a retention index of 0.635193 and a rescaled consistency index of 0.315717. Maximum likelihood analysis yielded one tree (–ln likelihood = 2 036.85) (Fig. 2). The phylogenetic result characterized the relationships between the *T. islandicus* AUMC 11391 (OQ108584) with other *Talaromyces* species. The strain located within *T. islandicus* clade at the same branch as *T. islandicus* NRRL 1036 and *T. islandicus* CBS 338.48 endorsing very strong bootstrap support of 99% ML/95% MP (Fig. 2).

3.2. Optimization of culture conditions for maximum amylase production

In order to maximize the production of amylase, changes were made to the pH, nitrogen supply, temperature, and fermentation time at OFAT. The current research has found that *T. islandicus* AUMC 11391 could generate 194.4 ± 20 U/mL of amylase activity and that amylase production was increased when the fermentation medium's pH was 6.0 at 25°C when sodium nitrate was provided as a source of nitrogen (Fig. 3A–B). Amylase production was most influenced by temperature, and at 30°C, it produced 229.14 ± 32 U/mL (Fig. 3C). During the eighth day of fermentation, the maximal amylase production (232 ± 36 U/mL) was achieved (Fig. 3D).

3.3. Yield and activity of amylase enzyme

T. islandicus AUMC 11391 was able to generate 3.48 g of amylase powder per liter of fermentation medium after 70%

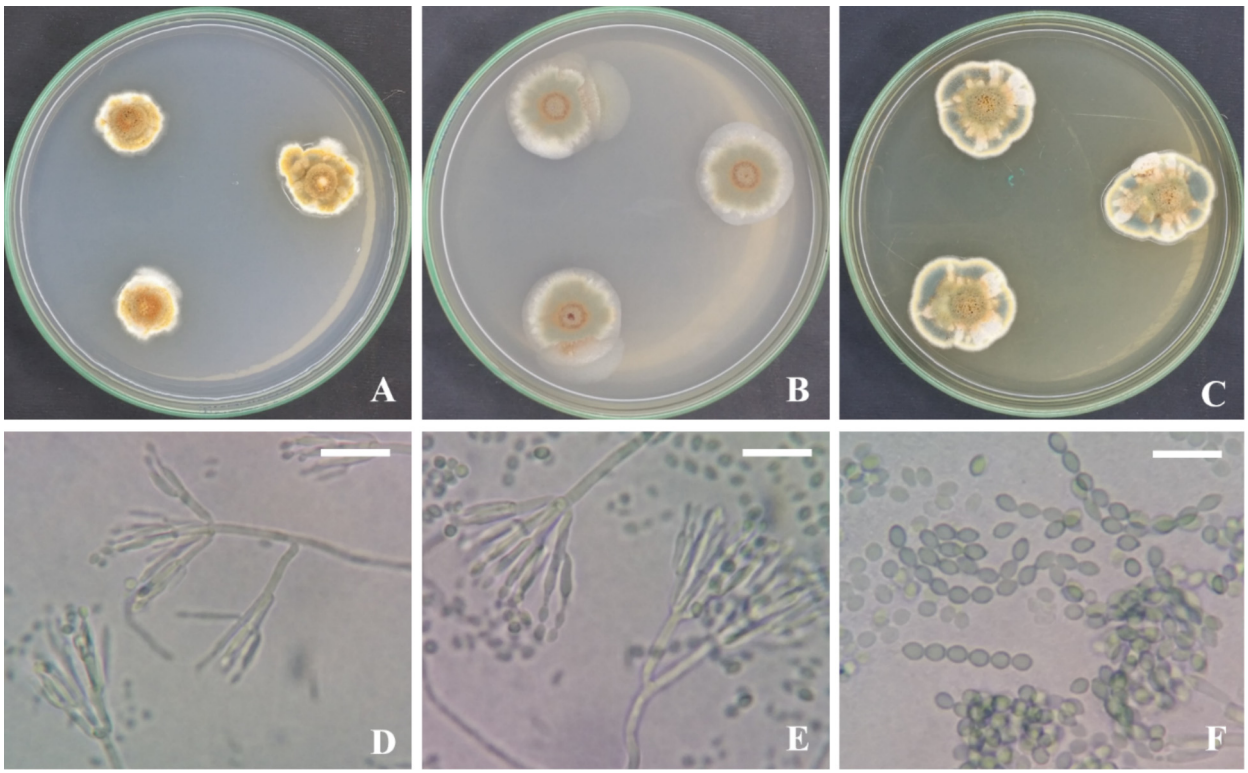

Fig. 1. *T. islandicus* AUMC 11391 (A–C), Seven-day-old colonies on Cz, MEA and CYA at 25°C. (D–E), Biverticillate, symmetrical penicilli. (F), Ellipsoidal conidia. Scale bars (D–E) = 20 μm (F) = 10 μm.

ammonium sulfate precipitation and dialysis. At pH 5.0 and 50°C, the enzyme had a specific activity of 722.6 ± 35 U/mg protein (Fig. 4), which rose to 992.14 ± 80 U/mg enzyme at 55°C (Fig. 5).

3.4. Effect of some ions and inhibitors on amylase activity

Cu, Co, Fe, Ni, Ca, and Zn had an activating effect on the activity of the amylase enzyme at the optimal conditions (pH 5.0 and 55°C), resulting in specific activity of 1335 ± 62, 1221 ± 48, 1146 ± 56, 1090 ± 50, 1046 ± 60, and 1023.8 ± 44 U/mg enzyme, which represented 134.57, 123.1, 115.4, 109.76, 105.43, and 103.2% residual activity, respectively, over the control (Table 1). The other ions and inhibitors tested exhibited an inhibitory impact, where the amylase-specific activity decreased to its least value reporting residual activity of 137.8 ± 20, 352.75 ± 28, 388.4 ± 32, and 637.8 ± 43 U/mg enzyme in case of SDS, K, Na, and Mn, respectively.

3.5. Kinetic parameters of amylase produced by T. islandicus AUMC 11391

Regarding the impact of various starch concentrations on enzyme activity, the findings demonstrated that α-amylase activity increased with the addition of soluble starch up to 1.0 mg/mL. The K_m and V_{max} were 132.1 mM and 60.6 μmol/min, respectively at 55°C and pH 5.0 (Fig. 6).

3.6. Substrate specificity

The substrate specificity of a-amylase varies from one microorganism to another. In the present study, *T. islandicus* AUMC 11391 α-amylase hydrolyzed the raw starch of corn, sorghum, wheat, rice, and oat at a relative rate of 12.3, 63.3, 32.25, 34.67, and 73.6%, compared to the soluble starch (Table 2).

4. Discussion

In recent years, the opportunity to use microorganisms as biotechnological repositories of industrially essential enzymes has given rise to an interest in exploring extracellular enzymatic behavior in many microorganisms [2,22,23,30,31,32,33,34]. Amylases are essential enzymes used in the starch manufacturing industry for the hydrolysis of polysaccharides such as starch into simple sugar constituents [3,6,35]. While amylases can be extracted from many sources such as plants and animals, the enzymes usually fulfill industrial demand from microbial sources [36,37,38,39]. In the starch processing industry, microbial amylases have successfully compensated for chemical starch degradation. They still have potential uses in a number of manufacturing processes, such as the dairy, baking, brewing, detergent, fabric and paper industries, in addition to their use in starch saccharification. The use of amylase has grown to include many other fields, such as clinical, medical and analytical chemistry, with the advent of modern biotechnology frontiers [38]. Several studies have been conducted to investigate the production of amylase enzymes from a wide range of fungi [1,2,40,41].

Amylases are found in a wide variety of bacteria and fungi. Most investigations on α-amylase production using fungi have used some mesophilic fungal species, with attempts made to specify culture conditions and select better fungal strains to manufacture in large quantities [42,43,44,45]. Exo-acting, endo-acting, and debranching enzymes are the three types of amylases. α-amylase is an endo-acting enzyme, whereas β-amylase is an exo-acting enzyme [46]. The two most common fungal sources for amylase synthesis are *Penicillium* and *Aspergillus* [47,48,49,50].

The optimization of media is a key factor to consider in the development of fermentation technology. However, there have been few publications on optimizing medium composition, particularly for fungal strains in amylase production [51,52]. The

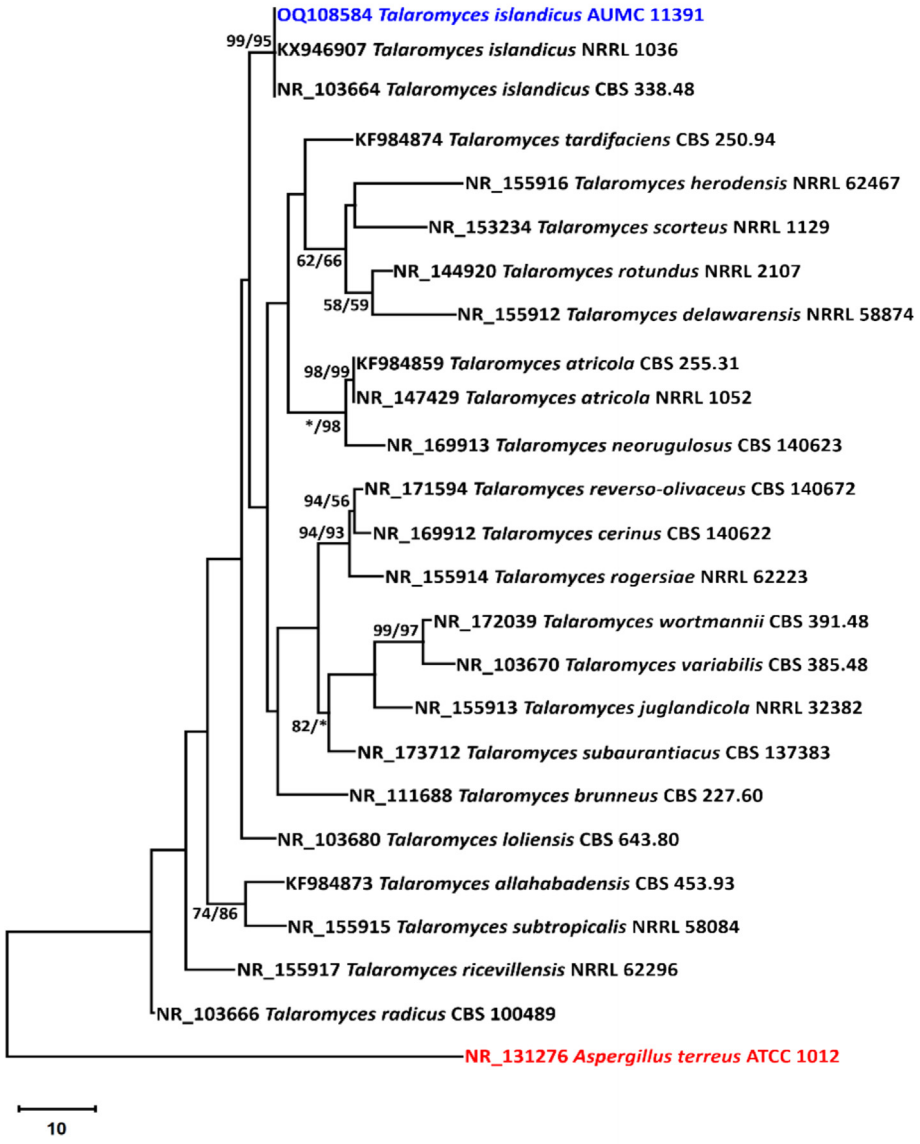

Fig. 2. The first of 1 000 equally most parsimonious trees obtained from a heuristic search (1000 replications) of *T. islandicus* AUMC 11391 (in blue color) compared to other closely ITS sequences belonging to genus *Talaromyces* in GenBank. Bootstrap support values for ML/MP ≥50% are indicated above/below the respective nodes. The tree is rooted to *A. terreus* ATCC 1012 as outgroup (in red color).

optimization of the amylase production conditions in this study revealed that *T. islandicus* AUMC 11391 was a potential amylase producer capable of generating the most amylase enzyme (232.1 4 ± 36 U/mL) following an 8 d incubation at pH 6.0 and 30°C using peptone as a nitrogen source. To the best of our knowledge, this is the first time *T. islandicus* has been used to produce amylase. However, *Penicillium pinophilum* strain MS20 has been used in the production of endoglucanase [53], and the strain F2 for the production of alkaline cold-active endoglucanase and pigment [54]. *Penicillium funiculosum* ATCC 11797 has been used to produce cellulase enzymes [55]. α-amylase synthesis by *Penicillium chrysogenum* peaked at 6–8 d with an initial pH of 4.0–5.0 at 30°C. According to the published studies, various fungal species have extremely varying optimal conditions. This means that the optimal culture conditions must be investigated specifically for each species.

According to the current findings, *T. islandicus* AUMC 11391 is reported herein as a promising amylase producer that is capable of producing 232.14 ± 36 U/mL at optimal conditions. This yield was considerably higher than that of each of *P. chrysogenum* which reported 155 U/mL [56], *P. janthinellum* NCIM 4960 which yielded 120 U/mL [57], *P. expansum* MT-1 that produced 25.3 U/mL [58], and *Rhizopus arrhizus* which produced 22.4 U/mL of amylase activity [59], and much more higher than the amylase activity produced by *Aspergillus clavatus* (1.29 U/mL), *Penicillium camemberti* (0.4 U/mL), *Penicillium citrinum* (0.27 U/mL), *P. chrysogenum* (0.48 U/mL) and *Penicillium* sp. (0.27 U/mL) [8]. pH and temperature of the growth media influence enzyme synthesis by altering the flow of components from the cell membrane [60].

The optimal pH and temperature for the enzyme activity were 5.0 and 55°C when the enzyme activity was evaluated at different pH buffer systems and temperatures in this study. Most fungal α-amylases have pH optima in the acidic to neutral range [36] as well as moderate temperatures. *Penicillium expansum*'s amylase showed its peak activity at pH 4.5 and 60°C [61]. The optimal pH for *Aureobasidium pullulans*'α-amylase was documented to be 5.0 at 55°C [62]. α-amylase from *Aspergillus flavus* was determined to be 7.0

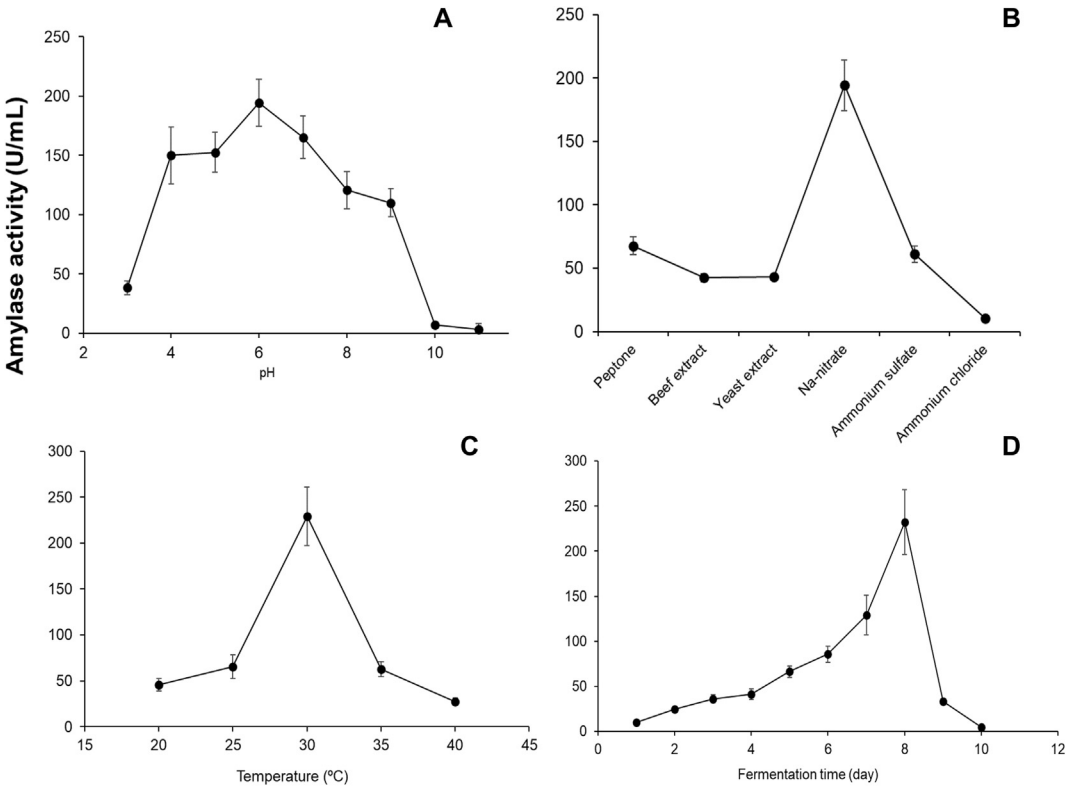

Fig. 3. Optimization of *T. islandicus* AUMC 11391′s amylase production conditions (A), Effect of pH of the medium (B), Effect of different nitrogen source (C), Effect of incubation temperature (D), Effect of fermentation time.

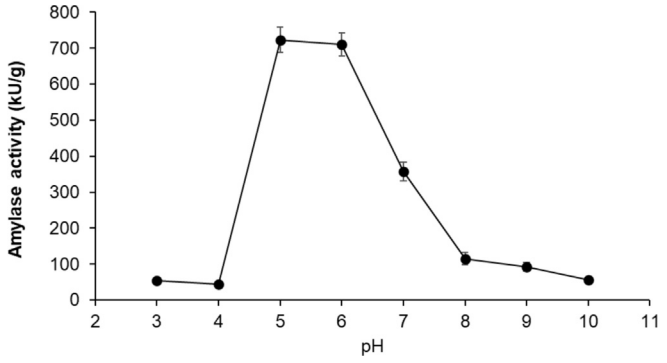

Fig. 4. Effect of pH on the activity of pure amylase produced by *T. islandicus* AUMC 11391.

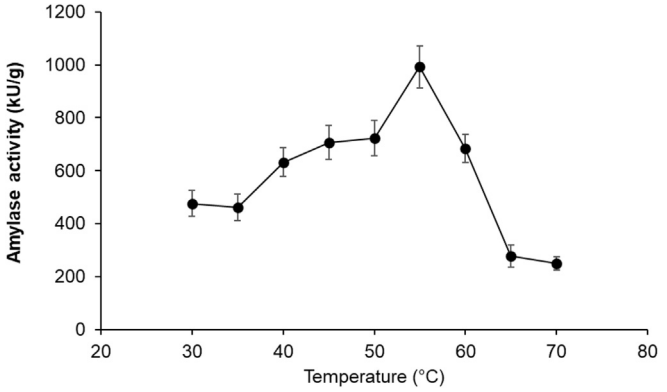

Fig. 5. Effect of temperature on the activity of pure amylase produced by *T. islandicus* AUMC 11391.

Table 1
Effect of metal ions and inhibitors (5 mM) on amylase activity produced by *T. islandicus* (mean ± SD, n = 3). The results are expressed as the residual activity (%) in the tested inhibitory conditions, from the amylase activity in the control without inhibitors.

Ions and inhibitors	Residual activity (%)
Control	100
Na^{+}	39.15
K^{+}	35.74
Ca^{2+}	105.43
Mg^{2+}	98.4
Mn^{2+}	63.0
Co^{2+}	123.1
Ni^{2+}	109.76
Cu^{2+}	134.57
Fe^{2+}	115.4
Zn^{2+}	103.2
EDTA	86.12
SDS	13.84
L.S.D. at 5%	1.27

at 30°C [63]. The optimum reaction pH and temperature were 5.0 at 30–40°C, respectively, for the amylase produced by *P. chrysogenum* [56]. The maximum activity of amylase produced by *Trichoderma harzianum* was determined at pH 4.5 and 40°C [64]. *Candida tropicalis* AUN-H100 produced its maximum amylase at pH 5 and 30°C [2]. *R. arrhizus* demonstrated the highest activity at pH 5.0 [59].

The ideal range of various parameters is contingent upon the fermentation process utilized, microbial sources, intended end product, and several more considerations. Two temperatures are necessary during manufacture within the optimal range. The temperature is essential for microbial growth and proper enzyme

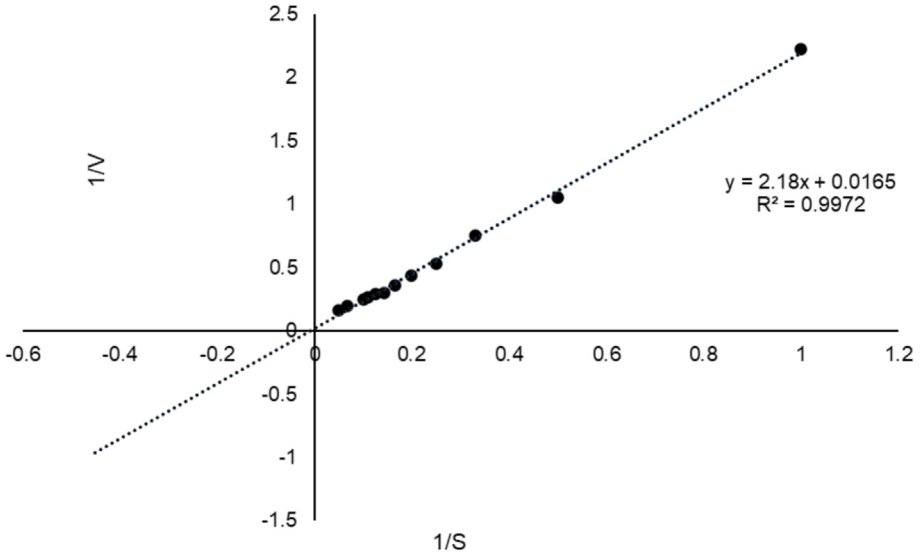

Fig. 6. Lineweaver-Burk plot of the reciprocal of initial velocities and starch concentration.

Table 2
Specific and residual activities of α-amylase produced by *T. islandicus* AUMC 11391 toward different raw starch substrates compared to the soluble starch at pH 5 and 55°C.

Starch type	Residual activity (%)
Soluble starch	100
Corn starch	12.3
Sorghum starch	63.3
Wheat starch	32.25
Rice starch	34.67
Oat starch	73.6

production. Viswanathan et al. [65] elucidated that *Bacillus* sp. exhibited optimal α-amylase production at 37°C by submerged fermentation (SmF). The optimal temperature for α-amylase production by *Bacillus subtilis* was 45°C [66]. Thermophilic α-amylases retain efficacy and proliferate at elevated temperatures. α-Amylases derived from *Thermococcus* and *Pyrococcus* have optimal activity at 80°C and 100°C, respectively [67].

The sensitivity and stability of enzymes in many industries are significantly influenced by pH and temperature; therefore, it is essential to regulate the pH and temperature of the production process at an optimal level. Viswanathan et al. [65] indicated that *Bacillus* sp. predominantly exhibited an optimum pH of 7.0 for maximal α-amylase production. The extracellular amylase generated by *Thermomyces lanuginosus* exhibits peak activity at pH 6.0 [68]. α-Amylase exhibits an optimal pH of 7.0, derived from *Bacillus amyloliquefaciens* [69]. Peak enzyme activity is achieved at an optimal duration. If the duration is less than optimal, it diminishes yield. In numerous instances, extending the incubation period beyond the optimal duration results reduced the enzyme synthesis due to nutrient depletion [70] and the accumulation of hazardous by-products in the medium [6].

α-amylase is the most effective industrial catalyst, exhibiting stability over extended periods under harsh conditions [71]. The relative activity of α-amylase is maximized at a slightly acidic pH of 6.0, exhibiting 100% relative activity. When the pH shifts from 7.0 to 9.0, the activity precipitously declines, retaining only 10% of the relative activity. When the pH ranges from 5.0 to 7.0, α-amylase maintains 80% of its relative activity. α-amylase functions optimally at slightly acidic to alkaline pH levels, maintaining stability across a broad pH range of 5.0 to 8.0, where approximately 60% relative activity is reported. Between pH 4.0 and 6.0, there is a significant reduction in relative activity of approximately 60%. At this acidic pH, the active sites of enzymes are hindered due to elevated hydrogen ion concentration [72].

In this study, the crude enzyme extract was utilized to purify amylase through ammonium sulfate precipitation followed by dialysis. At 70% ammonium sulfate, the highest activity was recorded. The protein was then dialyzed, and the activity was measured. The specific activity of the partly purified amylase enzyme was 992.14 ± 80 U/mg. Several previous studies demonstrated that the enzyme activity was improved by the ammonium sulfate partial purification. In this sense, *Penicillium camemberti* PL21 exhibited 154.2 U/mg protein after ammonium sulfate precipitation [40]. The cell-free dialyzed α-amylase from *A. terreus* NCFT4269 displayed 15.24 U/mg protein-specific activity [73]. The specific activity of *Aspergillus penicillioides* TISTR3639 amylase was improved to 43.3 ± 0.1 U/mg by ammonium sulfate precipitation [74]. The specific activity of the amylase enzyme generated by *A. clavatus* (131.4 ± 7.9 U/mg) and that produced by *P. citrinum* (121.84 ± 8.8 U/mg) was also improved [8].

The addition of various monovalent, divalent, and inhibitors had a significant effect on the activity of the amylase enzyme in this investigation. Cu, Co, Fe, Ni, Ca, and Zn had an activating effect on the activity of the amylase enzyme produced in this study. Many studies have confirmed the impact of microbial amylases on metals. In this sense, the enzyme activity of *Mucor* sp. was not affected by the addition of Na, Ca and Mg, but was strongly inhibited by EDTA [75]. The amylase enzyme of *Scytalidium thermophilum* was not activated by several metal ions tested, including Ca, but Hg and Cu inhibited its activity [76]. Metal ions Ca and Co increased the enzyme activity of *Aspergillus niger* JGI 24 [77]. Mn, Ca, Co, Fe, and Ba greatly increased the enzyme activity of *P. citrinum* HBF62–amylase [78]. The *Preussia minima*'s amylase was activated and stabilized mainly by the metal ions Mn and Ca [79]. *Penicillium oxalicum* amylase activity was somewhat enhanced by Mn and Fe, but hindered by Ag, Cu, and SDS, whereas calcium ions had no discernible effect on enzyme activity [80]. The α-amylase of *Streptomyces rochei* was improved by adding Cu, Zn, and Fe [52]. The presence of Ag, Ca, K, Mg, and Si ions resulted in greater dextranase activity of *P. funiculosum* TFZ.91, with Ca ions being the most effective [81]. A large number of enzymes have been shown to require metal ions in order to operate. A vast variety of metal ions are present in both the environment and the body. Although

they have many different roles in proteins, their most important ones include changing protein structures, enhancing the structural stability of the proteins in the configuration required for biological activity, or taking part in enzyme catalysis. Several enzymes must have metal ions present in order to function. In some circumstances, metal ions are likely to link to the enzyme's free cysteine amino acid sulfhydryl (—SH) groups, altering the enzyme's structure and rendering it incompatible with the substrate. This is what causes certain ions to have a deactivating effect on the enzyme.

When ions function as a co-substrate, substrate, or co-factor for an enzyme, they can bind or chelate with proteins to form complexes that affect protein stability. These ions may induce competitive or non-competitive inhibition of enzymes. In accordance with the Hofmeister series, ions can exert inhibitory or activating effects on enzymes. Halophilic enzymes, exemplified by menadione reductase from *Halobacterium cutirubrum*, necessitate elevated salt concentrations to preserve their optimal activity and stability. Numerous investigations have determined that kosmotropic anions and chaotropic cations enhance enzyme stability, whereas chaotropic anions and kosmotropic cations compromise it. Numerous other experiments corroborated this conclusion [82]. Water can be characterized as a distinctive hydrogen-bonded polymeric structure with low entropy, comprising two local structures: low-density water (LDW) and high-density water (HDW) [82]. An equilibrium exists between LDW and HDW. Kosmotropes concentrate in high-density water (HDW) and move the equilibrium to the left, whereas chaotropes preferentially partition into low-density water (LDW) and shift the equilibrium to the right [30,31]. Extensive research on proteins and other biological molecules has revealed that strong kosmotropic anions stabilize proteins, but strong kosmotropic cations destabilize them.

The current study indicated that amylase activity augmented with the incorporation of soluble starch. The K_m and V_{max} were 132.1 mg/mL (equivalent to 2.87 mmole/mL) and 60.6 μmol/min, respectively, at 55°C and pH 5.0. The K_m of the amylase in this investigation was found to be nearly equivalent to that of the commercial enzyme, Fungamyl (K_m = 2.9 mM), and significantly lower than that of *P. expansum* amylase (K_m = 0.76 mM) [61]. A number of studies established the K_m and V_{max} for fungus amylases. The K_m and V_{max} values for amylase derived from *Trichoderma harzianum*, during the hydrolysis of potato soluble starch and glycogen, were 6.53 mg/ml and 4.5 mg/ml, and 2 μmol reducing sugar/mL and 2.2 μmol reducing sugar/mL, respectively [64]. Using soluble starch as the substrate, α-Amylase from an obligate halophilic *A. penicillioides* isolate exhibited K_m and V_{max} values of 5.41 mg/mL and 1.05 μmol/min, respectively [74]. The purified α-amylase from *Penicillium camemberti* PL21 exhibited a K_m value of 0.92 mg/mL for soluble starch [40].

Enzyme-substrate affinities could be determined using K_m as an "apparent" equilibrium constant. Although this viewpoint is being contested, the mechanical interpretation of K_m has never been properly addressed. K_m is a useful quantity in enzymology due to the potential of being confident about the concentration of substrate yielding (*in vitro*) half maximum V_{max}. Another takeaway from this research is that K_m defines the steady state, which is the true condition that manifests in *in vitro* enzymatic experiments meant to determine K_m.

5. Conclusions

In this investigation, *T. islandicus* AUMC 11391 was used to produce the amylase enzyme in SmF in an augmented concentration at pH 6 and 30°C. The obtained enzyme exhibited its optimum activity at pH 5.0 and 55°C. Cu, Co, Fe, Ni, Ca, and Zn had an activating effect on the activity of the amylase enzyme at the optimal conditions. The K_m and V_{max} were 132.1 mg/mL and 60.6 U/min, respectively. In comparison to the soluble starch, *T. islandicus* AUMC 11391′s -amylase hydrolyzed the raw starch of corn, sorghum, wheat, rice, and oat at a rate of 12.3, 113.7, 32.25, 34.67, and 73.6%. With its improved output and advantageous characteristics, this improved α-amylase from *T. islandicus* AUMC 11391 appears to be a promising choice to fulfil the current amylase requirements of a number of industrial processes.

CRediT authorship contribution statement

Osama A.M. Al-Bedak: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Ahmed M. Moharram:** Writing – review & editing, Project administration, Conceptualization. **Fuad Ameen:** Funding acquisition, Data curation. **Steven L. Stephenson:** Writing – review & editing, Data curation. **Marwa M.M. Idres:** Methodology, Investigation. **Manal M. Yasser:** Project administration, Conceptualization.

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Declaration of competing interest

The authors declare no conflict of interest.

Supplementary material

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Data availability

No data was used for the research described in the article.

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