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Isolation of *Fusarium fujikuroi* AUMC16188 from Alexandria, Egypt as a novel tannase producer exhibiting potent antioxidant activity

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Abstract

Tannase represents a technically crucial enzyme with significant potential to replace hazardous chemicals in various industrial applications. This study reports the successful isolation and screening of novel tannase-producing fungi from Egyptian soils. Among 68 fungal isolates obtained from 24 soil samples, *Fusarium fujikuroi* AUMC16188 emerged as the most promising strain, demonstrating exceptional tannase production (1399.73 U/mL; 264.1 U/mg) under optimized conditions. Genetic identification through ITS sequencing confirmed its novelty as a previously unreported tannase source. Remarkably, the purified enzyme exhibited superior antioxidant activity, showing an IC_{50} of 1.18 µg/mL in 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays-approximately 6.5-fold more potent than Trolox. These findings establish *Fusarium fujikuroi* AUMC16188 as a dual-function biocatalyst with substantial biotechnological potential for sustainable industrial applications.

Keywords: Screening, isolation, application, soil fungi, *Fusarium fujikuroi*, tannase.

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1. Introduction

Since the earliest human civilizations, microbes have been used. The Sumerians and Babylonians are credited with the first commercial use of yeast to make alcoholic beverages (e.g. beer) from barley (malt) in 6000 BC. The prevalent usage of microbial enzymes in many industries, including chemicals, agriculture, energy, food, and medicine, has led to increased acknowledgment of these substances on a global scale. Because of their low energy requirements, shorter processing times, cost effectiveness, environmental friendliness, and non-toxicity, procedures mediated by enzymes from microbes are quickly attaining popularity (Arbige *et al.,* 2019). Fungi are chemo-organo-heterotrophs that can degrade organic materials (Devi *et al.*, 2020). Fungi create a variety of hydrolytic (glycolytic, proteolytic, and lipolytic) and oxidative enzymes for different purposes during their life cycle in nature (Afifi and Bayoumi, 2008; Dhevagi *et al.*, 2021; El-Ghomary *et al.*, 2021; El-Gendy *et al.*, 2021; El-Shall *et al.*, 2022; Kour *et al.*, 2019; Verma *et al.*, 2020). Tannins are hydrophilic polyphenolic compounds classified as secondary metabolites in plants. They represent the second most abundant class of plant phenolics after lignin. Their molecular weights vary depending on the nature of the molecules with which they form complexes (Aguilar and Gutierrez-Sanchez, 2001; Aguilar *et al.*, 2007; Jana *et al.*, 2014). Tannins are divided into two major classes: hydrolysable tannins-including gallotannins and ellagitannins-which are susceptible to enzymatic hydrolysis and serve as primary substrates for tannase, and condensed polyflavonoid tannins, which possess high structural stability and are largely resistant to hydrolytic degradation (Pizzi, 1983). Hydrolysable tannins, typically present in small amounts in plants, are simple derivatives of gallic acid and are classified into two main categories based on their hydrolysis products: gallotannins, consisting of gallic acid compounds and glucose; and ellagitannins, which comprise biaryl units and glucose. Although polyols exist in various forms, the majority of gallotannins isolated from plants contain a polyol residue produced from D-glucose. There are also two other categories: caffe-tannins, composed of quinic acid esterified with caffeic acids and glucose moieties; and tara gallotannins, which consist of quinic acid, gallic acid, and glucose (Giovando *et al.*, 2013; Radebe *et al.*, 2013). One of the most crucial components regarding hydrolysable tannins is tannic acid, which is present in nature as a combination of chemically related compounds, such as penta-(digalloyl)-glucose and tetra-(digalloyl)-glucose or tri-(digalloyl)-di-(galloyl)-glucose, etc. Ellagitannins differ structurally from gallotannins through the formation of additional chemical linkages via oxidative coupling between galloyl units. Their biosynthesis originates from the enzymatic oxidation of gallotannins, resulting in these complex structural features (Pasch and Pizzi, 2002; Radebe *et*

al., 2013). Condensed tannins, commonly referred to as proanthocyanidins, are polymers composed of flavonoid oligomers (such as flavan-3-ol or flavan-3,4-diol) with varying degrees of polymerisation and lacking a sugar core (Aracri *et al.*, 2019). These compounds may be cleaved under strong acidic conditions, yielding their corresponding anthocyanidins (Falcão and Araujo, 2018). In contrast, complex tannins are formed through the interaction between hydrolysable tannins, such as gallotannins or ellagitannins, and catechin or glucoside units, resulting in structurally diverse compounds (Khanbabaee and van Ree, 2001). Tannins serve as key defensive agents in plants, protecting against pathogens and herbivores, and are distributed throughout plant tissues including seeds, roots, bark, wood, and leaves. Their defensive role stems from their ability to form insoluble complexes with proteins (Jana *et al.*, 2014). In humans, tannins contribute to reducing the risk of cancer and cardiovascular disease when consumed through fruit- and vegetable-rich diets. They also support ruminant health by mitigating foam-induced fermentation associated with high-protein feeds such as alfalfa (Pizzi, 1983). Additionally, hydrolysable tannins have shown antiviral properties against Hepatitis B by targeting its covalently closed circular DNA (Liu *et al.,* 2016). They have also been used for centuries in Asian traditional medicine to treat tonsillitis, cancer, infections, diarrhea, and various other conditions (Chowdhury *et al.*, 2004). Tannin acyl hydrolase (E.C.3.1.1.20), also known as tannase, is an intracellular or extracellular inducible enzyme. It catalyzes the breakdown of ester and depside linkages in gallotannins, gallic acid esters, and epigallocatechin-3-gallate, producing glucose and phenolic acids like gallic acid (Biswas *et al.*, 2020; Tang *et al.*, 2024). It has the potential to impact various industries, including chemicals, drinks, dye-making, food, leather, and pharmaceuticals (Biswas *et al.*, 2020; Gezaf *et al.*, 2021). Owing to their exceptional catalytic efficiency and metabolic adaptability, fungal species—particularly *Penicillium, Fusarium,* and *Aspergillus*—are recognized as potent tannase producers and represent predominant candidates for industrial enzyme applications (Arbildi *et al.*, 2025; Govindarajan *et al.*, 2025; Jana *et al.*, 2014). This research aimed to isolate and identify a potent tannase-producing fungal strain from Egyptian soils and evaluate its potential as a natural antioxidant source. The present study successfully established *F. fujikuroi* AUMC16188, isolated from Alexandria soils in Egypt, as a novel tannase producer. The purified enzyme demonstrated exceptional antioxidant efficacy, with an IC_{50} value of 1.18 µg/mL—approximately 6.5-fold superior to the Trolox standard. This finding highlights the dual biotechnological significance of this fungal strain, serving both as an efficient tannase producer and a source of potent natural antioxidants.

2. Materials and methods

2.1 Collection of soil samples

In sterile containers, several soil samples were taken from the surface and a depth of 10 to 20 cm at various sites in the Egyptian governorates of Al-Gharbia, Alexandria, Asyut, and Suhag (Table 1). All samples were brought to the lab, either for immediate use or to be preserved at 4 °C until needed.

2.2 Isolation and identification of fungi

All collected soil samples were serially diluted. On modified Czapek Dox's agar (Pitt and Hocking, 2009), aliquots of the suspension containing 0.1 mL were equally distributed. The plates were incubated for 5-7 days at 28±2 °C. By sub-culturing, produced fungal colonies were separated and purified. The morphology of fungal colonies was investigated, along with microscopic features, and further screening was performed.

2.3 Preservation of fungal isolates

Single colonies of tannase-producing isolates were cultured on modified potato dextrose agar (PDA) slants supplemented with 0.01% tannic acid, stored at 4°C under sterile mineral oil (paraffin oil), and sub-cultured annually (Stanbury *et al.*, 2003). The working isolate was sub-cultured monthly and maintained at 4 °C without mineral oil addition (Bajpai and Patil, 1997).

2.4 Qualitative screening of tannase-producing fungi

Czapek Dox minimal agar medium with tannic acid as sole carbon source was used to screen fungal strains that can produce tannase enzyme. Tannic acid (1% w/v), KCl (0.05% w/v), $MgSO_4$.7H2O (0.05% w/v), $NaNO_3$ (0.3% w/v), K_2HPO_4 (0.1% w/v), yeast extract (0.05 % w/v), and 22 g/L agar-agar were all included in the basal medium, which has a final pH adjustment of 7.0. Separately, the tannic acid solution was sterilized by passing through a 25 mm diameter, 0.45 µm pore size cellulose nitrate membrane filter. Point inoculations were done, and plates were kept at a temperature of 28±2 °C for 5-7 days. After 5 and 7 days of incubation, the colonies were observed (Bradoo *et al.*, 1996; Murugan *et al.*, 2007). The plates were saturated with 0.01 M $FeCl_3$ in 0.01 N HCl and left at room temperature for 5-10 min to produce brown colored clear zone by the tannase-producing fungal colonies (Aguilar *et al.*, 2007). The size of the clear zone was then measured.

2.5 Quantitative screening of tannase-producing fungi

Production of tannase from promising isolates was carried out under submerged fermentation in the modified Czapek Dox's medium with tannic acid as the sole carbon source (Batra and Saxena, 2005; Bradoo *et al.*, 1996). Tannic acid (1% w/v), $NaNO_3$ (0.3% w/v), KH_2PO_4 (0.1% w/v), $MgSO_4$.7H2O (0.05 % w/v), KCl

(0.05 % w/v), and yeast extract (0.05 % w/v) were the components of the medium, which has a final pH adjustment of 5.0. Fungal isolates were used to inoculate the submerged fermentation medium, which was then incubated for 7 days at 28±2 °C under shaking at 120 rpm. Following incubation, the medium was filtered and the extracellular tannase activity was evaluated (El-Fouly *et al.*, 2012; 2015).

Table (1): Sources of isolation of microorganisms producing tannase.

Fungal ID	Number of isolates	Isolation source	Location	Isolation date
A1, A2 (1), HOS. 2	3	Moist sandy soil	Hanoville Beach, Al-Ajami, Alexandria Governorate, Egypt	02/05/2021
A2 (2), A5(2, 4, 5, 8), A (5.4), A6 (2,3,4,5,7,8,9,10)	14	Desert soil sample	Burj Al-Arab city, Alexandria Governorate, Egypt	18/05/2021
B1 (1,2,3), B2 (1,1a,2,3)	7	Moist sandy soil sample	New Damietta, Mediterranean coast, Damietta Governorate, Egypt	11/04/2021
C1 (1,2,3,4,5,6)	6	Moist clay agricultural soil sample	Sohag Center, Sohag Governorate, Egypt	25/04/2021
D4 (3,4,5)	3	Moist clay soil sample under guava trees	Kafr Sunbat Village, Zefta Center, Al-Gharbia Governorate, Egypt	25/04/2021
D4 (5a)	1	Moist clay soil under guava trees	Kafr Sunbat Village, Zefta Center, Al-Gharbia Governorate, Egypt	02/05/2021
E (1,2,3), F (1,2,3,4a,4b), G (1)	9	Moist clay soil under citrus trees		18/05/2021
H (1,2), HOS. 1	3	Sandy soil sample	Kafr Sunbat Village, Zefta Center, Al-Gharbia Governorate, Egypt	02/05/2021
H (3a).	1			18/05/2021
I (1,2,3,4,5,6)	6	Moist clay agricultural soil sample		02/05/2021
J (1,5)	2	Moist clay agricultural soil sample next to wastewater		18/05/2021
J (2,3,4)	3			02/05/2021
K (1a,1b,3,4,5)	5	Clay agricultural soil sample	Juhayna Center, Sohag Governorate, Egypt	18/05/2021
L (1,5)	2	Moist clay agricultural soil next to the fish farm	Faculty of Science (Boys), Al-Azhar University, Assiut Governorate, Egypt	02/05/2021
L (2,3,6)	3			18/05/2021

2.6 Tannase activity assay

The methanolic rhodanine method was used to measure tannase activity (with modifications) (Sharma *et al.*, 2000). The reaction mixture, which contained 0.25 mL of the enzyme sample and 0.25 mL of methyl gallate (0.01 M methyl gallate in 0.05 M citrate buffer, pH 5.0), was incubated for 5 min at 30 °C. To stop the reaction and form a complex between gallate and rhodanine, 0.3 mL of methanolic rhodanine solution (0.67 % w/v) was added. For 5 min, the tubes were kept at 30 °C. After that, 0.2 mL of KOH solution (0.5 M) was added, and the tubes were once more kept at 30 °C for 5 min. A set of blank and control reactions was kept, and a standard curve was produced with gallic acid. A spectrophotometer was used to read the pink color that was formed at 520 nm. Under assay conditions, one unit of tannase activity (U) was defined as the quantity of enzyme necessary to liberate 1 µmol of gallic acid per min (Figure 1). By applying bovine serum albumin (BSA) as a standard, Folin's reagent was used to evaluate the enzyme protein concentration (Lowry *et al.*, 1951). The protein concentration was

indicated as mg of protein per mL of sample utilized (Figure 2).

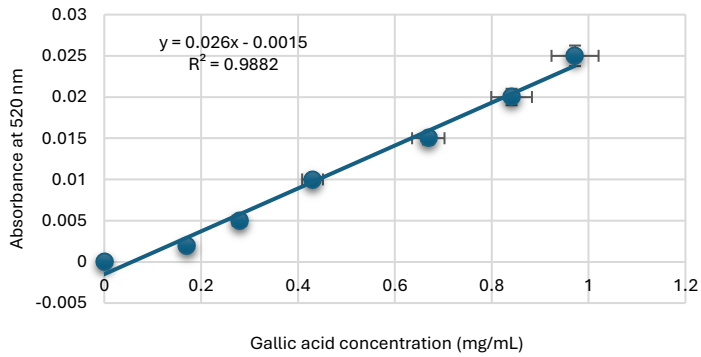

Figure (1): Standard calibration curve of gallic acid.

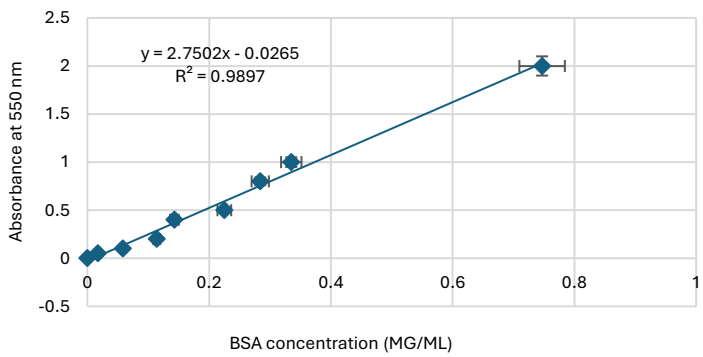

Figure (2): Standard calibration curve of BSA.

2.7 Molecular identification of the most potent fungal isolate for tannase production by ITS examination

To obtain a fresh culture, the fungal isolate was sub-cultured on CYA medium at 28°C for 5 days (Pitt and Hocking, 2009). Extraction of DNA was done using Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). Polymerase chain reaction (PCR) and sequencing were carried out by SolGent Company (Daejeon, South Korea). Specific regions of 18S rRNA sequence were amplified using the universal forward primer ITS1(5'- TCCGTAGGTG AACCTGCGG-3') and ITS4 reverse primer (5'- TCCTCCGCTTATTGATATGC-3'). The purified PCR product was sequenced with the same primers (White *et al.*, 1990). The found sequence was studied using Basic Local Alignment

Search Tool (BLAST). Phylogenetic tree was established with MegAlign (DNA Star) software version 5.05.

2.8 Tannase applications

2.8.1 Tannase antioxidant activity determination using DPPH radical scavenging activity

The ability of tannase to scavenge free radicals such as DPPH was used to determine its antioxidant activity. Purified tannase at a concentration of 8.3 mg/mL was diluted in methanol to 0.13, 0.26, 0.52, 1.04, and 2.08 µg/mL final concentrations. A stock solution of 100 µg/mL concentration of Trolox (positive control) was prepared in methanol, from which 5 concentrations were prepared including 1.95, 3.91, 7.81, 12.00, and 15.63 µg/mL. The DPPH free radical assay was performed as described previously (Boly *et al.*, 2016; Elkholy *et al.*, 2023). Briefly, in a 96-well plate, 100 µL of sample was mixed with 100 µL of freshly prepared DPPH reagent (0.1% in methanol). The reaction was incubated in the dark at room temperature for 30 min, and the resulting decrease in color intensity of DPPH was evaluated at 540 nm. Data were expressed as means ± SD of three replica. Inhibition ratio for purified tannase was calculated using the equation below:

$$\text{Inhibition ratio} = \left(\frac{\text{mean absorbance of blank} - \text{mean absorbance of test}}{\text{mean absorbance of blank}}\right) \times 100$$

Data were normalized using Microsoft Excel and the IC_{50} value was calculated using GraphPad Prism 9., the concentrations of enzyme and Trolox were converted into logarithmic values, and a non-linear inhibitor regression equation was selected: (log (inhibitor) vs. normalized response − variable slope equation).

3. Results

3.1 Morphological identification of the most potent fungal isolate for tannase production

A total of 68 fungal isolates were isolated from 24 soil samples and characterized. Figure (3) describes colonies of *F. fujikuroi* AUMC16188 isolated from moist sandy soil in Alexandria, Egypt after growth on CYA and potato dextrose agar (PDA) at 28±2 °C. Meanwhile, its light microscopic images are demonstrated in Figure (4).

3.2 Qualitative screening of tannase-producing fungi

Qualitative screening of soil fungal isolates on Czapek Dox minimal agar medium revealed their tannase activity. Tannic acid was used as the sole carbon source in the medium, with $FeCl_3$ as an indicator that reacted with the metabolites to produce a brown color as a clear zone (appear from the back of the petri dish) (Figure 5c).

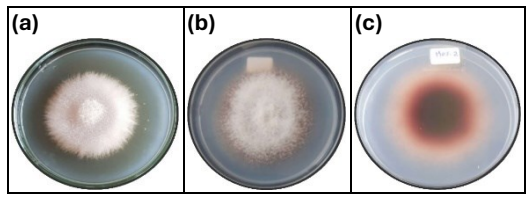

Figure (3): *F. fujikuroi* AUMC16188 cultivated on CYA and PDA at 28±2 °C. (a) *F. fujikuroi* AUMC16188 on PDA after 5 days; (b) *F. fujikuroi* AUMC16188 on CYA after 7 days; (c) *F. fujikuroi* AUMC16188 on CYA after 7 days (Back of the plate).

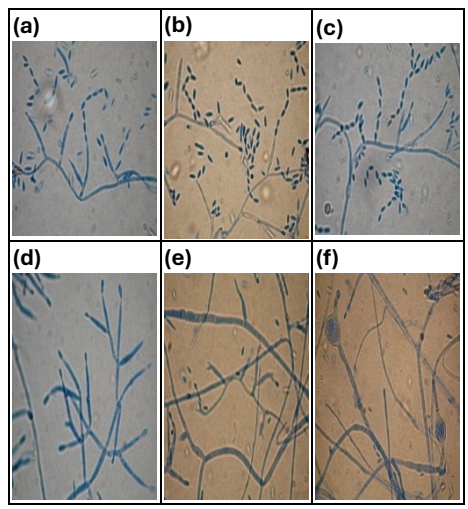

Figure (4): Light microscopic images showing *F. fujikuroi* AUMC16188 at 1000 X. (a), (b), and (c) showing conidial chains of the fungus; (d) showing simple phialides of the fungus; (e) showing polyphialides of the fungus; and (f) Chlamydospores of the fungus.

Table (2) showed qualitative screening results of 68 soil fungal isolates for production of tannase. Twenty-six fungal strains out of 68 isolates had brown (clear) zones (appear from the back of the petri dish) either after 5 or 7 days of incubation. Hence, these strains were quantitatively screened for the most potent tannase producer.

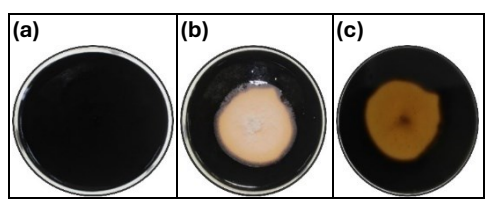

Figure (5): Tannase activity assay for *F. fujikuroi* AUMC16188 on Czapek Dox minimal agar plates using tannic acid as the sole carbon source. (a) Negative control; (b) The fungus after 5 days; (c) The fungus after 5 days (back of the plate).

Table (2): Qualitative screening of 68 soil fungal isolates for production of tannase.

Fungal ID	Scientific name	Result
A (1)-2/5	*Aspergillus ochraceus*	++ve
A2 (1)-25/4	*A. niger*	+++ve
A2 (2)-18/5	*F. verticillium*	-ve
A5 (2)-18/5	*F. verticillium*	-ve
A5 (4)-18/5	*F. nygamai*	+++ve
A5 (5)-18/5	*A. terreus*	-ve
A5 (8)-18/5	*Mucor circinelloides*	-ve
A (5.4)-18/5	*F. proliferatum*	++ve
A6 (2)-18/5	*Penicillium chrysogenum*	-ve
A6 (3)-18/5	*P. brevicompactum*	-ve
A6 (4)-18/5	*Mucor racemosus*	+++ve
A6 (5)-18/5	*P. griseofulvum*	-ve
A6 (7)-18/5	*P. verrucosum*	-ve
A6 (8)-18/5	*A. versicolor*	-ve
A6 (9)-18/5	*A. niger*	-ve
A6 (10)-18/5	*A. nidulans*	+++ve
B1 (1)-11/4	*P. brevicompactum*	-ve
B1 (2)-11/4	*P. citrinum*	-ve
B1 (3)-11/4	*A. niger*	+++ve
B2 (1)-11/4	*F. semitectum*	-ve
B2 (1a)-11/4	*F. fujikuroi*	-ve
B2 (2)-11/4	*A. niger*	-ve
B2 (3)-11/4	*Alternaria alternata*	-ve
C1 (1)-25/4	*P. chrysogenum*	++ve
C1 (2)-25/4	*A. flavus*	-ve
C1 (3)-25/4	*A. terreus*	-ve
C1 (4)-25/4	*F. semitectum*	-ve
C1 (5)-25/4	*P. brevicompactum*	-ve
C1 (6)-25/4	*Mucor circinelloides*	-ve
D4 (5a)-2/5	*A. versicolor*	++ve
D4 (3)-25/4	*A. niger*	+++ve
D4 (4)-25/4	*Scopiolariopsis brevicaulis*	-ve
D4 (5)-25/4	*A. sydowii*	-ve
E (1)-18/5	*A. niger*	+++ve
E (2)-18/5	*F. oxysporum*	-ve
E (3)-18/5	*F. verticillium*	-ve
F (1)-18/5	*F. semitectum*	+++ve
F (2)-18/5	*F. verticillium*	-ve
F (3)-18/5	*F. proliferatum*	-ve
F (4a)-18/5	*Mucor racemosus*	+++ve
F (4b)-18/5	*P. chrysogenum*	-ve
G (1)-18/5	*A. niger*	+++ve
H (1)-2/5	*F. verticillium*	-ve
H (2)-2/5	*A. terreus*	+ve
H (3a)-18/5	*F. semitectum*	-ve
HOS. 1	*A. niger* AUMC16187	+++ve
HOS. 2	*F. fujikuroi* AUMC16188	+++ve
I (1)-2/5	*P. corylophilum*	-ve
I (2)-2/5	*Alternaria alternata*	-ve
I (3)-2/5	*P. citrinum*	-ve
I (4)-2/5	*F. nygamai*	-ve
I (5)-2/5	*A. niger*	+++ve
I (6)-2/5	*F. verticillium*	-ve
J (2)-2/5	*P. citrinum*	-ve
J (3)-2/5	*Mucor racemosus*	+++ve
J (4)-2/5	*A. niger*	++ve
J (1)-18/5	*F. proliferatum*	++ve
J (5)-18/5	*F. nygamai*	-ve
K (1a)-18/5	*Mucor circinelloides*	++ve
K (1b)-18/5	*P. verrucosum*	-ve
K (3)-18/5	*A. niger*	+++ve
K (4)-18/5	*A. terreus*	+ve
K (5)-18/5	*F. nygamai*	-ve
L (1)-2/5	*F. verticillium*	-ve
L (5)-2/5	*A. niger*	++ve
L (2)-18/5	*F. nygamai*	-ve
L (3)-18/5	*F. proliferatum*	-ve
L (6)-18/5	*Mucor circinelloides*	++ve

+++ve: High productivity indicated by very large clear zones around the colonies, ++ve: Moderate productivity indicated by moderate-sized clear zones around the colonies, and +ve: Low productivity indicated by small clear zones around the colonies.

3.3 Quantitative screening of tannase-producing fungi

Twenty-six fungal isolates were selected attributed to their capacity to produce tannase. These isolates were quantitatively screened for production of tannase under shaking incubation for 7 days. *F. fujikuroi* AUMC16188 was found to be the most potent producer of tannase, displaying activity of 1399.73 U/mL and specific activity of 264.1 U/mg as demonstrated in Table (3). Thus, *F. fujikuroi* AUMC16188 was selected for the following experiments.

Table (3): A quantitative assay of tannase production using different fungal isolates selected from qualitative screening.

Fungal ID	Scientific name	Tannase absorbance after dilution (mean ± SD)	Tannase activity before dilution (U/mL/min)	Tannase specific activity before dilution (U/mg)	Protein absorbance (mean ± SD)	Protein content before dilution (mg/mL)
A (1)-2/5	*A. ochraceus*	2±0.004	327.33	55.48	2.12±0.002	5.9
A2 (1)-25/4	*A. niger*	0.08±0.005	13.33	47.6	0.75±0.029	0.28
A5 (4)-18/5	*F. nygamai*	2.2±0.025	634.65	115.39	1.98±0.003	5.5
A (5.4)-18/5*	*F. proliferatum*	0.75±0.008	122.9*	292.63*	1.14±0.009	0.42*
A6 (4)-18/5	*Mucor racemosus*	2.07±0.027	597.29	97.92	2.21±0.005	6.1
A6 (10)-18/5	*A. nidulans*	0.15±0.001	24.78	63.53	1.07±0.008	0.39
B1 (3)-11/4	*A. niger*	0.36±0.002	59.12	125.79	1.27±0.008	0.47
C1 (1)-25/4	*P. chrysogenum*	2.46±0.008	402.56	71.89	2.03±.003	5.6
D4 (3)-25/4	*A. niger*	0.09±0.004	14.96	44.01	0.92±0.02	0.34
D4 (5a)-2/5	*A. versicolor*	2.38±0.003	389.48	67.15	2.08±.003	5.8
E (1)-18/5	*A. niger*	0.35±0.007	57.49	185.44	0.85±0.01	0.31
F (1)-18/5	*F. semitectum*	0.11±0.003	18.24	52.1	0.95±0.003	0.35
F (4a)-18/5	*Mucor racemosus*	1.75±0.005	286.45	98.78	1.07±0.004	2.9
G (1)-2/5	*A. niger*	0.1±0.001	16.6	48.82	0.92±0.04	0.34
H (2)-2/5	*A. terreus*	0.05±0.008	8.42	23.4	0.97±0.003	0.36
HOS. 1*	*A. niger* AUMC16187	0.69±0.006	113.09*	353.41*	0.86±0.004	0.32*
HOS. 2#	*F. fujikuroi* AUMC16188	2.46±0.006	1399.73#	264.1#	1.9±0.006	5.3#
I (5)-2/5	*A. niger*	0.07±0.005	11.69	41.76	0.75±.003	0.28
J (1)-18/5	*F. proliferatum*	2.05±0.006	335.51	58.86	2.06±0.002	5.7
J (3)-2/5	*Mucor racemosus*	1.93±0.004	556.76	94.37	2.11±0.005	5.9
J (4)-2/5	*A. niger*	0.15±0.004	24.77697	72.87	0.93±.007	0.34
K (1a)-18/5	*Mucor circinelloides*	2.23±0.012	364.95	59.83	2.18±0.005	6.1
K (3)-18/5*	*A. niger*	1.12±0.002	183.42*	764.23*	0.64±0.004	0.24*
K (4)-18/5	*A. terreus*	0.12±0.005	19.87	56.77	0.96±0.001	0.35
L (5)-2/5	*A. niger*	0.08±0.002	13.33	47.6	0.76±0.004	0.28
L (6)-18/5	*Mucor circinelloides*	2.48±0.006	405.84	72.47	2.02±0.005	5.6

* The increase in specific activity of tannase with isolates K (3)-18/5 (*A. niger*) 764.23 U/mg-183.42 U/mL, HOS. 1 (*A. niger* AUMC16187) 353.41 U/mg-113.09 U/mL, and A (5.4)-18/5 (*F. proliferatum*) 292.63 U/mg-122.9 U/mL was due to the lower amount of protein with these isolates (0.55 mg/mL) in contrast to #HOS. 2 (*F. fujikuroi* AUMC16188) 264.1 U/mg-1399.73 U/mL. #HOS. 2 (*F. fujikuroi* AUMC16188) was the most potent isolate for tannase production.

3.4 Molecular Identification of the most potent fungal isolate for tannase production via ITS examination

ITS sequence of *F. fujikuroi* AUMC16188 submitted to GenBank (Accession: OR447708; bases 1 to 528) was as follows:

```
  1 cggagggatc attaccgagt ttacaactcc caaacccctg tgaacatacc aattgttgcc
 61 tcggcggatc agcccgctcc cggtaaaacg ggacggcccg ccagaggacc cctaaactct
121 gtttctatat gtaacttctg agtaaaacca taaataaatc aaaactttca acaacggatc
181 tcttggttct ggcatcgatg aagaacgcag caaaatgcga taagtaatgt gaattgcaga
241 attcagtgaa tcatcgaatc tttgaacgca cattgcgccc gccagtattc tggcgggcat
301 gcctgttcga gcgtcatttc aaccctcaag cccccgggtt tggtgttggg gatcggcgag
361 cccttgcggc aagccggccc cgaaatctag tggcggtctc gctgcagctt ccattgcgta
421 gtagtaaaac cctcgcaact ggtacgcggc gcggccaagc cgttaaaccc ccaacttctg
481 aatgttgacc tcggatcagg taggaatacc cgctgaactt aagcatat
```

Figure (6) revealed phylogenetic tree based on ITS sequence of rDNA of *F. fujikuroi* AUMC16188 aligned with closely related strains in the GenBank. According to morphology, in addition to microscopic and molecular identification, the most potent fungal isolate for tannase production was identified as *F. fujikuroi* AUMC16188 (Figures 3, 4, and 6).

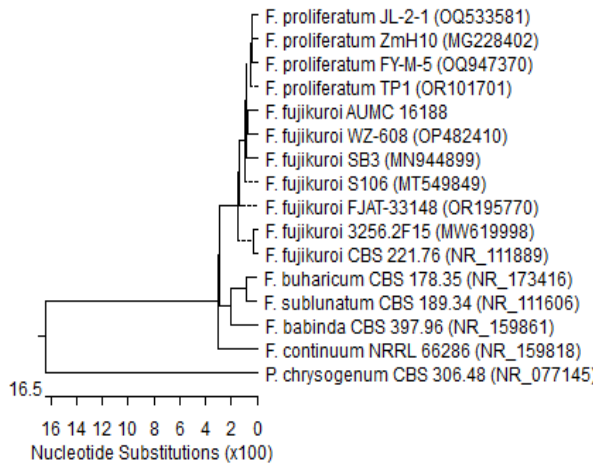

Figure (6): Phylogenetic tree built from ITS rDNA sequence of *F. fujikuroi* AUMC16188 (arrowed). This strain exhibited 99.81% - 100% identity and 97% - 100% coverage with several strains of the same species including the type of material *F. fujikuroi* CBS221.76 (gb: NR_111889). *P. chrysogenum* represents an outgroup strain.

3.5 Tannase applications

3.5.1 Tannase antioxidant effect determination using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of purified tannase was studied using DPPH free radical scavenging activity. When the concentration of the purified tannase was raised, the scavenging activity significantly increased. DPPH free radical inhibition by fungal tannase and IC_{50} values are demonstrated in Table 4. DPPH inhibitory concentration 50 graph of fungal tannase is shown in Figure (7). The results indicated that the purified tannase exhibited significantly higher antioxidant activity against DPPH compared to Trolox, as evidenced by its much lower IC_{50} value (1.18 µg/mL) compared to Trolox (7.65 µg/mL) (Table 4 and Figure 7). This suggests that tannase is a more effective antioxidant, making it a promising green antioxidant candidate.

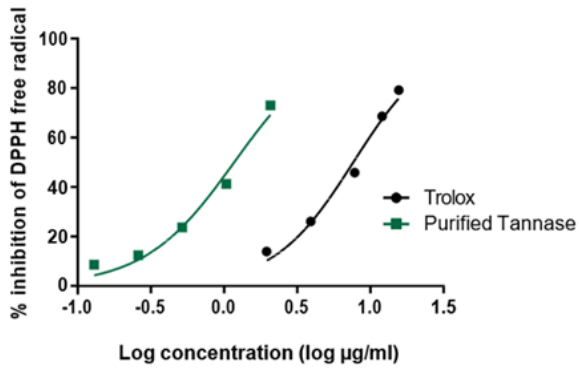

Figure (7): DPPH inhibitory concentration 50 graph of fungal tannase.

Table (4): Purified fungal tannase antioxidant activity against DPPH free radicals.

Standard antioxidant (Trolox)					
Concentration (µg/mL)	1.95	3.91	7.81	12.00	15.63
Inhibition (%)	13.98	26.17	45.69	68.44	79.01
	13.83	24.70	47.16	68.29	79.16
	13.54	26.90	44.22	68.59	78.86
Purified tannase					
Concentration (µg/mL)	0.13	0.26	0.52	1.04	2.08
Inhibition (%)	8.22	13.33	23.80	41.08	72.61
	8.46	11.63	23.68	41.21	73.10
	8.70	11.99	23.19	40.96	72.85
Sample	IC_{50} (µg/mL)			Standard error (SE)	
Trolox	7.65			1.03	
Purified tannase	1.18			1.04	

4. Discussion

Obtaining bioactive enzymes from microorganisms that can be applied in food production, washing processes, degradation of effluent, or biofuel production is very important for developing sustainable green biotechnology. There are usually two major ways to achieve such goal, either engineered or natural enzymes. The first one is by working on an existing wild type (native) enzyme to improve its properties. Such approach could be accomplished through protein engineering by using random (Amara *et al.*, 2002) and/or directed mutagenesis (Amara and Rehm, 2003), strain improvement through chemical mutagenesis and the like (Amara, 2013). This can successfully lead to obtaining new feature and property that might be applied for a new application(s) and/or in a new environment (Amara, 2013). Nevertheless, nature simply provides us with amazing biological structures that fulfill our demand without any side effect in a shorter time and lesser cost (Amara, 2012; 2013). Usually, natural products are

safer than hazardous chemicals, sustainable, degradable, applicable, bioavailable, compatible with biodiversity, cheap, etc. (Amara, 2013). Tannase, which hydrolyzes tannic acid to gallic acid and glucose, is appreciated for its applications in various industries (Biswas *et al.*, 2020; Gezaf *et al.*, 2021). In this study, a tannase enzyme was isolated from different soil fungal strains. The best producer was identified as *F. fujikuroi* AUMC16188. Additionally, the enzyme was purified, and its properties and applications were investigated. In this work, we successfully produced tannase from soil fungal isolate that has potent natural antioxidant activity. The use of soil fungi for tannase production aligns with recent studies (Saad *et al.*, 2023; Thakur *et al.*, 2024). These studies highlighted the capability of soil fungi to produce tannase. The purified tannase from *F. fujikuroi* AUMC16188 exhibits exceptional antioxidant efficacy, as quantitatively determined by a remarkably low IC_{50} of 1.18 µg/mL in the DPPH assay-representing approximately 6.5-fold greater potency than the Trolox standard (7.65 µg/mL). This robust activity must be contextualized within the limited literature on the direct antioxidant application of purified tannase. While Hidayathulla *et al.* (2018) documented only marginally superior DPPH activity for fully purified *A. nidulans* tannase relative to synthetic antioxidants Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA), our findings demonstrate substantially greater efficacy. The stark contrast between these previously reported marginal enhancements and our robust quantitative results underscores a critical methodological and biological distinction. The pronounced enhancement in antioxidant performance not only highlights the pivotal influence of biological origin but also suggests fundamentally distinct structure-activity relationships among tannase isoforms across fungal genera. Furthermore, the exceptional stability and potency of *F. fujikuroi* tannase position it as a viable natural alternative to conventional chemical antioxidants, particularly in stabilizing lipid-rich matrices for food preservation. This combination of validated bioactivity, sustainable origin, and superior functionality establishes *F. fujikuroi* AUMC16188 as a premier microbial source for biocatalytic applications demanding potent antioxidant functionality.

5. Conclusion

This comprehensive study identified *F. fujikuroi* AUMC16188 as the most effective tannase-producer among soil samples from four Egyptian governorates. Notably, the enzyme showed superior antioxidant properties compared to Trolox, with significantly lower IC_{50} values for DPPH assay. These findings highlight the potential of *F. fujikuroi* tannase for practical applications as a powerful antioxidant. Building on these promising results, future work will be directed toward the biotechnological exploitation of this enzyme, including

scaling up production, structural analysis to understand its catalytic mechanism, and exploring its efficacy in relevant industrial models such as food preservation or nutraceuticals.

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