

## Research Article

# Purification, Characterization, and Biocatalytic and Antibiofilm Activity of a Novel Dextranase from *Talaromyces* sp.

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Received 17 March 2020; Revised 6 September 2020; Accepted 2 November 2020; Published 11 November 2020

Academic Editor: Karl Drlica

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Dextranase is a useful enzyme that catalyzes the degradation of dextran to low-molecular-weight fractions, which have many critical commercial and clinical applications. Endophytic fungi represent a source of both high heat-stable and pH-stable enzymes. In this study, from *Delonix regia* bark by plate assay, out of 12 isolated fungal strains, hyaline zones were detected in only one strain. By using the standard ITS rDNA sequencing analysis, the isolated strain was identified as *Talaromyces* sp. In the case of carbon source, in a medium containing 1% dextran T2000 as the sole carbon source, the maximum dextranase activity reached approximately 120 U/ml after incubation of 2 days where the optimum pH was 7.4. Peptone addition to the production medium as a sole nitrogen source was accompanied by a significant increase in the dextranase production. Similarly, some metal ions, such as  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$ , increased significantly enzyme production. However, there was no significant difference resulting from the addition of  $\text{Cu}^{2+}$ . The crude dextranase was purified by ammonium sulfate fractionation, followed by Sephadex G100 chromatography with 28-fold purification. The produced dextranase was 45 kDa with an optimum activity at 37°C and a pH of 7. Moreover, the presence of  $\text{MgSO}_4$ ,  $\text{FeSO}_4$ , and  $\text{NH}_4\text{SO}_4$  increased the purified dextranase activity; however, SDS and EDTA decreased it. Interestingly, the produced dextranase expressed remarkable pH stability, temperature stability, and biofilm inhibition activity, reducing old-established biofilm by 86% and biofilm formation by 6%.

## 1. Introduction

Dextranase,  $\alpha$ -1,6-d-glucan-6- glucanohydrolase, is an inducible enzyme that cleaves the  $\alpha$ -1,6-glycosidic linkages in dextrans resulting in the hydrolysis of high molecular weight dextrans to low-molecular-weight fractions [1–3]. Dextranases produced by various fungi, bacteria, and other microorganisms are extensively used for research purposes [4]. In medicine, the partial dextranase hydrolysis of native dextran generates specific molecular weight fractions used in preparing blood substitutes to restore blood volume in shocked patients due to extreme blood loss [5–8]. On the other hand, dextranase plays an efficient role in food industries, such as molasses and beverage processing [9]. In the sugar industry, dextranase could minimize the dextran residues in sugarcane juice, which interferes with the sugar

manufacturing process [10], avoiding the tremendous loss in sucrose quantity and improving the quality of produced sugar [11–13].

In oral hygiene, dextranase can be used in oral care products such as dental paste and mouthwashes to remove dental plaque and prevent dental caries. Different dextranase-producing microorganisms, such as bacteria, mold, and yeast, have been previously reported in various studies [4, 14–17]. As previously documented, fungi are the most common commercial sources of dextranase with high enzymatic activity and low immunological effects, compared to those produced by bacteria and other microorganisms [2, 18]. Endophytic fungal enzymes have great pharmaceutical, industrial, and agricultural importance and demonstrate various biological activities resulting from their endophytic natural products [19, 20].

Previous studies have shown that most isolated dextranases have been characterized by low heat and pH stability [1, 21]. Generally, fungal endophytes improve the performance of plants and resistance against different stresses and afford them unique adaptation via the production of bioactive compounds [22, 23]. Consequently, endophytic fungi may produce fungal enzymes highly beneficial in the enzyme industry due to their reasonable stability at high-temperature levels and extreme pH values. The main objectives of this study were to isolate endophytic fungi producing dextranase and to optimize the production condition of this enzyme.

## 2. Materials and Methods

**2.1. Isolation of Endophytic Fungal Strains and Primary Screening for Dextranase-Producing Strains.** Several fungal strains were isolated from the *Delonix regia* plant, which grows in Egypt's Delta region. A total of 12 endophytic fungal strains were collected. The resulting fungal strains were streaked for purification on yeast peptone dextrose agar (YPD) plates and incubated at 28°C for 3–8 days. Afterwards, a separate colony of each isolate was subcultured on dextranase production-screening medium (pH 5.5) containing 1% dextran T2000 [(2000 kDa) Pharmacia products (Uppsala, Sweden)], 0.3% NaNO<sub>3</sub>, 0.05% KCl, 0.1% K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.05% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.001% FeSO<sub>4</sub>·7H<sub>2</sub>O, and 1.5% agar powder, and the results were detected after 5 days of incubation at 28°C [1]. Positive dextranase-producing strains produced a hyaline (clear) zone against the turbid background of this medium.

**2.2. Identification of Dextranase-Producing Endophyte.** For identification of the dextranase-producing fungal strain, the ribosomal internal transcribed spacer (ITS) was amplified using the universal ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers (Biosearch Technologies, UK). The target PCR product (581 bp) was used as a template in sequencing reactions using the ABI PRISM® BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Bio-Systems, Foster City, USA). The reaction mixtures were analyzed on an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, USA). Based on the DNA gene sequences of dextranase-producing strain and related organisms from the GenBank, multiple DNA sequence alignment was performed using the software package MEGA4 version. For the detection of isolated fungal strain, a phylogenetic tree was constructed using the bootstrap test of neighbor-joining algorithm after 1000 times.

**2.3. Culture Conditions for Crude Dextranase Production and Optimization of Incubation Time.** For the production of extracellular dextranase, the production liquid culture medium was composed of (g%) 1.5% dextran T2000, 0.1% K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.4% yeast extract, 0.05% MgSO<sub>4</sub>·7H<sub>2</sub>O, and pH 7.4 [24]. The spore count was adjusted to 10<sup>7</sup> spores/ml using a direct microscopic counting

hemocytometer [10]. For submerged culture fermentation, one milliliter of the adjusted spore suspension of the dextranase-producing strain was suspended in this medium (50 ml) followed by incubation in a rotary shaker (200 rpm) at 30°C for 7 days. Afterward, the content of each flask was filtered, followed by centrifugation at 6000 rpm for 20 min at 4°C. The supernatant was then tested for enzyme activity [1, 25] and stored at –80°C until further analyses of dextranase production.

**2.4. Assay of Dextranase Production.** Dextranase activity was determined by measuring the reducing sugar liberated during enzyme-substrate interaction via the microplate colorimetric assay developed by Somogyi and Nelson with slight modifications [26–28]. In a 96-well microplate, 25 µl of enzyme sample was incubated with 25 µl of 1% dextran T2000 solubilized in 0.1 M citrate buffer (pH 5) in each well at 37°C. After 24 h of incubation, 50 µl of freshly prepared Somogyi copper reagent was added; then the wells were sealed with the acetate sheet followed by incubation in a water bath at 80°C for 30 min. Plate cooling was performed with running cooled water for 5 min followed by the addition of 50 µl of arsenomolybdate reagent (2.5 g ammonium molybdate, 3 g sodium arsenate, 2.1 ml concentrated sulfuric acid, and 45 ml water) in each well. Colorimetric measurements were performed at 500 nm using microplate spectrophotometer reader ELx808™ (Biotek Instruments Inc., Winooski, VT). The standard curve was constructed using maltose at concentrations ranging from 100 to 2000 µg/ml. One unit (U) of dextranase enzyme activity was defined as the amount of enzyme required to catalyze the formation of 1 mol of maltose in 1 min at standard assay condition (U/ml). Other values were calculated as follows:

The specific activity (U/mg) = total activity (U)/total protein amount (mg), where total activity (U) = enzyme activity (U/ml) × volume of enzyme solution, while total protein = protein concentration (mg/ml) × volume of protein solution [29].

**2.5. Determination of Protein Concentration.** The protein concentration (mg/ml) was determined using the Bradford method using Bradford reagent (Thermo Fisher Scientific, USA) [30]. The standard curve of bovine serum albumin (BSA) was constructed by plotting different concentrations of bovine serum albumin (BSA) (Sigma-Aldrich, USA), which was used as standard protein against their absorbance values at 595 nm as read by the microplate spectrophotometer reader. Each protein concentration was calculated in mg/ml based on absorbance measurements at 595 nm compared to standard.

**2.6. Optimization of Different Factors Affecting Dextranase Activities.** Different factors affecting dextranase activities were investigated in the inoculated dextranase production media as previously described conditions for submerged culture fermentation.

**2.6.1. Optimization of Carbon Source for Dextranase Production.** For maximum dextranase production, various carbon sources, such as glucose, starch, and sucrose, were added at a concentration of 1.5% in growth medium instead of dextran T2000.

**2.6.2. Optimization of Nitrogen Source and Concentration for Dextranase Production.** For maximum dextranase production, various organic and inorganic nitrogen sources substituting yeast extract such as peptone, tryptone, urea, potassium nitrate, sodium nitrate, and ammonium sulfate were supplemented at a concentration of 0.4%.

**2.6.3. Optimization of Medium pH for Dextranase Production.** The effect medium pH was tested within a wide range from 3 to 8.5 using 1 N HCl and 1 N NaOH before sterilization. By the standard assay procedure, the enzymatic activity of each pH medium was calculated to determine optimum pH for enzyme production.

**2.6.4. Effects of Metal Ions and Other Reagents on Dextranase Production.** The effect of some metal ions was estimated separately at a concentration of 0.05% under standard assay conditions using  $\text{Cu}^{2+}$  ( $\text{CuSO}_4$ ),  $\text{Zn}^{2+}$  ( $\text{ZnSO}_4$ ), and  $\text{Fe}^{2+}$  ( $\text{FeSO}_4$ ) in  $\text{Mg}^{2+}$  ion ( $\text{MgSO}_4$ ) free production medium.

**2.7. Dextranase Enzyme Purification.** Crude enzyme extract was treated with saturated ammonium sulfate solutions ranging from 20 to 90%, followed by incubation at 4°C with magnetic stirring. After overnight incubation, the precipitated proteins were collected by centrifugation at 10,000 rpm for 20 min at 4°C, followed by dissolution in 0.01 M potassium phosphate buffer (pH 7). The resulting extracted proteins were dialyzed in the same buffer overnight at 4°C. Further purification of the concentrated dialyzed enzyme was performed on a Sephadex G100 column preequilibrated with potassium phosphate buffer (pH 7) at low temperature with a flow rate of 0.2 ml/min. One hundred fractions (2 ml each) were collected after column separation, followed by absorbance measurements at 280 nm to monitor the concentration of separated proteins [31].

After determining the enzyme activity, fractions with high dextranase activity were pooled together, followed by determining their molecular weight by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation (SDS-PAGE) followed by Coomassie Brilliant Blue staining [32].

**2.8. Effect of Different Parameters on Activity and/or Stability of Dextranase Enzyme.** The incubation temperature effect on dextranase activity was measured at 25, 30, 37, 40, 50, and 60°C. Relative activity was expressed as a percentage of the highest activity, while the highest dextranase activity was 100%. The thermostability of dextranase was tested without substrate addition by treating for 1 h at 20–90°C in 0.01 M potassium phosphate buffer (pH 7). The control sample at 100% activity was used as the maximum enzyme activity.

For studying the effect of pH, the purified dextranase enzyme activity was determined at a pH range of 3.0–10.0 (citrate-sodium citrate buffer, pH 3.0–6.0; potassium phosphate buffer, pH 7.0–8.0; carbonate-bicarbonate buffer, pH 9–10). Relative activity was expressed as a percentage of the highest activity, while the highest dextranase activity was calculated as 100%. The pH stability of dextranase activity was tested at 3.0–10 pH range for 1 h at 37°C using the same buffers. Relative activities were expressed as percentages of the maximum activity. Furthermore, the effects of several metal ions and reagents, namely,  $\text{MgSO}_4$ ,  $\text{NH}_4\text{SO}_4$ ,  $\text{FeSO}_4$ , NaCl, SDS, and EDTA, influencing dextranase activity were investigated at 3 different concentrations, 1 mM, 5 mM, and 10 mM.

**2.9. Quantitative Detection of Biofilm Activities.** For quantitative determination of biofilm production, standard strain *P. aeruginosa* PAO1 (Microbiology and Immunology Department culture collection) TSBG (tryptic soy broth supplemented with 1% glucose) overnight culture was adjusted to 0.2–0.25 at 600 nm ( $\text{OD}_{600\text{nm}}$ ) using a spectrophotometer (WPA colorwave CO7500 Colorimeter) [33]. Afterward, 100  $\mu\text{l}$  aliquots of preadjusted culture were inoculated in four adjacent vertical wells, in addition to the negative control (TSBG only), followed by incubation at 37°C for 24 h. After overnight incubation, the content of each well was removed and washed three times with 200  $\mu\text{l}$  phosphate buffered saline (PBS) [0.01 M, pH 7.4]. Adherent cells of each well were fixed by treatment with 150  $\mu\text{l}$  absolute methanol for 15 min, followed by aspiration of methanol. After drying, each well was stained by 150  $\mu\text{l}$  of 1% (w/v) crystal violet and left for 20 min; then the plate was rinsed three times with distilled water and air-dried. The resulting bound biofilm was resolubilized by adding 150  $\mu\text{l}$  33% (v/v) glacial acetic acid per well. At 540 nm, measurements were performed using ELx808™ Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT) [34–37].

**2.9.1. The Effect of the Enzyme on Biofilm Production.** For determination of the antibiofilm enzyme activity, 100  $\mu\text{l}$  of the purified enzyme (5 U) in 0.01 M potassium phosphate buffer was added in 4 replicates to 100  $\mu\text{l}$  of OD-adjusted bacterial suspension, followed by incubation at 37°C for 24 h without shaking. As a negative control, 100  $\mu\text{l}$  0.01 M potassium phosphate buffer was used to substitute the purified enzyme. After incubation, biofilm activity was determined as described above.

**2.9.2. The Effect of the Enzyme on Old Established Biofilm.** For the investigation of the dextranase impact on the 24 h old-established biofilm of PAO1 strain, 100  $\mu\text{l}$  of the purified enzyme (5 U) in 0.01 M potassium phosphate buffer (pH 7) was added into 4 wells harboring 24 h old-established biofilms of the standard strain PAO1, followed by incubation 24 h at 37°C. Negative controls were prepared by adding 100  $\mu\text{l}$  potassium phosphate buffer (0.01 M, pH 7) into 4 wells harboring 24 h old biofilms of the same strain PAO1.

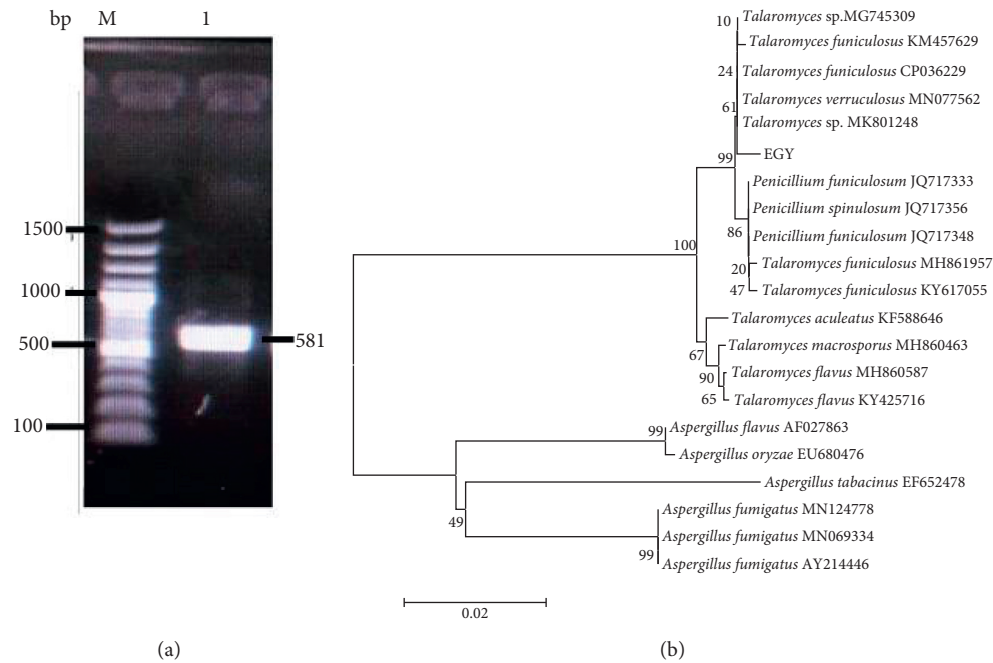


FIGURE 1: Molecular identification of dextranase-producing strain (*Talaromyces* sp.) based on ITS region sequence analysis. (a) PCR amplification of DNA of the fungal strain (EGY) using universal primers (ITS1/ITS4). Lane 1: PCR products were resolved by 1% agarose gel electrophoresis. M: 100 bp DNA ladder (Thermo Scientific GeneRuler 100 bp Plus). (b) Phylogenetic tree based on ITS rRNA gene sequences.

Biofilm reducing activities were quantified after overnight incubation. Relative biofilm reducing activities were calculated as percentages relative to the negative controls (set as 0 % reduction percent).

**2.10. Statistical Analysis.** Data were analyzed using One-Way ANOVA followed by Bonferroni's test using SPSS software.

### 3. Results and Discussion

**3.1. Screening and Identification of Dextranase-Producing Strains.** Under the aerobic conditions, only one isolate (named EGY) formed a hyaline zone on the screening media plate by degrading dextran T2000. The phylogenetic tree was constructed, including the ITS rDNA sequence (581 bp) of the isolated strain and some sequences obtained from the gene bank (Figure 1). As a result, the isolated strain was identified as *Talaromyces* sp.

**3.2. Effect of Incubation Time on Dextranase Production.** Generally, the incubation period differs according to the microbial growth rate and the pattern of the enzyme production [10]. After 48 h of incubation, the dextranase activity increased to its maximum production reaching approximately 120 U/ml (Figure 2). Similarly, in 2014, Cai et al. found that the optimal incubation time for dextranase production from *Catenovulum* sp. was within the range of 0 and 48 h [38]. In contrast, maximum yields of dextranase were attained after 5 days of *Paecilomyces lilacinus* and 7

days of incubation of *Fusarium* sp. and *Penicillium aculeatum* [10, 39].

**3.3. Optimization of Carbon Source for Dextranase Production.** Different carbon sources such as dextran, glucose, starch, and sucrose were added as a sole carbon source in the dextranase production medium. The maximum dextranase production was achieved when only dextran T2000 was incorporated in the culture medium, while no enzyme production was detected in media supplemented with glucose, sucrose, or starch (Figure 3). These results suggested that dextranase enzyme production is highly substrate-specific and inducible. Some studies previously reported that increases in microbial growth and dextranase production could be observed when using dextran as a carbon source [40–43].

**3.4. Optimization of Nitrogen Source for Dextranase Production.** Some organic and inorganic nitrogenous sources were tested for their role in the production of the dextranase enzyme. The yeast extract, peptone, and tryptone supported higher enzyme production in the 2<sup>nd</sup> day of incubation but interacted differently; the peptone resulted in a significant increase in enzyme production compared to yeast extract. In contrast, no significant difference between tryptone and yeast extract could be traced (Figure 4). Similar results were detected in another study, starting from *Bacillus* sp. as a dextranase-producing microorganism [41]. Additionally, yeast extract and peptone were associated with high dextranase production levels from *Catenovulum* sp., while



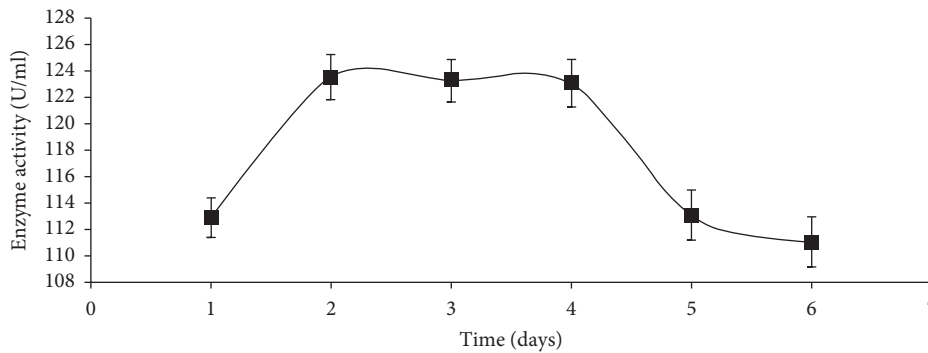


FIGURE 2: Effect of incubation time on dextranase production from *Talaromyces* sp.

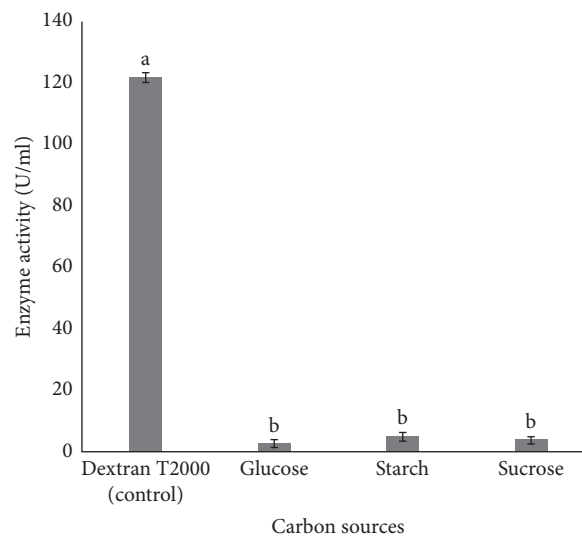


FIGURE 3: Effect of carbon sources on dextranase production from *Talaromyces* sp. at the second day of fermentation. Symbols (mean  $\pm$  SE,  $n = 6$ ) having similar letters are not significantly different from each other (Bonferroni,  $P < 0.0125$ ).

soybean powder, cornmeal, and casein decreased dextranase production. Moreover, the inorganic nitrogen sources, such as ammonium nitrate, urea, potassium nitrate, and ammonium phosphate, had no significant effects on dextranase production. The same observation was previously detected using inorganic nitrogen sources such as ammonium sulfate and ammonium nitrate [38].

**3.5. Optimization of pH for Dextranase Production.** The effect of the initial pH of culture media on dextranase production was studied over a pH range of 3 to 8.5. The maximum yield of the enzyme was attained at pH 7.4; however, a remarkable decline in enzyme production was observed when the pH of the media gradually decreased (Figure 5). Some studies have indicated that maximal dextranase enzyme yield from *Penicillium funiculosum* and *Catenovulum* sp. could be reached at an initial pH of 8.0 [17, 38]. In contrast, the optimum dextranase production by different fungal species could be achieved at the pH range from 5.5 to 6.0 [10, 16, 39, 44].

**3.6. Effect of Metal Ions on Dextranase Production.** The effects of some metal ions on dextranase production compared to  $Mg^{+2}$  were estimated. The addition of either  $Fe^{+2}$  or  $Zn^{+2}$  resulted in a significant increase in enzyme production. However, there was no significant effect resulting from the addition of  $Cu^{+2}$  (Figure 6).

**3.7. Identification of Purified Enzyme.** *Talaromyces* sp. crude dextranase enzyme was primarily concentrated by 70% ammonium sulfate treatment. The specific activity of this partially purified enzyme was 385 U/mg (Table 1). The dextranase enzyme was further purified by gel filtration chromatography using Sephadex G100. Samples from tubes 19, 20, 21, and 22 were merged and analyzed by SDS-PAGE. The purified dextranase in the merged fractions appeared as a single band (Figure 7(a)), suggesting a monomer separated by an efficient purification process, purified to 28-fold concentration with a 6% yield and 3429 U/ml specific activity (Table 1). The molecular weight of the purified dextranase was about 45 kDa (Figure 7(b)), which is different

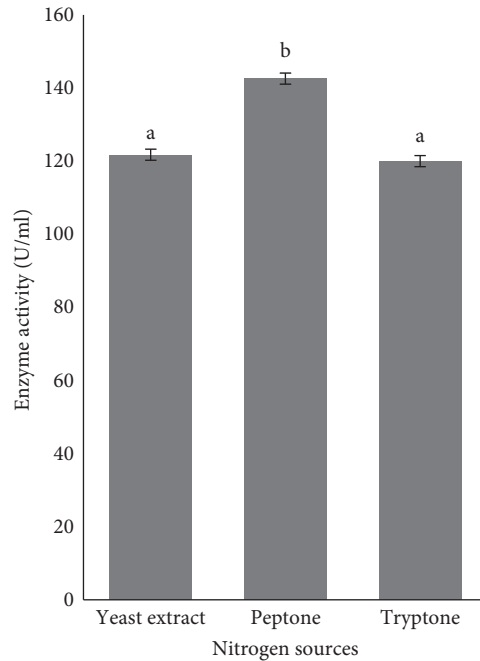


FIGURE 4: Effect of nitrogen sources on dextranase production from *Talaromyces* sp. at the second day of fermentation. Symbols (mean  $\pm$  SE,  $n = 6$ ) having similar letters are not significantly different from each other (Bonferroni test,  $P < 0.017$ ).

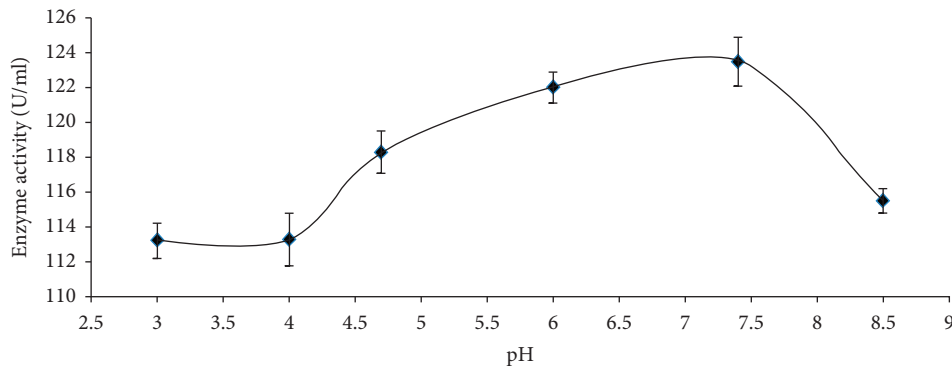


FIGURE 5: Effect of initial pH in the medium on the dextranase production from *Talaromyces* sp. at the second day of fermentation.

from other previously reported fungal dextranases such as *Aspergillus allahabadii* X26 (66 kDa) [12], *Talaromyces pinophilus* H6 (58 kDa) [45], *Penicillium aculeatum* (66.2 kDa) [21] *Penicillium cyclopium* (66 kDa) [46], and *Chaetomium globosum* (53 kDa) [31].

### 3.8. Dextranase Enzymatic Properties

**3.8.1. Effects of Temperature and pH on Dextranase Activity and Stability.** The effect of pH and temperature was studied on *Talaromyces* sp. dextranase activity. An optimum temperature of 37°C was obtained. The thermal stability assessment of dextranase revealed that the enzyme retained greater than 80% residual activity after storage at 20–70°C for 1 h. Furthermore, a pH value of 6 was required for maximum dextranase activity (Figures 8 and 9). The produced enzyme was remarkably stable in the pH range of

5.0–8.0 (Figure 9). Therefore, the produced dextranase was highly thermal-stable and pH-stable. However, for dextranase from *Penicillium aculeatum*, the optimum pH and temperature were found to be 4.5 and 45°C, respectively [21]. The highest dextranase activity of *Penicillium cyclopium* was reported at 55°C and 5.0 pH [46]. Interestingly, *Chaetomium globosum* dextranase could withstand a high temperature of 60°C and a low pH of 5.5 [31].

**3.8.2. Effects of Several Factors and Compounds on Dextranase Activity.** The effect of several compounds on dextranase activity was investigated considering the control (the reaction without the addition of such compounds) 100% activity (Table 2). The enzyme activity was increased in the presence of FeSO<sub>4</sub> (1 mM), MgSO<sub>4</sub> (10 mM), and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (10 mM) by 17.7%, 12.6%, and 18.8%, respectively. Contrarily, NaCl (1 mM) addition was followed by decreased

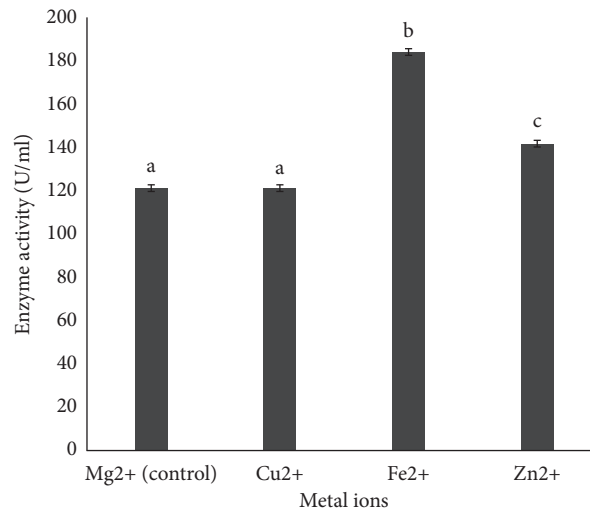


FIGURE 6: Effect of metal ions on dextranase production from *Talaromyces* sp. at the second day of fermentation. Symbols (mean  $\pm$  SE,  $n = 6$ ) having similar letters are not significantly different from each other (Bonferroni,  $P < 0.0125$ ).

TABLE 1: Parameters related to purified dextranase from EGY strain.

	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Fold of purification	Yield (%)
Crude dextranase	8647	70	124	1	100
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation (70% saturation)	1020	2.7	385	3	12
Sephadex G-100	480	0.1	3429	28	6

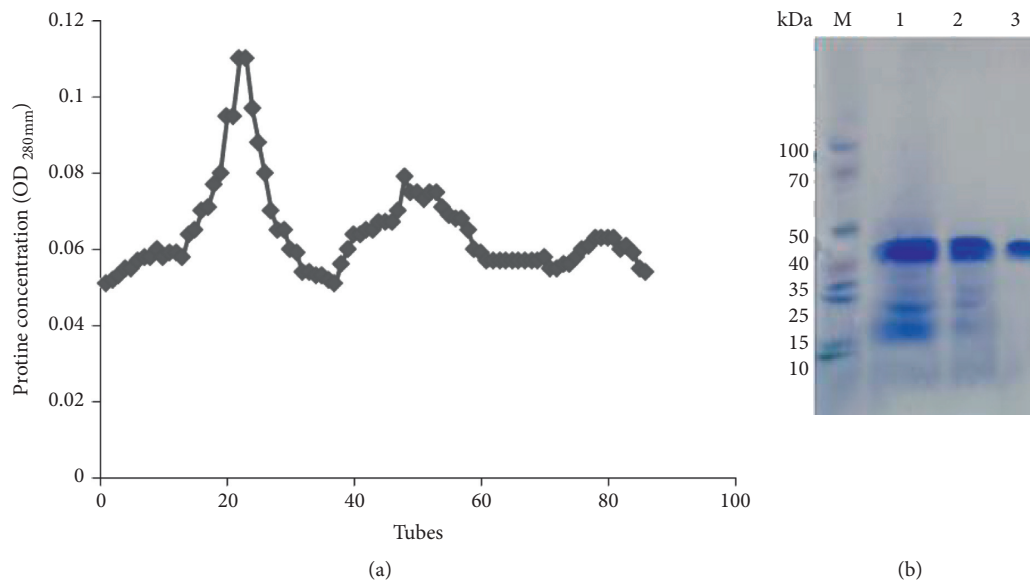


FIGURE 7: Purification of dextranase by Sephadex G-100 chromatography. (a) The protein concentrations of different tube fractions eluted from the Sephadex G-100 column. (b) Analysis of purified dextranase (tube fractions 19–22) by 12% SDS-PAGE. Lane M: protein molecular mass marker (Thermo Scientific Spectra Multicolor Broad Range Protein Ladder); Lane 1: crude dextranase produced after optimization of production conditions; Lane 2: partially purified dextranase obtained by precipitation with 70% ammonium sulfate saturated solution; Lane 3: purified dextranase obtained by collecting of fraction tubes (19–22) after gel filtration chromatography.

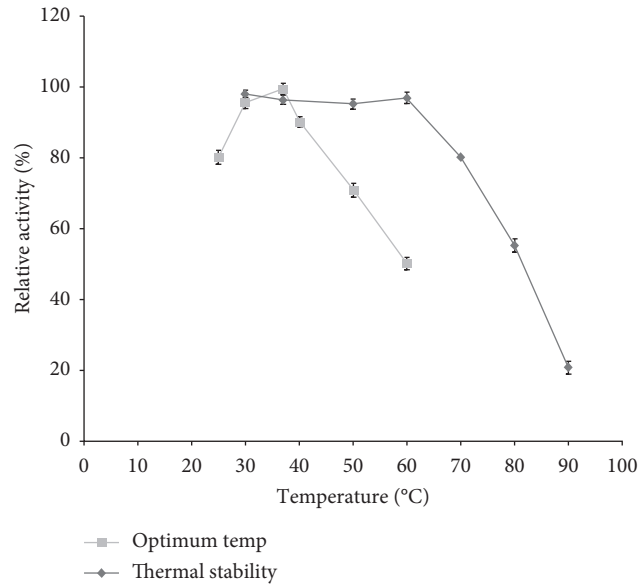


FIGURE 8: Effect of temperature on the activity and stability of dextranase from *Talaromyces* sp. Optimum temperature: activity was assayed by the standard method at different temperature values. Thermal stability: activity was assayed by the standard method after incubating the purified dextranase enzyme at different temperatures for 1 h without substrate addition. Error bars represent standard errors.

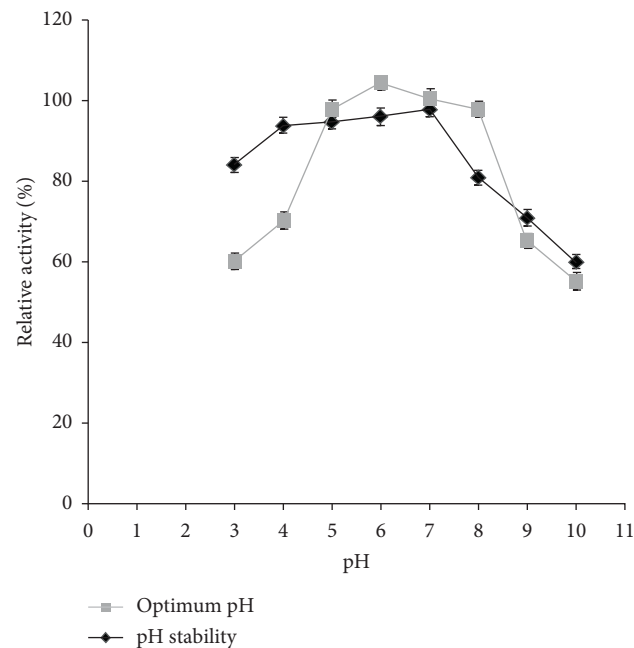


FIGURE 9: Effect of pH on the activity and stability of dextranase from *Talaromyces* sp. optimum pH : activity was assayed by the standard method at various pH values. pH stability: activity was assayed by the standard method after incubating the purified dextranase enzyme at different pH values for 1 h at 37°C without substrate addition. Error bars represent standard errors.

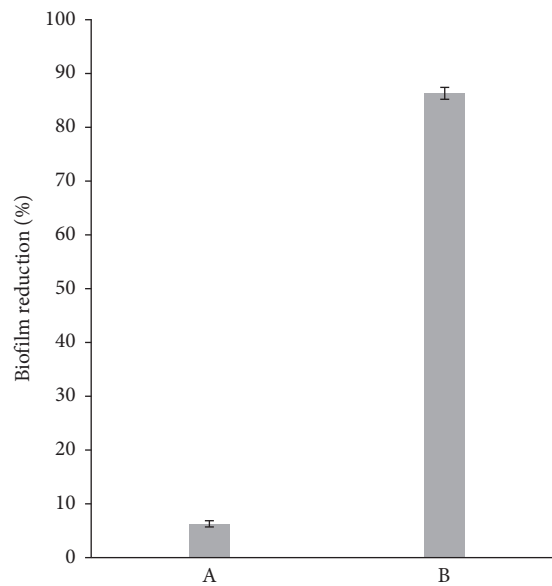
enzyme activity by 5.6%. However,  $\text{Na}^+$  and  $\text{Fe}^{2+}$  improved the enzymatic activity of *Penicillium cyclopium* [46]. In contrast, some results indicated that  $\text{FeCl}_2$  (1 mM) strongly inhibited the dextranase activity of *Penicillium aculeatum* and *Chaetomium globosum* [21, 31]. In the current study, the

addition of EDTA (10 mM) and SDS (5 mM) was followed by reducing the enzyme activity to 9% and 12.6%, respectively. Similar observations were detected for the enzyme activities of *Chaetomium globosum* and *Talaromyces pinophilus* that were slightly decreased by EDTA (10 mM) by 8% and 17%,



TABLE 2: Effects of some metal ions and selected compounds on the activity of *Talaromyces* sp. dextranase enzyme.

Compound	Relative activity %		
	1 mM	5 mM	10 mM
MgSO <sub>4</sub>	104	113	99
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	111	119	110
FeSO <sub>4</sub>	118	95	94
NaCl	94	101	95
SDS	101	88	89
EDTA	92	101	91
Control	100	100	100

FIGURE 10: The effect of purified dextranase enzyme on biofilm formation of *P. aeruginosa* standard strain PAO1. (A) The dextranase-inhibitory effect on biofilm formation during bacterial cultivation. (B) The dextranase-reducing effect on 24 h old-established biofilm.

respectively [31, 45]. In contrast, *Talaromyces pinophilus* dextranase activity could be increased by 9% in SDS (5 mM) presence [45].

**3.9. Effect of Dextranase Enzyme on Biofilm Formation of *P. Aeruginosa* Standard Strain PAO1.** The effect of enzyme addition on old-established biofilm was previously documented [47]. In our study, the use of 5 units of the enzyme had 86% reducing activity on 24 h old-established biofilm of *P. aeruginosa* strain PAO1, adhering to the wall of the microtiter plate (Figure 10). Moreover, the biofilm formation was reduced by 6% when *P. aeruginosa* is cocultured with dextranase from *Talaromyces* sp. (Figure 10). A similar consequence was observed in the case of *Catenovulum* sp. as 40 units of catenovulum dextranase impeded biofilm formation in BHI media and reduced the cell population of *S. mutans* adhering to the glass coverslips [38].

#### 4. Conclusion

In summary, one fungal strain identified as *Talaromyces* sp. could produce a thermal-stable enzyme expressing high

enzyme activity (approximately 120 U/ml). Studying the factors affecting the enzyme production resulted in the conclusion that the best carbon source was dextran T2000 and that the optimum pH was 7.4. Likewise, the maximum enzyme yield was detected on the 2<sup>nd</sup> day of incubation. Some variation in production conditions could be traced; the peptone used as a nitrogen source increased the enzyme production. Fe<sup>2+</sup> and Zn<sup>2+</sup> addition resulted in a significant increase in enzyme activity while Cu<sup>2+</sup> addition had no significant difference in such activity. The crude enzyme extract was partially purified (3-fold) by precipitation with ammonium sulfate (70% saturation) and then further purified (28-fold) by gel filtration chromatography. The purified dextranase was 45 kDa with an optimum temperature of 37°C and an optimum pH of 6.0. Dextranase activity increased in the presence of MgSO<sub>4</sub>, FeSO<sub>4</sub>, and NH<sub>4</sub>SO<sub>4</sub>. In contrast, SDS and EDTA inactivated the enzyme. Furthermore, the purified dextranase reduced the 24 h old-established biofilm of *P. aeruginosa* standard strain PAO1 by 86% and inhibited the formation of biofilm to a lower extent (6% reduction) when cocultured with such bacterium. Finally, because of the high heat-stability and pH stability of the *Talaromyces* dextranase, endophytic fungi could be considered promising sources of

dextranase enzyme having different applications in the industries.

### Data Availability

All datasets generated or analyzed during this study are available upon reasonable request from the corresponding author.

### Disclosure

This work was performed at the Microbiology Department, Faculty of Pharmacy, Mansoura University, Egypt.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Acknowledgments

The authors would like to thank Professor Dr. Ashraf Taha Khalil, Professor of Pharmacognosy, Faculty of Pharmacy, Mansoura University, for providing plant samples and his help in plant work. The authors would like to thank Mr. Mohamed Eladawy, Demonstrator in the Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, for helping in the biofilm chapter. Our special thanks are extended to Miss Rana Sherif, Former Researcher in Biochemistry Department, College of Arts and Science, Texas Tech University, for helping with English usage.

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