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الملخص

تهدف هذه الدراسة إلى توصيف تحلل العفص الفعال في الكائنات الحية الدقيقة من خلال انتاج التاناز Tannase. تم فحص 10 عزلات فطرية في وسط حمض التانيك. تم اختيار وتحديد أعلى مُنتج للتاناز وهو الكريبتوكوكس. *Cryptococcus* sp. NRC10 بمستوى تماثل 99.9% ومتوسط 11 على 45 درجة مئوية لمدة 24 ساعة على رقم هيدروجيني pH5 وهذه هي الشروط المطلوبة والأكثر ملائمة لأقصى انتاج للإنزيم. أشار البحث إلى أن الجلوكوز والأمنيوم كلورايد NH_4Cl هما أفضل مصادر الكربون والنيتروجين على التوالي. كما أوضح البحث أن أفضل ظروف الإنزيم المُنَقى هي 0.20% من حمض التانيك و0.15 ml من إنزيم التاناز على درجة حرارة 45 مئوية بعد عشر دقائق مع pH 6. حَفّز إضافة Mn^{2+} نشاط الإنزيم. أشار البحث إلى استخدام الإنزيم النقي في تحلل العفص في مياه البحر الأحمر وعينات أوراق الشاي الأخضر. أوضحت الدراسة انخفاض كمية العفص عقب إضافة التاناز. وفي الختام يشير البحث إلى أن التاناز له دور في إزالة العفص من المياه والتربة الملوثة.

Biodegradation of Tannins from polluted sources using natural enzymes

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Abstract

This study aimed to characterize an efficient tannin-degrading microorganism through tannase production. Ten fungal isolates were screened on tannic acid medium. The highest tannase producer was selected and identified as *Cryptococcus* sp. NRC10 with homology level 99.9%. Medium II at 45°C for 24 h at pH 5 were the most favourable conditions required for maximum enzyme production. Glucose and NH_4Cl were the best carbon and nitrogen sources, respectively. The best conditions for the purified enzyme were 0.20 % of tannic acid and 0.15 ml of tannase enzyme at 45°C after 10 minutes with pH 6. The addition of Mn^{2+} stimulated the enzyme activity. The purified enzyme was used for the degradation of tannins in Red Sea water and green tea leaves samples. The amount of tannins after tannase addition was decreased. In conclusion, tannase has a role in the removal of tannins in contaminated water and soil.

Keywords: 18S rRNA; *Cryptococcus*; SDS-PAGE; tannase; tannins.

1. Introduction

Tannins are natural water-soluble polyphenolic compounds of plant origin, particularly shrubs and leguminous plants (Chowdhury *et al.* 2004; Frutos *et al*. 2004). Tannins are used as dyes or in food industries (Beena 2010; Chowdhury *et al*. 2004; Khanbabaee & van Ree, 2001; Rodriguez *et al.* 2008). They are medicinally important and involved in the treatment of many diseases (Alvi *et al*. 2018; Rangari, 2007). However, they have toxic effects on human when consumed in large quantities, causing hepatotoxicity and cancer (Chung *et al*, 1998; Rodriguez *et al*. 2008). Sometimes they pollute the environment because of their large molecular weights and complex composition, which are difficult to be degraded by microorganisms (Pepi *et al.* 2010; Singh, 2002). Decomposition of organic matter in soil contaminated with abundant tannins is delayed due to inhibition of bio-degradative enzymes of the involved microorganisms (Parales & Haddock, 2004; Pepi *et al*. 2010). However, several bacteria, fungi, and yeasts are resistant to tannins and utilize them as a carbon source (Deschamps *et al*. 1983).

Tannin acyl hydrolase (tannase, E.C.3.1.1.20) belongs to the esterase superfamily that catalyzes the hydrolysis of ester bonds of tannins into gallic acid and glucose (Aguilar *et al*. 2007; Banerjee *et al*. 2012). Tannase has many applications in food, feed, beverages, and the pharmaceutical and chemical industries (Madeira *et al*. 2011; Mohapatra *et al*. 2009). It is involved in the production of gallic acid for several pharmaceutical and cosmetic products (Aithal & Belur, 2013). Tannase is found in plants, animals, and microorganisms, whereas microbial tannase is more stable and is produced commercially (Beniwal *et al.* 2010).

The current work aimed for isolation and identification of tannase producing fungus, optimization of factors affecting tannase production, purification of the enzyme, and biodegradation of tannins in some samples.

2. Materials and methods

2.1. Samples collection

A contaminated soil sample was collected from rhizosphere soil of Madinah, KSA by sharp spade at 5 cm deep, kept in a sterile plastic bag, transferred to the lab. and air dried. Three contaminated water samples were taken from different deeps (1, 3 and 9 m) of Red Sea, North Kornesh, Jeddah, KSA. A green tea sample was purchased from a supermarket in Makkah, KSA.

2.2 Isolation of microorganisms

One gram from each air drying sample was resuspended in 20 ml of tannic acid medium (Jana *et al*. 2012) and incubated with shaking (120 rpm) at 37°*C* for 24 h., and 200 µl from the preculture was transferred into new flasks containing fresh medium (Chowdhury *et al*. 2004). Sterile saline (0.89% NaCl) was used for serial dilution and pure cultures were obtained on nutrient agar plates and incubated at 37°C for 48 h (Chowdhury *et al*. 2004). Separated colonies on tannic acid plates surrounded by clear zone were selected, and the highest clear zone isolate was chosen (Jana *et al*. 2012) and stored at -80°C until used (Sambrook, 2001). Growth was estimated at 550 nm using UV spectrophotometer (Pharmacia LKB-Novaspec II, 80-2088-71) (Vidotto *et al*, 1996).

2.3. Enzyme assay

Tannase activity was determined by the method described by Dave *et al* (2011). From freshly prepared substrate solution (175 mg of tannic acid, 50 ml of citrate buffer 50 mM at pH 5.5), 1 ml was incubated with 0.25 ml of crude enzyme solution at 30°C for 15 minutes; the reaction was stopped by adding five ml of 90 % ethanol. The absorbance was determined at 380 nm using UV spectrophotometer (Pharmacia LKB-Novaspec II, 80-2088-71). One unit (U) is identified as the quantity of enzyme which hydrolyses 1 micromole of the ester bond in tannic acid per min at 30°C and pH 5.5 (Dave *et al*. 2011). Enzyme activity was calculated by using the following formula:

$$Activity(U/ml) = \frac{(\mathrm{A0} - \mathrm{As}) \times 20.3 \times 1.0\ (\mathrm{ml}) \times 1.04 \times df}{0.71 \times 0.25(\mathrm{ml}) \times 15\ (\mathrm{min})} = \Delta A * 7.93 * df$$

20.3: Micromoles of tannic acid in 1.0 ml of substrate solution.

0.71: Change in absorbance after complete hydrolysis of 20.3 µmol of tannic acid under the assay conditions

1.04: A factor for correction (Dave *et al*. 2011)

df: Dilution factor.

2.4. Taxonomical studies

The morphological characters of the selected isolate were recorded after incubation for 4 days. Furthermore, after growth for 2 days on Sabouraud agar plates, microbial morphology and capsule formation were examined using the light microscope (Nikon 860507, Nikon, edipse E400). Many physiological characters were also determined.

DNA was extracted using a QIAamp kit (Qiagen, Germany).PrimerI(5'GTTAAAAAGCTCGTAGTTG-3') and primer II (5'TCCCTAGT CGGCATAGTTTA-3') were designed based on the highly-conserved regions of the 18S rRNA gene of many pathogenic fungi, such as *Cryptococcus neophormans* (Bialek *et al*. 2001). The purified amplicon of 429-bp was sequenced using an ABI PRISM310 genetic analyzer (Perkin Elmer, USA). Data were submitted to the GenBank database and compared using the BLAST program.

2.5. Optimization of tannase production

The preculture was prepared in 50 ml of Sabouraud medium and incubated at 37°C under shaking (120 rpm) for 24 h. Tannin broth medium (50 ml) was inoculated with 2 ml of the tested microbe. After growth, the supernatant was assayed for tannase activity (Dave *et al*. 2011; Mondal *et al*. 2001). The isolate was grown in different media: nutrient broth, medium I (Selective tannic acid medium (STA) (Jana *et al*, 2012), medium II (Mondal *et al*. 2001), medium III (Sivashanmugam & Jayaraman, 2011), medium X (Dave *et al*. 2011). The supernatant was assayed for enzyme production (Mondal *et al*. 2001). Effect of pH on cell growth and tannase production was estimated by cultivating the isolate NRC10 in medium II broth with different pH values (4.0 - 8.0) (Dave *et al*. 2011) at 37°C with shaking (120 rpm) for 24 h, (Sivashanmugam & Jayaraman, 2011). Numerous carbon sources like glucose, fructose, sucrose, maltose, lactose, and galactose were added to medium II broth at 0.5 g/l. Likewise, several nitrogen sources including yeast extracts, meat extract, peptone, and casein, $NaNO_3$ and NH_4Cl were added to medium II broth at 1 g/l. The inoculated flasks were incubated with shaking (120 rpm) at 37°C for 24 h, and tannase activity was estimated (Reddy & Kumar, 2011).

To determine the optimum incubation period for maximal tannase production, the inoculated production medium (pH 5) was incubated at 37 °C with shaking (120 rpm). Samples were withdrawn periodically at every 16 hours up to 144 hours and evaluated for tannase activity. Similarly, the effect of temperature was estimated by varying the growth temperature from 20 to 55 °C at pH 5.0 with shaking (120 rpm), tannase activity was assayed (Reddy & Kumar, 2011).

2.6. Enzyme purification and characterization

Enzyme purification involved three main steps: microbial cells removal by centrifugation, enzyme precipitation from cell-free supernatant at 4 ºC using 60 % ammonium sulfate, and dialysis process to remove any low molecular

weight impurities (Anitha & Arunkumar, 2013). Purification was done using Sephadex G-200 column (2x60 cm), equilibrated with 20 mM acetate buffer, pH 5.0, and anion exchange DEAE cellulose column, equilibrated with 20 mM phosphate buffer, pH 7. The active fractions were collected, lyophilized and stored at -20 °C. The molecular mass of tannase was determined (Laemmli, 1970) on a polyacrylamide slab gel and estimated with reference to high molecular mass protein standard (Amersham Pharmacia) ranged from 5-250 kDa. The impact of substrate concentration, various incubation periods (5 - 40 min.) and various incubation temperatures (20 to 55 °C) on tannase activity was evaluated (Lal & Gardner, 2012; Sabu *et al*. 2005). The effect of pH was studied using different pH varying from 3 to 8 using a citrate- buffer (0.05 M) for pH 3.0-6.0, sodium-phosphate buffer (0.05 M) for pH 6.5-7.0, and Tris–HCl buffer (0.05 M) for pH 7.5–8 (Anitha & Arunkumar, 2013; Nadaf & Ghosh, 2011; Sabu *et al.* 2005).Various metal ions like Mg^{2+}, Zn^{2+}, Ca^{2+} and Mn^{2+} were dissolved in a 0.05 M citrate buffer (pH 5) at a concentration of 0.01 M. The different metal ions were added to the reaction mixture before incubated at 30°C for 15 min (Nadaf & Ghosh, 2011; Sabu *et al.* 2005).

2.7. Enzyme application

One gram of contaminated soil or tea sample transferred into a flask containing 75 ml distilled water was boiled for 30 min. and filtered. The supernatant volume was brought up to 100 ml with distilled water. To the flask containing 1ml of the sample extract and 75 ml distilled water, 5 ml of Folin-Denis reagent (Negi A, 2012) and 10 ml of sodium carbonate solution were added and brought up to 100 ml with distilled water. Likewise, to a flask containing 75 ml distilled water, 1 ml of a contaminated water sample, 5 ml of the Folin-Denis reagent and 10 ml of sodium carbonate solution were added and brought up to 100 ml of distilled water. The mixture was shaken for 30 min and absorbance (700 nm) was read. Then, tannin content was calculated from the standard curve prepared by using gradient concentrations of tannic acid (supplemented data).

To calculate tannase activity, another standard curve was prepared using different concentrations of tannic acid (0.0 – 1.30 %) dissolved in a citrate buffer of 50 mM (pH 5.5). One ml of each concentration was transferred to a tube containing 0.25 ml water. To stop the reaction, 5 ml of 90 % ethanol was added; this mixture was measured at 380 nm with the blank reagent (Dave *et al*. 2011).

Tannic acid concentration was determined before and after applying tannase enzyme as described above. Absorbance was recorded after applying 0.25 ml of tannase enzyme to the reaction mixture instead of water.

2.8. Statistical analysis

Each test had three replicates. Means of variable and standard deviation were documented. Data were analyzed using statistical methods and differences between mean values evaluated by the Student's *t*-test. Differences were considered significant when probability was less than 0.05.

3. Results

3.1. Selection of tannase producing microorganisms

Out of ten isolated microorganisms, four isolates (40%) were tannase producers. The highest enzyme producer was isolate NRC10 which was selected for more studies.

3.2. Identification of the selected yeast strain

Morphological characteristics were defined on selective medium after 24 h. The creamy colored colonies were smooth with a crimpy edge and round to irregular in shape and had a moderate diameter (3-4 mm) with undulate to fimbriae margins. The cells had capsules, appeared singly or in pairs with a pointed bud scar and had a diameter of 3.5±2.2 mm (Figure 1A). The isolate was like *Cryptococcus neoformans*. The physiology and biochemical characters of NRC10 were studied and the results were recorded in Table (1). The isolate was aerobic, and the growth temperature and pH were 20-55ºC and 4-8, respectively.

Identification was achieved using molecular events. The purified amplicon representing 429 bp of 18S rRNA (nucleotide position: 617-1045) was sequenced and compared to GenBank database using Geneious v5.5 program (*www.geneious.com*). This isolate was found belonging to *Cryptococcus* sp. with homology level (99.9 %) as depicted in the phylogenetic tree (Figure 1B). It was identified as *Cryptococcus* sp. NRC10. The partial 18S rRNA sequence of NRC 10 showed high similarity levels (99.9 %) with *Cryptococcus gattii WM276* (NC_014939), C. *neoformans* var. neoformans JEC21 (NC_006684), *C. gattii* WM276 (CP000287), *C. gattii* strain CBS6289 (KF036677) and *C. neoformans var. neoformans B-3501A* (NC_009178). The partial nucleotide sequence of 18S rRNA of NRC10 was deposited in GenBank database (accession number: MF577081).

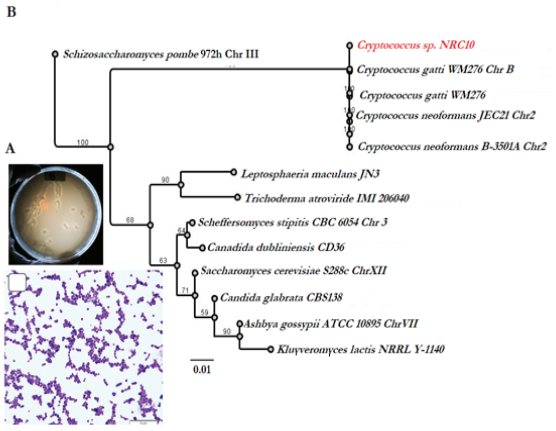

Fig. 1. A: The microbial NRC10 colonies on a selective plate at 37°C for 2 days, the cell shape under light microscope (400X). B: Phylogenetic tree based on 18S rRNA sequence comparisons of *Cryptococcus sp.* NRC10, using neighbor joining tree method, and maximum sequence difference =0.001.

Table 1. Physiological and biochemical characters of the isolate NRC10.

Character	Result
Growth temperature	20-55 °C
Growth pH	4-8
D-glucose	+
Glycerol	-
Calcium 2-Keto-Gluconate	+
L-Arabinose	+
D-xylose	+
Adonitol	-
Xylitol	+
D-Galactose	+
Inositol	+
D-Sorbitol	+
Methyl-α-Glucopyranoside	-
N-Acetyl-Glucosamine	+
D-Cellobiose	+
D-Lactose (bovine origin)	-
D-Maltose	+
D-Saccharose (sucrose)	+
D-Trehalose	+
D-Melezitose	+
D-Raffinose	-

-: negative results, +: positive results

3.3. Optimization of tannase production

The highest growth (1.46±0.05) and the maximum enzyme activity (0.035±0.016) were shown when the yeast NRC10 was grown in medium II (Figure 2A). Thus, medium II was selected for optimization of tannase production. The highest enzyme production was observed at pH 5 (Figure 2B) in medium containing glucose as a sole carbon source (0.023±0.02), whereas the highest growth (1.44±0.01) was found in medium containing maltose (Figure 2C). The maximum yeast growth and tannase production (0.021±0.002 U/ml) were recorded with NH_4Cl (Figure 2D). Furthermore, 24 h was the most suitable period for maximum tannase production (Figure 2E). The maximum tannase production was also found at 45°C (0.047±0.01) and gradually decreased with increasing incubation temperature. The optimum temperature for growth was 37°C (Figure 2F).

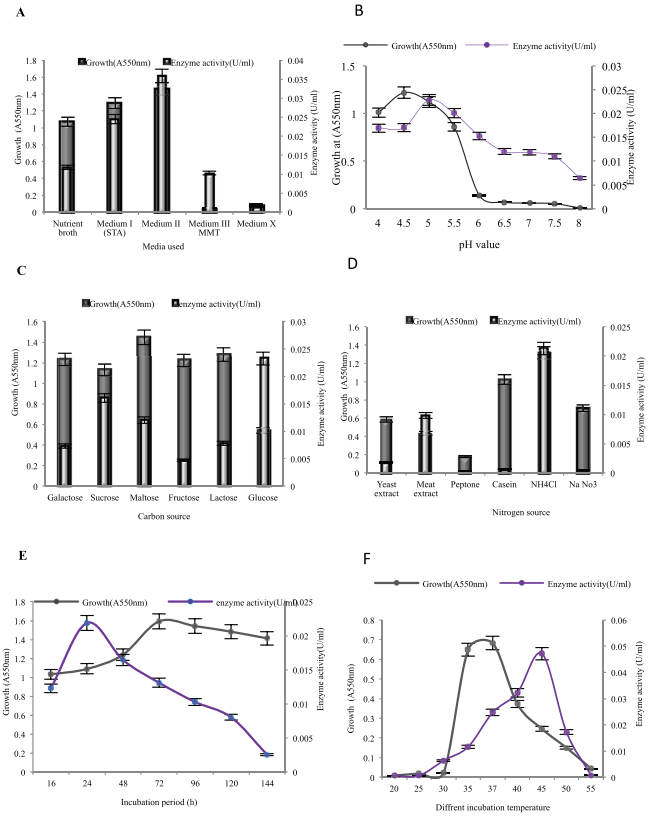

Fig. 2. Effect of different media (A), pH (B), carbon source (C), nitrogen source (D), incubation period (E) and incubation temperature (ºC) (F) on growth (absorbance) and enzyme activity (U/ml) of the selected yeast NRC10.

3.4. Purification and characterization of tannase

A single peak was recorded in the elution profile (Figure 3A) and found to be a monomeric protein, gave a single band with a molecular weight of 50 kDa (Figure 3B).

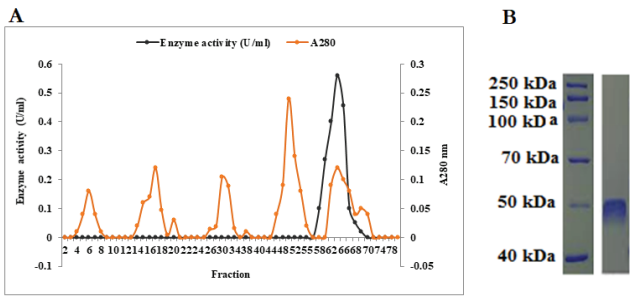

Fig. 3. A: Elution profile of NRC10 tannase on an anion exchange DEAE cellulose column chromatography. B: Molecular weight of the purified tannase. lane 1: Standard, Lane 2: tannase.

Tannic acid was used as an inducer for enzyme production. The maximum enzyme activity was found with 0.20 % of substrate concentration (Figure 4A), after 10 min of the incubation period (Figure 4B), with pH 6 (Figure 4C) and 0.15 ml of enzyme (Figure 4D). The highest relative enzyme activity was also recorded at 45°C (Figure 4E), a sudden drop at 50 and 55°C was observed. The relative enzyme activity was recorded (40.3%) with distilled water only when the citric acid buffer was used as a control. It was shown that among the metal ions, Ca^{2+} and Zn^{2+}greatly inhibited the enzyme activity (7.4, 2.6 % respectively) while Mg^{2+} moderately inhibited it (25.12 %). It is remarkable that Mn^{2+}strangely stimulated the enzyme activity (212.5 %) (Figure 5).

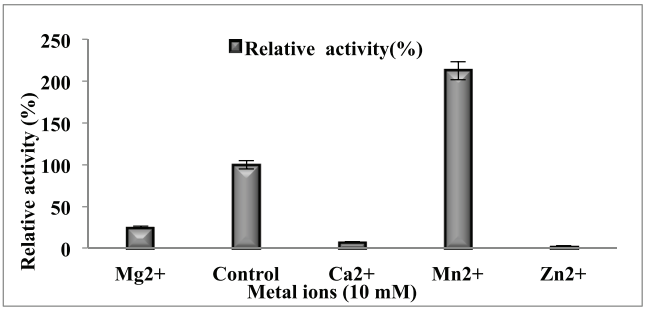

Fig. 5. Effect of different metal ions on relative enzyme activity.

3.5. Tannase application

A linear relation between tannic acid concentrations and absorbance at 700 nm was mentioned (Supplemented data). The absorbance of different weights of both soil samples had average values between 0.0373-0.039

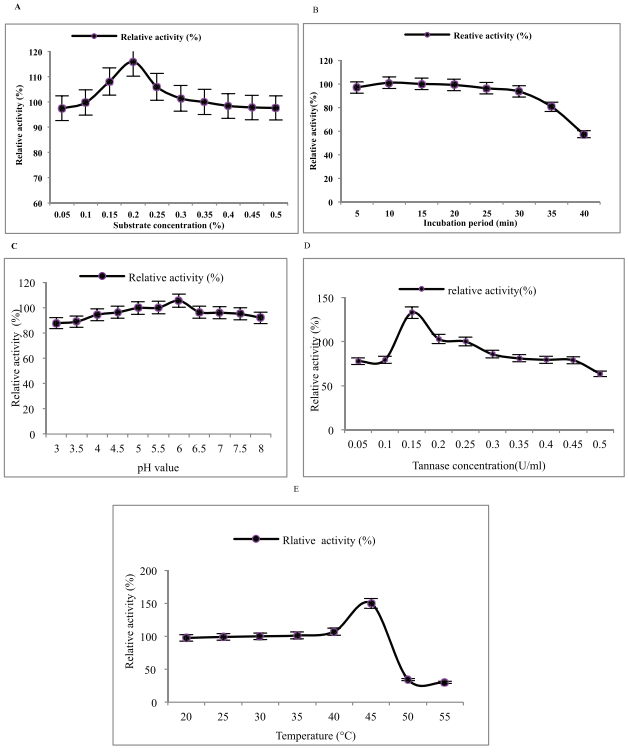

Fig. 4. Effect of different substrate concentration (A), incubation period (B), pH (C), tannase concentration (D) and incubation reaction temperature (E) on relative enzyme activity.

Table 2. Concentration of Tannic acid present in soil, green tea and water samples, collected from different depths

Tested sample	Quantity of Tannic acid	
	Absorbance (700nm)	Concentration mg/l
Soil	0.0376±0.0005	0.6
Water (1m)	0.042±0.0104	0.9
(3m)	0.047±0.0076	3.0
(9m)	0.056±0.0081	16.0
Green tea	0.053±0.009	12.0

Table 3. Determination of tannic acid concentration in water before and after applying enzyme

Water Sample	Before applying enzyme		After applying enzyme		The difference (mg/ml)
	Absorbance	Tannic acid concentration (mg/ml)	Absorbance	Tannic acid concentration (mg/ml)	
Sample 1	0.099	2.8	0.061	1.9	0.09
Sample 2	0.114	3.6	0.072	2. 0	1.5
Sample 3	0.308	10	0.278	8.8	1.2

Table 4. Tannic acid concentration in green tea different weights before and after applying enzyme.

Green tea sample	Before apply enzyme		After apply enzyme		
	Absorbance (380nm)	Tannic acid concentration (mg/ml)	Absorbance (380nm)	Tannic acid concentration (mg/ml)	The difference (mg/ml)
Sample 1	0.163	5.2	0.117	3.6	1.6
Sample 2	0.295	9.6	0.241	8.0	1.6
Sample 3	0.342	11.2	0.300	9.8	1.4
Sample 4	0.383	12.6	0.349	11.6	1.0

which is equivalent to 6 mg/ml of tannic acid concentration (Table 2). Likewise, the concentration of tannic acid in green tea and water samples of different water depths (1, 3, 9 m) were determined by the absorbance at 700 nm. It is obvious that the increase in tannic acid concentration matched with the elevation of water depth. Therefore, increasing the concentration of tannic acid was correlated with rising in the absorbance.

Table 3 summarizes the absorbance and tannic acid concentration of different water samples before and after applied tannase enzyme. Before applied enzyme, it was observed that the tannic acid concentration was different between water samples; tannic acid content was 2.8, 3.6 and 10 mg/ml, respectively. However, after applying tannase enzyme, the tannic acid content was estimated to be 1.9, 2.1, 8.8 mg/ml respectively. It was reported that the tannin content was decreased by 0. 9, 1.2 and 1.5 mg/ml for water samples which reflected the efficiency of yeast tannase enzyme in degradation of tannins in contaminated wastewater. Similarly, Table 4 shows absorbance (380 nm) and tannic acid concentration of different green tea samples. After applying tannase enzyme, tannic acid concentration gradually decreased in green tea. The tannin content was 3.6, 8, 9.8, and 11.6 respectively. The difference of tannic acid concentration (mg/ml) of the green tea was decreased gradually.

4. Discussion

In the present work, the yeast strain, isolated from agriculture soil and produced the maximum yield tannase enzyme, belonged to the genus *Cryptococcus* with a high homology level (99.9 %); it was identified as *Cryptococcus* sp. NRC10.

The maximum tannase production was obtained in medium II which agreed with the finding of Mondal *et al* (2001). The best temperature and pH were 45°C and 5, respectively. Our finding was agreed with that conducted by Lal and Gardner (2012). In contrast, Reddy & Kumar (2011) documented that the highest yield of tannase from *Aspergillus terreus* was at 30°C and pH 3.5. Glucose and NH_4Cl were the best carbon and nitrogen sources, respectively. This result was consistent with the finding obtained by Akroum (2014). However, it did not agree with the studies conducted by Lal & Gardner (2012) and Reddy and Kumar (2011). Other studies illustrated that degradation of tannic acid produced glucose which can be efficiently used by the organism for its growth as a carbon source. Hence, any disaccharide or polysaccharide that produced glucose on degradation will serve as a better carbon source for tannase production (Bradoo *et al.* 1997). Moreover, inclusion of yeast extract resulted in low NRC10 tannase production due to the complexity of the nitrogen source in the fermentation media. Finally, it might be concluded that microorganisms required a low level of nitrogen for producing enzymes because nitrogen could be a limiting factor (Patel *et al*. 2005). Furthermore, 24 h was the optimum incubation period for tannase production. This agreed with the finding obtained by Dave *et al*. (2011), Gupta *et al*. (1997), Mondal *et al.* (2001), and Nadaf and Ghosh (2011).

The purification process of tannase and the obtained cell-free culture filtrate was dark brown in color due to the possible existence of residual tannic acid (Anitha & Arunkumar, 2013). Treatment with aluminum oxide removed the color, leaving the enzyme crude extract colorless and transparent. Tannase from NRC10 is a monomer with a native molecular mass of 50 kDa. In contrast, most known tannases are multimeric proteins. That supported the suggestion of a new group of microbial tannases (Ueda *et al*. 2014). Monomeric enzymes have commonly been reported for bacterial tannases, like *Lactobacillus plantarum* ATCC14917 (Iwamoto *et al*.

2008; Tork *et al*. 2019). Otherwise, *A. niger* MTCC2425 produced extracellular tannase with two different polypeptides of 102 kDa and 83 kDa, (Bhardwaj *et al.* 2003). Also, trimeric and tetrameric enzymes were documented (Barthomeuf *et al.* 1994; Mata-Gomez *et al*. 2009). The best substrate and tannase concentration for high tannase activity were 0.2 % and 0.15 ml, respectively. These results did not agree with those obtained by Dave *et al*. (2011). However, the maximum activity was reported at 1.0 % tannic acid concentration (Iqbal, 2012; Lal & Gardner, 2012). The best reaction incubation period was 10 min for the highest enzyme activity. The optimum reaction time was reported at 15 – 20 min. (Sabu *et al*. 2005). The maximum enzyme activity was at 45°C with pH 6. Aoki *et al*. (1976) in closer results reported the best enzyme activity from *Candida* sp. at 50°C with pH 6. Other studies reported the maximum enzyme activity from *A. niger* LCF8 at 35°C and from *Rhodococcus* NCIM 2891at 30°C (Barthomeuf *et al*. 1994; Nadaf & Ghosh, 2011).

Finally, the divalent metal ions, Mg^{2+}, $Ca^{2+}$and Zn^{2+}strongly inhibited the enzyme activity (25.12 %, 7.5 %, and 2.6 % respectively), while the activity significantly increased with the addition of Mn^{2+} (21.5 %). This result did not agree with that obtained by Iqbal (2012). Kar *et al*. (2003) and Mata-Gomez *et al*. (2009), who found that Ca^{2+} enhanced *Aspergillus* GH1 tannase activity, while Fe^{2+}, Cu^{2+}, and Zn^{2+} inhibited it. Lal and Gardner (2012) documented that Zn^{2+}, Al^{3+}, Mn^{2+}, Cu^{3+}, Li^{3+} metal ions had an inhibitory effect on tannase activity. Metals ions may be required for enzyme activation as a cofactor.

It is important to estimate the degradation efficiency of the purified tannase from NRC10 on tannin compounds. Total tannin content was estimated before and after applying tannase enzyme. Our results demonstrated the contamination of soil by tannins. However, the tannin concentration was increased with an increase in water depth. It seems that at high aeration rates the efficiency of tannin degradation increased significantly, so the elevated content of tannins was found in water collected from depth.

To sum up, the enzyme produced by NRC10 showed distinctive characters, as a monomeric structure, activation by Mn^{2+} and inhibition by Mg^{2+}, Ca^{2+}, Zn^{2+}, suggestive of a new kind of microbial tannase. A suitable pH 5 and temperature 45°C considered an interesting characteristic for its application in biotechnology.

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