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Optimization study for β-mannanase production from locust bean gum by a local *Aspergillus tamarii* NRC 3 isolate.

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ABSTRACT

A survey of forty locally isolated fungal isolates from Egyptian soil was carried out for the extracellular β-mannanase production by using both static and shaking cultures. *Aspergillus tamarii* NRC 3 recorded the highest β-mannanase activity (30.89 Um l^{-1}) when grown statically on a medium contains locust bean gum. No aflatoxins were detected in the culture filtrate of *Aspergillus tamarii* NRC 3. Molecular identification of the isolate was carried out 5S rRNA using fungal ITS primer. Factors affecting β-mannanase Production were studied. From the obtained results, sodium nitrate at a concentration of 0.2%, inoculum age (6 days), inoculum size (one disk, 4 mm diameter, equal $2x10^7$ spores), aeration ratio (4:1), 7 days of static incubation, pH 5.0 and 25 ºC were the favorable parameters for β-mannanase production (31.00 Uml^{-1}, 31.60 Uml^{-1}, 31.67Uml^{-1}, 31.70Uml^{-1}, 31.75Uml^{-1}, 31.75 Uml^{-1}, 31.88 Uml^{-1}, respectively). Surfactants used were, tween 20, 80 and SDS had no effect on β-mannanase production.

Keywords: β-mannanase activity, *Aspergillus tamarii*, locust bean gum, Protein content 5S rRNA, aflatoxin.

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INTRODUCTION

Filamentous fungi are good source for the industrial enzyme production. They are able to produce large amounts of extracellular enzymes [1 and 2]. β- Mannanases are very important enzymes for the digestion of hemicelluloses, one of the most abundant groups of polymers in nature. β- Mannanases hydrolyze mannan yielding mannotriose and mannobiose [3]. β-Mannanases can be produced by a number of yeasts, bacteria, and marine algae, as well as from germinating seeds of terrestrial plants, various invertebrates [4,5,6 and 7] and some fungi like *A. tamarii* [8], *A. oryzae* [9], *A. niger* [10], *Sporotrichum cellulophilum* [11], *Thielavia terrestris* [12] and *Trichoderma reesei* [6]. In recent years, mannanases have gained increased attention because of their various biotechnological applications as upgrading of animal feed stuff [13, 14 and 15], oil drilling, [16, 17, 18, 19 and 20], and can be used to reduce the viscosity of coffee extracts [21and 22]. Mannanases are useful in many fields including bio bleaching of pulp and detergent industry [23, 24and 25], the production of mannooligosaccharides, which were reported to be excellent prebiotics stimulating growth of beneficial intestinal microorganisms [26], and bioconversion of biomass wastes to fermentable sugars [27, 28 and 22]. Mannanase alone or with other enzymes can be used for the economical production of mannose which becomes important substances in medical field because it provides fast dissolving and structure forming properties to the tablets [29]. Also, D-mannose powder can be used for prophylaxis of recurrent urinary tract infections in women [30].

The aim of the present work is to evaluate the local fungal isolates for production of β-mannanase. Also, study the ability of factors affecting the enzyme production by the most potent fungal strain.

MATERIALS AND METHODS

Microorganisms

Isolation and characterization of fungal strains for mannanase production

A serial dilution technique was used to isolate some fungi from Egyptian soil by using modified Czapek's Dox agar medium supplemented with 1% locust bean gum. Fungal isolates which grown on agar plates were quantitatively screened on the same liquid medium. [31 and 10].

Detection of aflatoxins in the culture filtrates of the selected fungal isolate

Aflatoxins extraction was carried out according to the method described by Murakami, and Suzuki [32]. High-performance liquid chromatography (HPLC Model 1525, USA) was used to determine the presence of aflatoxins B1, B2, G1, and G2 in the purified extract.

Identification of the selected fungal isolate

The primers ITS 1 5' (TCC GTA GGT GAA CCT GCG G) 3' and ITS 4 5’ (TCC TCC GCT TAT TGA TAT GC) 3' were used for the PCR. The PCR reaction was performed with 20 mg of genomic DNA as the template in a 30μℓ reaction mixture by using a *EF-Taq* (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2minutes, 30 cycles of 95 °C for denaturation 1minutes, 55°C annealing, and 72 °C extension for 1minutes each were performed, finishing with a 10-minute step at 72 °C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA. The 5S rRNA PCR amplification sequence was matched with previously published 18 S rRNA and 5 SrRNA sequences of *Aspergillus* species in the NCBI databases using BLAST. Selected sequences of other microorganisms with greatest similarity to the 18S rRNA sequences of the fungi isolate were extracted from the nucleotide sequence databases and aligned using MEGA4 generating the phylogenetic tree.

Statistical analysis

Data are expressed as the mean ± S.E. of three independent culture preparations performed in triplicate. Statistical analysis achieved using prism software programmed one-way ANOVA.

Media

PDA medium. Potato dextrose agar medium (PDA) was used for maintenance of the isolated fungi. It contains (g /l): potato 300, glucose 20, and agar 20. Final pH was adjusted to 5. The inoculated slants were incubated for 7 days at 30^{o}C then stored at 4^{o}C until used.

***Aspergillus tamarii* NRC 3 cultivation**

Different five media were used to select the best β-mannanase producing fungal strain. The media were prepared and sterilized in autoclave at 121ºC for 15 min.

Medium (A): modified Czapek's dox medium contained the following ingredients (g/l): locust bean gum 10.0, $NaNO_3$ 2.0, K_2HPO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ traces and agar-agar 20. Final pH was adjusted to 5 [31].

Medium (B): guar gum 21.3, bacto-peptone 57.0, NH_4NO_3 2.5, $MgSO_4.7H_2O$ 1.5, KH_2PO_4 1.2, KCl 0.6 and trace elemental solution 0.3ml/l. Final pH was adjusted to 5.5. [33].

Medium (C): copra meal 10.0, $NaNO_3$ 2.0, KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5 and FeSO4.7H2O traces. Final pH was adjusted to 6.8 [34].

Medium (D): palm kernel cake (PCK) 10, $MgSO_4.7H_2O$ 0.5, KH_2PO_4 1.0 and yeast extract 1.0. Final pH was adjusted to 5.5 [35].

Medium (E): locust bean gum 10.0, KH_2PO_4 2.0, $NaNO_3$ 5.0, $MgSO_4{\cdot}7H_2O$ 0.3, $CaCl_2$ 0.3, yeast extract 1.0, tween 80 1 ml/l and trace elements solution 1.0 ml/l. Final pH was adjusted to 5.5 [36].

Preparation of the crude enzyme

Fifty ml aliquots of the production medium were dispensed in 250 ml Erlenmeyer flasks. The medium was adjusted to pH 5 and then sterilized at 121^{o}C for 15 min. After sterilization, each flask was inoculated with 1 ml fungal spores suspension, obtained from 7 days old culture; the flasks were then incubated statically at 30°C for 7 days. At the end of incubation period, the fungal mats were separated from the culture by filtration and the clear supernatant was considered as the crude enzyme source [31].

Enzyme assay

The reaction mixture containing 1 ml of 1% locust bean gum dissolved in 0.05 M acetate buffer at pH 5 and 1 ml enzyme solution was incubated in water-bath at 40^{o}C for 5 min, after incubation time 1.0 ml dinitrosalicylic acid (DNS) was added to 1 ml reaction mixture [37]. This mixture was boiled for 10 min. The released mannose due to the enzyme activity was determined by measuring the absorbance of the cooled mixture at 550 nm using spectrophotometer (Cary 100 UV-vis). One unit of mannanase activity is defined as the amount of enzyme needed to produce 1.0 µmol ml^{-1} of released mannose.

Estimation of protein content

The protein content of the enzyme preparation was determined by the method of Bradford and the developed color was measured at 595 nm with bovine serum albumin as standard [38].

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Determination of dry weight

The fungal biomass was separated from the fungal culture by filtration, washed twice with distilled water and dried at 70°C till reach a constant weight.

RESULTS AND DISCUSSION

Isolation of a high level β–mannanase producer strain

Among 40 fungal strains grown on solid Czapek's Dox medium supplemented with 1.0% locust bean gum as a substrate for β-mannanase production, 35 strains can able to grow in plates and the qualitative mannanase activity was determined on the basis of clear zones formed around the colonies. The fungi in plates showing the highest clear zone were selected by visual observation followed by a second selection using liquid modified Czapek's Dox medium. The production of mannanase activity on locust bean gum had been demonstrated by Dahawan *et al.,* and Sae-lee [17 and 39]. The formation of clear zone by these isolated fungal strains on agar medium supplemented with locust bean gum could be attributed to the ability of their genetic make up to secrete active mannanase with high diffusion rate as reported by Sae-lee [39].Of the thirty five isolates grown statically on the fermentation medium (pH, 5.0) at 30ºC for 7 days, a fungal strain NRC 3 grown on static culture was found to be the highly active organism for β-mannanase activity (27.64 u/ml with specific activity, 13.82 u/mg), whereas the extracellular β-mannanase produced in shaken culture was generally lower than that of static one (7.76 U/ml with specific activity 0.06 U/mg). These results in agreement with results of Artupin and Alsarani [34 and40].The highest β-mannanase activity in the static culture compared to shaken one could be attributed to the oxygen limitation that could be a serious problem in the shaken cultivation due to the highly non-Newtonian medium caused by the filamentous growth of the fungus [41].

Aflatoxin test of NRC 3 isolate culture filtrate

No aflatoxins (B1, B2, G1 and G2) were detected in fungal isolate NRC 3 culture filtrate as shown in Fig.1b in comparison with a standard as indicated in Fig.1a. Murakami and Suzuki [32] suggested that relatively few studies have compare aflatoxin in producing and non-producing isolates of *Aspergillus*.

Identification of NRC 3 isolate

Aspergillus tamarii was identified by 5S rRNA using ITS primer 1 and 4 by PCR-amplification for further characterization. The nucleotide sequence was compared to existing sequences in the databases. A dendrogram showing the result of 18S rRNA and 5S rRNA analysis is shown in Fig. 2. The result showed highest matching of fungi isolate to members of the *Aspergillus* group. As presented, the 18S rRNA sequence of the fungi isolate is greatest closely associated to *Aspergillus tamarii* with similarity of 99% which identified by ITS primer also. This result was identical with the conclusion of the cultural, morphological, biochemical and physiological characterization. In *Aspergillus* sp., 5S rRNA sequences are identical in several complexes of critical mycotoxigenic, industrial, and medically important species, and additional markers are necessary [42]. Although the ITS region is a potentially effective DNA barcode in several lichenized lineages [43].

Optimization of some critical cultural conditions affecting the β–mannanase production by *A. tamarii* NRC3

Effect of different media on β–mannanase production

Five different media (A, B, C, D and E) were used for testing the productivity of β–mannanase by fungal strains. The results presented in Table (1) indicated that a high β–mannanase activity (30.78 U/ml) was observed when the fungus *A. tamarii* NRC3 was cultivated in a medium (A) which contained locust bean gum (pH, 5.0) followed by a medium (B) (25.54 U/ml) and the lowest activity was occurred in a medium (E) (1.18 U/ml). The pH values of *A. tamarii* NRC3 culture filtrates were alkaline pH (7.89-8.65) after 7 days of incubation. These results were in agreement with the results of [25, 33 and 44].

Effect of different natural substrate and different carbohydrates on β– mannanase production

Nine different natural substrates (locust bean gum, wheat germ, soy bean, coconut pulp, milled carob, wheat bran, rice bran, rabbit feed and fenugreek) were used in this test. In addition to 13 sources of carbohydrates (glucose , fructose , sucrose , arabinose , galactose , lactose , sorbose , maltose , mannose, cellobiose , starch , xylose and locust bean gum as control). Upon testing the influence of some natural substrates and various carbohydrates on the β–mannanase production by *A. tamarii* NRC3. Different substrates were separately added to medium (A), (pH, 5.0) in such amount that the final concentration 1.0% of these substrates in the medium remained unchanged. The result in Table (2) showed that slight decreases in β–mannanase production were occurred by using wheat bran (30.54 U/ml) followed by Coconut pulp (30.46 U/ml) in comparison with locust bean gum (30.81 U/ml). Juliet *et al*., [44] found that the value obtained for orange peels was significantly lower than that of locust bean gum (control).On the other hand, the other various sugars used gave the lowest β–mannanase activity (Table, 3). It was noticed that the final pHs were increased after 7 days of incubation. (6.4-8.29)These results agreed with most of researchers who worked on β–mannanase production from different sources and used galactomannan-rich substrate locust bean gum widely as an inducer of β-mannanase [45, 46 and 44].

Effect of different concentration of locust bean gum on β-mannanase production

Different concentrations of locust bean gum ranging from (0.25%- 2.0%) were individually supplemented to the medium (A). Data presented in Table (4) showed that 1. 0% locust bean gum was the best concentration for β-mannanase activity (30.80 U/ml); the lowest β-mannanase activity was found at 0.25% and 0.5% locust bean gum (13.16 U/ml, 15.78 U/ml). The pH values in *A. tamarii* culture filtrate were increased (6.43-6.81). The extracellular protein content of the culture filtrates ranged from 1.12 mg/ml – 2.34 mg/ml). The highest protein content was obtained at 1.5% locust bean gum. Also, the growth of *A. tamarii* NRC3 as dry weight ranged from 0.03 g/50ml- 0.37 g/50ml). It was generally observed that there was no direct relationship between the protein content or fungal growth and the mannanase activity. These observations were in agreement with the observations of Abd-El Aziz and El-Naggar [10 and 31].

Effect of different nitrogen sources on β-mannanase production

A total of 19 different nitrogen sources were tested including 8 inorganic nitrogen sources (ammonium phosphate, mono, di, and tri-ammonium sulphate, sodium nitrate, potassium nitrate and ammonium chloride) and 11 organic nitrogen sources (urea, casein light, casein hydrate, meat extract, malt extract, yeast extract, beef extract, peptone, soy bean, ammonium oxalate , ammonium acetate and ammonium citrate). Regarding the effect of inorganic and organic nitrogen sources on β-mannanase production by *Aspergillus tamarii* NRC3. Therefore, nitrogen source of medium was replaced by other nitrogen in such amount that final concentration of nitrogen in medium remained unchanged. The results in table (5-6) showed that sodium nitrate was the best nitrogen source for β–mannanase activity (30.95 U/ml). Sodium nitrate promoted maximum production of mannanase by different types of fungi [47]. While, potassium nitrate gave β–mannanase activity (30.60 U/ml). On other hand, meat extract as organic nitrogen source recorded β–mannanase activity (28.16 U/ml) as shown in Table (6). It was reported that the enzyme activity of fungus *Arthrographis* sp. was higher on using inorganic nitrogen sources than organic nitrogen sources [44].

Effect of different concentration of $NaNO_3$ on β-mannanase production

The results indicated that sodium nitrate was found to be the best inorganic source for β-mannanase production by *Aspergillus tamarii* NRC3, according sodium nitrate was tested in production medium at different concentrations ranging from (0.25- 4 g/l). β-mannanase production was recorded in Fig., 3. The results showed that 2.0 g/l of sodium nitrate induced maximum β-mannanase production (30.95 U/ml).

Effect of initial pH of the culture medium on mannanase production

The tested pH of the production medium ranged from (3-8). The optimal pH for mannanase production by *Aspergillus tamarii* NRC3 was 5.0 (Fig., 4). Generally, the organism prefers the acidic range and this was also, reported by other researchers [31 and 41].

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Effect of inoculum age of *Aspergillus tamarii* NRC3 on β- mannanase production

Inoculum age was tested at regular intervals (2-4-6-8-10-12 days). Data presented in Fig., 5 showed that the 6-days old of inoculum gave the highest β-mannanase activity (31.67 U/ml).

Effect of inoculum size of *Aspergillus tamarii* NRC3 on β- mannanase production

Spore suspension ranging from (0.5-4x10^7 spore/ml) obtained from a 6-day old of *Aspergillus tamarii* NRC3 were used in this test. An increased of mannanase activity was noticed on raising the inoculum size from 0.5 to 2.0 x 10^7 spores/50ml medium. The decrease of the enzyme activity with increasing the inoculum size may be due to the lack of nutrients which led to decrease in enzyme production [10].

Effect of aeration on mannanase production

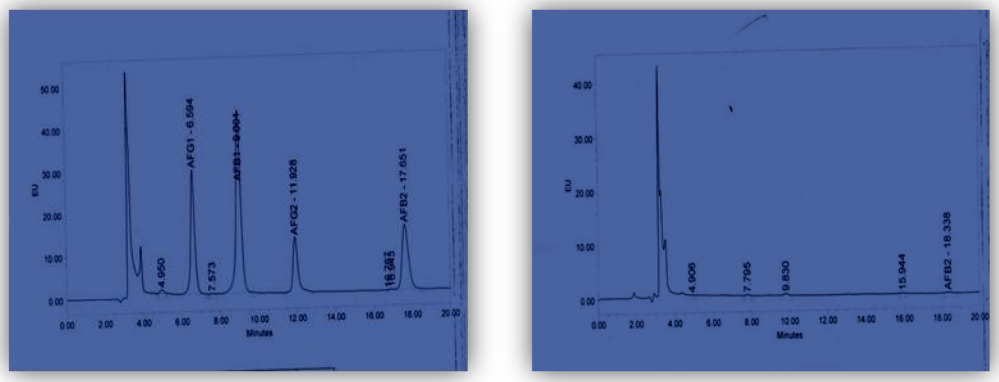
By using different volumes of production medium ranged from (25-150 ml medium / 250 ml conical flask), noticed that the highest volume for mannanase activity (31.70 U/ml) was obtained on using 50 ml of production medium after 7 day of incubation at 30ºC. β- mannanase activity was decreasing with increasing the volume of the production medium Table (8).

Effect of incubation period on mannanase production

β- mannanase activity was increased regularly during the first 7 days of incubation. The highest mannanase activity was obtained at 7^{th} day of incubation (31.75 U/ml) as indicated in Table (9). Gradually decreases in enzyme activity were occurred as incubation periods increased. The decrease in the β-mannanase activity at long incubation periods might be due to the depletion of nutrients and accumulation of other by-products like proteases in the fermentation medium initiating autolysis of cells [48 and 49].

Effect of incubation temperature on mannanase production

The inoculated flasks were incubated for 7 days at different temperatures ranging from 20 to 45 °C (Fig. 6). The maximum mannanase activity was achieved at an incubation temperature of 25°C. At higher or lower temperatures, the enzyme activity was decreased. Regarding the temperature effect and according to the theory proposed by Christgau *et al.,* [50] who reported that the synthesis of this enzyme can be induced. However, when the inducer is removed or the cell is in stationary phase, the enzyme synthesis will stop immediately. This observation would suggest that the mRNA of this kind of enzyme is unstable. An appropriate decrease in temperature would enhance the stability of the mRNA and prolong the duration of enzyme production. However, the operating temperature cannot be too low as biochemical reaction rate will decrease with decreasing temperature.



(a) **(b)**

Fig 1: Detection of aflatoxins on a standard sample (a) and in *A. tamarii* culture filtrate (b) using HPLC

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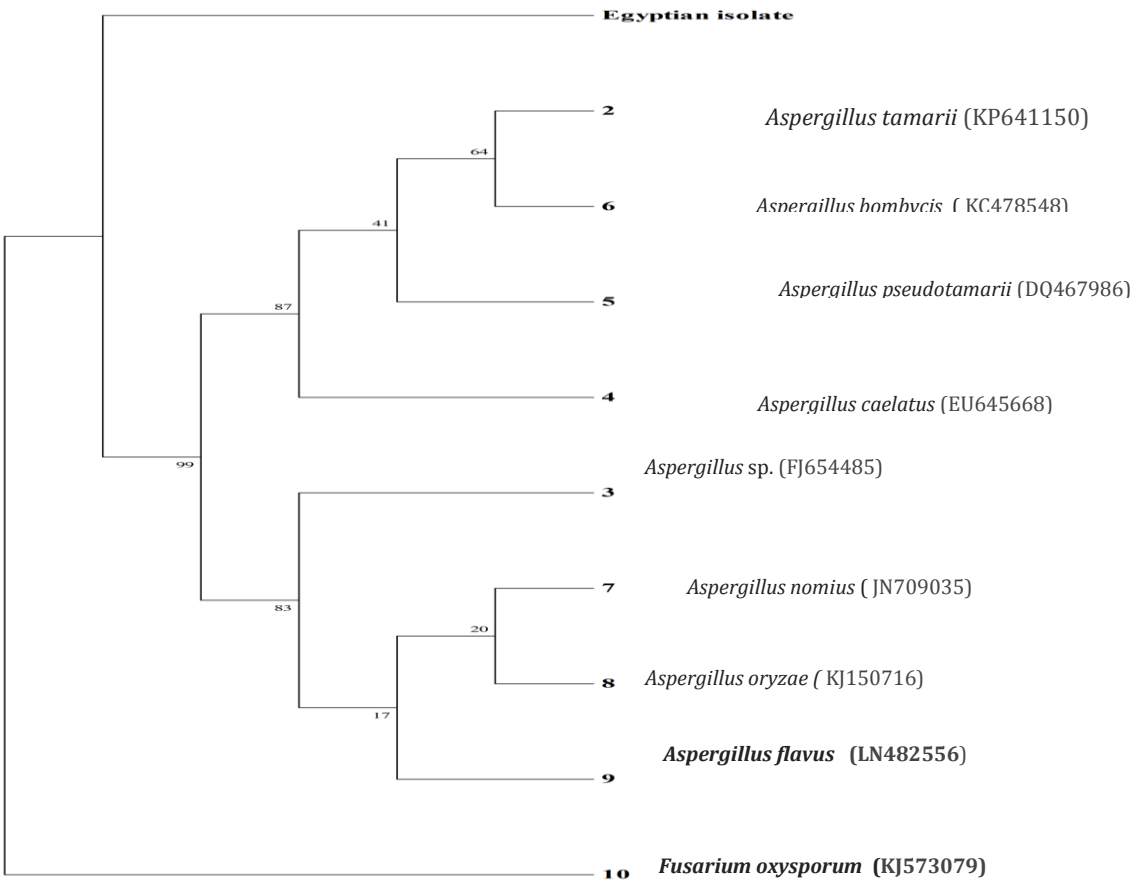

Fig 2: Phylogenetic tree of *Aspergillus tamarii*

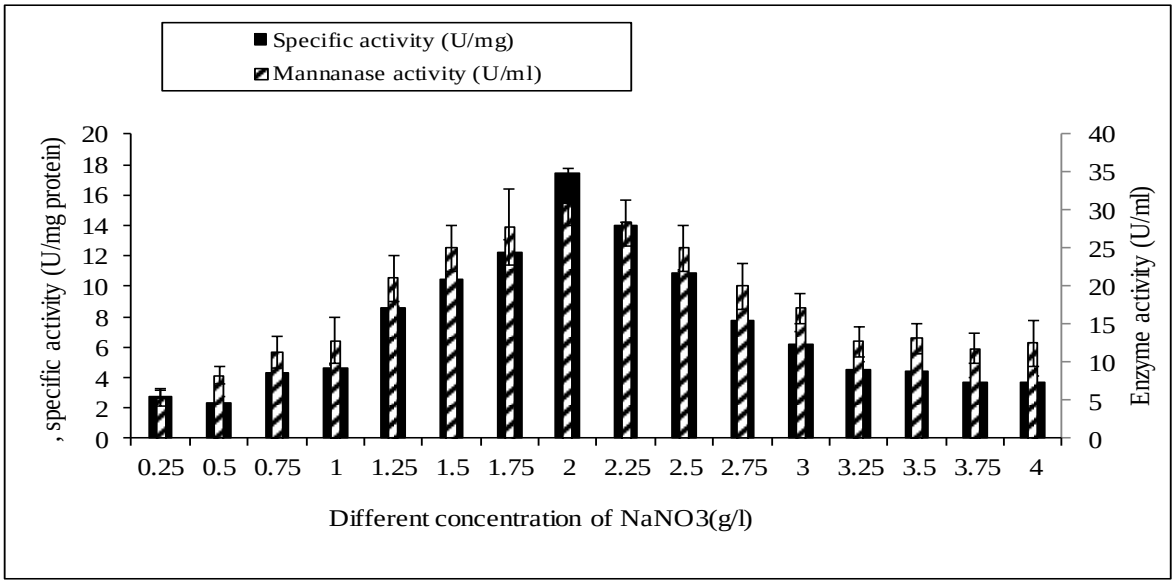

Fig 3: Effect of different concentrations of $NaNO_3$ on β-mannanase production by *Aspergillus tamarii* NRC3

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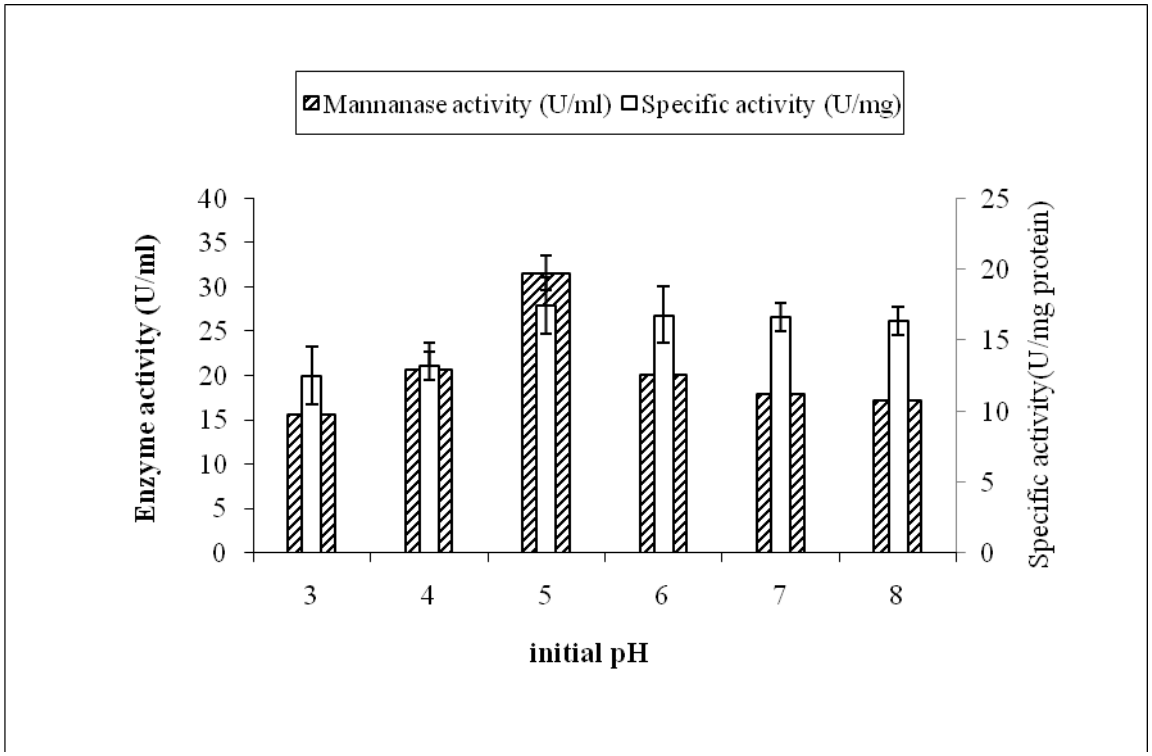

Fig 4: Effect of initial pH on mannanase production by *Aspergillus tamarii* NRC3

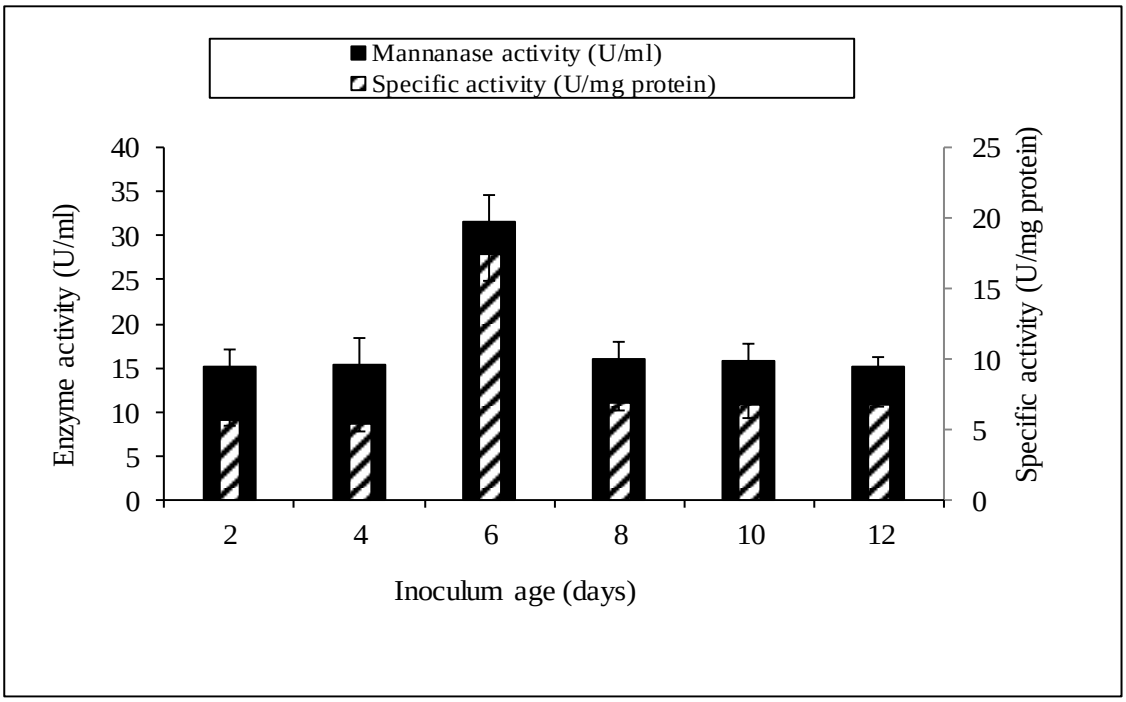

Fig 5: Effect of inoculum age of *Aspergillus tamarii* NRC3 on mannanase production

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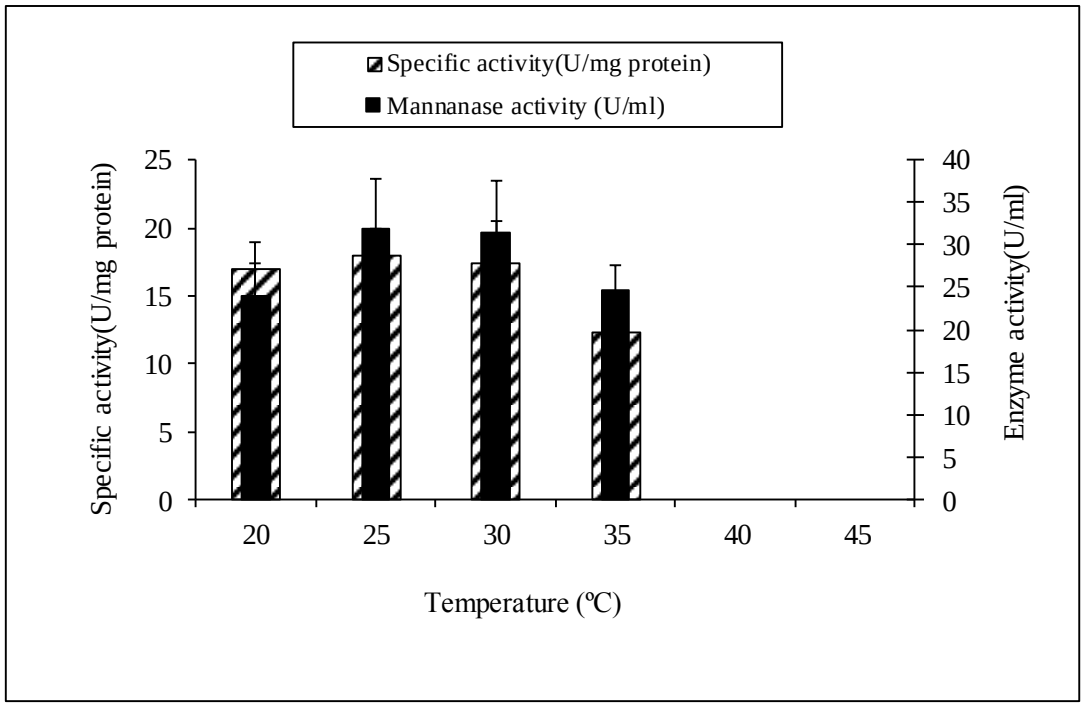

Fig 6: Effect of incubation temperatures on mannanase production by *Aspergillus tamarii* NRC3

Table 1: Effect of different media on β-mannanase production by *Aspergillus tamarii* NRC3

Different Media	Initial pH	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
A	5.00	8.63	0.18±0	1.97±0.2	30.78±4	17.00±3
B	5.47	7.89	1.05±0.2	11.48±2	25.54±5	3.10±0.5
C	6.80	7.96	0.20±0	2.16±0.2	23.92±4	12.00±2
D	5.50	8.65	0.28±0	3.94±0.4	15.66±3	3.98±0.6
E	5.50	7.51	0.22±0	3.61±0.4	1.18±0.4	0.32±0

Table 2: Effect of different natural substrates on β-mannanase production by *Aspergillus tamarii* NRC3

Different substrates	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
Locust bean gum	6.45	0.19±0	1.79±0.3	30.80±6	17.20±3
Wheat germ	7.89	0.14±0	2.62±0.4	14.60±2	5.58±1
Soy bean	8.05	0.12±0	4.26±0.9	21.18±2	4.78±1
Coconut pulp	6.40	0.14±0	2.53±0.5	30.46±4	12.04±2
Milled carob	7.09	0.13±0	2.89±0.6	2.64±0.6	0.92±0.1
Wheat bran	7.12	0.13±0	3.61±0.9	30.54±5	8.46±1
Rice bran	6.82	0.11±0	1.08±0.4	1.26±0.6	1.16±0.5
Rabbit feed	7.18	0.07±0	1.71±0.6	22.90±3	13.40±3
Fenugreek	8.29	0.12±0	3.61±0.5	29.08±3	8.06±1

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Table 3: Effect of various carbohydrates sources on β-mannanase production by *Aspergillus tamarii* NRC3

Different carbohydrates sources	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
Locust bean gum	6.45	0.17±0	1.81±0.4	30.81±4	17.02±3
Glucose	7.33	0.12±0	1.02±0.2	0.80±0.1	0.78±0.1
Fructose	6.80	0.13±0	0.69±0.1	0.40±0	0.60±0.1
Sucrose	7.10	0.18±0	1.77±0.3	0.72±0.1	0.40±0
Arabinose		No growth			
Galactose					
Lactose					
Sorbose	6.70	0.10±0	1.12±0.2	5.00±1	4.46±0.5
Maltose	7.07	0.14±0	0.69±0.1	0.54±0	0.78±0.2
Mannose	7.63	0.15±0	1.02±0.4	0.76±0.1	0.74±0.1
Cellobiose	6.62	0.15±0	0.66±0.1	3.68±0.4	5.58±1
Starch	7.66	0.14±0	0.43±0	0.76±0.1	1.74±0.5
Xylose	7.01	0.16±0	0.52±0.1	0.68±0.1	1.32±0.6

Table 4: Effect of different concentrations of locust beam gum on β-mannanase production by *Aspergillus tamarii* NRC3

Different concentration of Locust bean gum%	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
0.25%	6.46	0.03±0	1.12±0.3	13.16±4	11.96±2
0.50%	6.57	0.08±0	1.28±0.4	15.78±6	12.34±2
0.75%	6.79	0.13±0	1.67±0.5	21.46±3	12.86±3
1.00%	6.45	0.20±0	1.78±0.4	30.89±2	17.35±3
1.25%	6.46	0.23±0	2.23±0.6	25.66±5	11.50±2
1.50%	6.43	0.27±0	2.34±0.5	23.68±4	10.12±1
1.75%	6.71	0.26±0	2.13±0.5	21.18±3	9.94±1
2.00%	6.81	0.37±0	2.13±0.4	20.86±2	9.78±1

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Table 5: Effect of different inorganic nitrogen sources on β-mannanase production by *Aspergillus tamarii* NRC3

Different Inorganic Nitrogen sources	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
Ammonium phosphate	3.09	0.19±0	0.52±0.1	1.02±0.3	1.98±0.4
Di-Ammonium phosphate	3.76	0.18±0	0.66±0.1	0.10±0	0.15±0.0
Tri-Ammonium phosphate	3.34	0.19±0	1.15±0.2	0.16±0	0.14±0
Ammonium sulphate	3.05	0.14±0	2.20±0.2	0.44±0	0.20±0
Sodium nitrate	6.50	0.18±0	1.78±0.3	30.95±5	17.39±3
Potassium nitrate	6.73	0.18±0	2.72±0.3	30.60±6	11.25±2
Ammonium nitrate	3.71	0.19±0	1.38±0.3	3.96±0.5	2.88±0.6
Ammonium chloride	3.02	0.12±0	2.79±0.3	0.64±0.1	0.24±0

Table 6: Effect of different organic nitrogen sources on β-mannanase production by *Aspergillus tamarii* NRC3

Different organic Nitrogen sources	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
urea	6.85	0.17±0	4.07±0.4	20.26±4	5.78±1
Casein light	6.52	0.20±0	1.61±0.2	5.14±1	3.18±0.6
Casein hydrate	6.40	0.20±0	2.33±0.2	3.42±0.6	1.46±0.5
Meat extract	6.11	0.20±0	2.79±0.2	28.16±3	10.10±0.9
Malt extract	3.80	0.08±0	1.28±0.3	7.50±1	5.86±1
Yeast extract	6.61	0.20±0	3.78±0.3	15.40±3	4.08±1
Beef extract	7.04	0.19±0	3.44±0.3	26.18±5	7.62±1
peptone	6.52	0.20±0	2.36±0.4	15.52±2	6.58±1
Soya bean	3.76	0.10±0	1.77±0.2	8.02±1	4.54±0.5
Ammonium citrate	6.82	0.17±0	2.69±0.5	12.50±3	4.64±0.3
Ammonium oxalate	6.40	0.20±0	3.51±0.5	11.18±2	3.18±0.4
Ammonium acetate	6.01	0.19±0	1.94±0.3	10.00±1	5.16±0.3

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Table 7: Effect of inoculum size on mannanase production by *Aspergillus tamarii* NRC 3

Inoculum's size (spore/ml)x10^7	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
0.50	7.04	0.15±0	2.49±0.4	21.72±4	8.72±1
1.00	7.18	0.16±0	2.36±0.4	26.06±5	11.04±1
1.50	7.24	0.16±0	2.26±0.3	27.64±5	12.22±2
2.00	7.09	0.19±0	1.807±0.3	31.67±6	17.53±2
2.50	7.14	0.18±0	2.56±0.4	28.56±5	11.16±1
3.00	7.27	0.18±0	2.62±0.4	26.84±4	10.24±1
3.50	7.10	0.18±0	2.72±0.4	26.72±3	9.82±1
4.00	6.88	0.16±0	2.62±0.5	25.40±3	9.70±0.8

Table 8: Effect of aeration on β-mannanase production by *Aspergillus tamarii* NRC3

Different volumes of production medium	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
25	7.69	0.06±0	2.29±0.3	20.14±3	8.78±1
50	7.30	0.18±0	1.80±0.4	31.70±4	17.61±2
75	6.97	0.21±0	2.00±0.3	23.30±3	11.64±2
100	7.38	0.20±0	1.94±0.5	20.92±3	10.78±1
125	7.29	0.19±0	1.84±0.4	19.08±3	10.38±1
150	6.70	0.15±0	1.74±0.3	16.18±2	9.30±0.8

Table 9: Effect of incubation period on β-mannanase production by *Aspergillus tamarii* NRC3

Incubation period (days)	Final pH	Dry weight(g/50ml) mean±SD	Protein (mg/ml) mean±SD	Mannanase activity (U/ml) mean±SD	Specific activity (U/mg) mean±SD
3	6.91	0.06±0	1.31±0.2	17.50±3	13.36±2
5	6.69	0.14±0	1.48±0.3	23.16±4	15.64±2
7	7.09	0.17±00	1.79±0.3	31.75±5	17.74±3
9	7.27	0.10±0	1.87±0.3	27.50±3	14.70±2
11	7.26	0.10±0	1.67±0.3	24.08±3	14.46±2

CONCLUSION

This work collectively showed a high ability of the locally isolated *A. tamarii* for production of an extracellular mannanase enzyme which plays a significant role towards the enhancement of the industrial solubilization of lignocellulose under the optimized environmental and nutritional conditions.

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