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PROTOPLAST FUSION IN *PENICILLIUM FUNICULOSUM* MUTANTS FOR ENHANCING DEXTRANASE PRODUCTION

[4]

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ABSTRACT

Two induced mutant strains, obtained from the fungus *Penicillium funiculosum* after UV treatments, were used for protoplast fusion. These mutants (12 and 18) were selected according to their dextranase activity and stability. Seven fusants having dextranase over-yield than their parental strain were obtained. The best dextranase producing fusant (No. 2) was selected for further studies. Dextranase was purified from cell-free culture of fusant 2 by consecutive column chromatography using Q-sepharose FF, Superose 12 prep. grade 60/600 and Mono Q-FPLC. The purification was estimated by SDS-PAGE as well as isoelectric focusing. Four dextranase components (I, II, III and IV) were separated with an estimated molecular weights of 67 kDa. The pI of the all components were found to be around 3.0 as estimated by gel electrophoresis using both broad and low pI calibration protein kits. The dxetranase components showed pH and optimum temperature of 5 and 55°C, respectively. Dextran was the sole carbon source for dextranase production by the dextranase components. The effect of the dextranase components on different types of dextrans were also studied.

INTRODUCTION

Dextranase, ∝ 1,6-D-glucan-6-glucanohydrolase (E.C.3.2.1.11), which hydrolyse the ∝ 1,6-linkage in dextran, was found in different fungi (**Madhu and Prabhu, 1984; Galvez-Mariscal and Lopez-Munguia, 1991; Petronijevic *et al* 1993; Szczodrak *et al* 1994**); yeast (**Webb and Spencer-Martins, 1983; Koenig and Day, 1988**); bacteria (**Wanda and Curtiss, 1994; Hoster *et al* 2001 and Igarashi *et al* 2004**); Kidney, Liver, intestine and spleen (**Preobrazhenskaya *et al* 1974**) and also in higher plants (**Heyn, 1981**).

Dextranase enzyme has many industrial applications such as removing dextrans from sugar syrup in sugar mills (**Lee and Fox, 1985; Barfoed and Molgaard, 1987; Sun *et al* 1988; Brown and Inkerman, 1992**). It is also useful in preventing the formation of dental caries (**Fitzgerald *et al* 1968; Caldwell *et al* 1971; Murayama *et al* 1973; Hamada and Slade, 1980**) and in the production of clinical size dextran (**Day and Kim, 1992**).

Protoplast fusion is an efficient tool for inducing new genetic recombination. Protoplasts are structures derived from vegetative cells whose their entire walls have been removed or modified forming what so called "Spheroplasts". Protoplast fusion leads to a remarkable increase in the frequency of genetic recombination and results in introducing hybrid products. i.e., when mating closely related strains that make different compounds, novel substances were obtained (**Demain and Solomon, 1981**).

This work was aimed to isolate a potent dextranase-producing fusant strain from two mutant strains of *P. funiculosum* NRRL 6014 having a high dextranse productivity and to purify it with a series of chromatographic processes.

MATERIALS AND METHODS

The organism

Upon the treatment of *P. funiculosum* NRRL 6014 with UV light, different mutants are obtained.

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These mutants were evaluated for their dextranase productivity and the best enzyme producers (12 and 18) were chosen for protoplast induction.

Media and growth conditions

The fusants were maintained on a medium containing (g/l): glucose, 30; Peptone, 5; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; yeast extract, 0.5 and agar 15-20. The pH was adjusted to 6 and this is considered as complete medium (CM). The antifungal medium contains the same components of the complete medium with addition of one or more antifungal. The used antifungals were; griseofulvin (100 μg/ml), cycloheximide (250 μg/ml) and nystatin (50 units). These antifungals were produced by Sigma. The commercial fungicide, topsin (2.5 μg/ml), was also used. All antibiotics were dissolved in dimethylsulfoside (DEMSO) before adding the sterilized medium. The production medium has the following constituents (g/l): Dextran, 15; $NaNO_3$, 2; $MgSO_4.7H_2O$, 2.5 and yeast extract, 2. The pH of the culture medium was adjusted to 7. The strain was incubated at 30°C for 8 days on a rotary shaker (125 rpm). The culture was centrifuged at 16,000 g for 20 min. and then used for purification **(Abdel-Aziz, 2005)**.

Protoplast fusion technique

According to their dextranase productivity and resistance or sensitivity to one or more anifungal agents; equal numbers from protoplasts of the two mutants were mixed and centrifuged, the pellets were then resuspended in 1ml of prewarmed (30°C) solution of 20% (w/v) polyethylene glycol 6000 (PEG) containing 0.01M CaCl2 and 0.5M glycine buffer (pH 7.0). PEG-treated protoplast suspension were plated onto an antifungal selective medium. Colonies were considered as complementary fusants **(Reymond and Fevre, 1986; Prabavathy *et al* 2006; El-bondokly and Talkhan, 2007)**.

Enzyme and protein assay

Dextranase activity was measured by incubating the enzyme with a buffered dextran (2.5% w/v) using 0.05M acetate buffer pH 5 at 55°C **(Koenig and Day, 1988; Szczodrak *et al* 1994)**. The enzyme activity was measured by detecting the produced reducing sugar by DNS (3,5-dinitorsalicylic acid) according to **Sumner (1924)**. One enzyme unit (IU) is the amount of enzyme which liberates 1μ mole reducing sugar (glucose equivalent) per minute under the standard conditions. Protein was measured according to **Lowry *et al* (1951)**.

Dextranase purification

Different purification steps were used to purify dextranase enzyme from the culture filtrate of *Penicillium funiculosum* NRRL 6014 (fusant strain). A sample of 500 ml of the culture filtrate, the myclia have been removed by centrifugation at 16,000 g, was diluted to about 4000 ml to adjust the conductivity to be similar to the 20 mM Bis-Tris buffer and the pH was adjusted to 4.8 for both. The sample was then loaded to Q-Sepharose FF column (225ml) equilibrated with 20 mM Bis-Tris buffer and eluted with the same buffer containing 0.0-0.2M NaCl for 5 column volumes and the dextranase activity was measured for the eluted fractions. The dextranase active fractions were collected and concentrated to be 25 ml by using Amicon Ultrafiltration cell through a 10 pk Amicon Millipore membrane and this sample was then loaded to a gel filtration column (Superose 12 prep. grade 60/600) equilibrated with 20 mM Phosphate buffer pH 7.0 containing 0.2M NaCl, then the dextranase active fractions (one peak) were also collected and concentrated and desalted and this sample (30ml) was then loaded to Mono Q-FPLC column (10 ml) equilibrated with 20 mM Bis-Tris buffer pH 4.8 and elution has been done by the same buffer containing 0.0-01M NaCl (20column volume, CV). These chromatographic processes were carried out using ÄKTA FPLC Amershamphaimacia biotech (Uppsala, Sweden).

Enzyme Characterization

The purity and the molecular weight of the purified enzyme were estimated by SDS/PAGE electrophoresis using Phast Gel 8-25 with the use of low molecular weight calibration protein kit. The pI of the purified enzyme was also estimated by using IEF electrophoresis using Phast Gel IEF 3-9 with broad pI protein kit.

The optimum pH was measured by incubating the enzyme at 55°C for 10 min. in 2.5% (w/v) dextran dissolved in buffers with different pHs (3-9) using different buffers. pH stability was measured by incubating the enzyme alone in different buffers with different pHs (3-11) for 1h. Temperature optimum was measured by incubating the enzyme with 2.5% (w/v) dextran at pH 5 for 10 min. for different incubation temperatures ranging from 40 to 75°C.

The thermal stability was also determined by incubating the enzyme alone at different temperatures (30-55°C for 60 min). Different carbon sources (dextran, xylan, starch, cellulose, DEAE-Sephadex, CM-Sephadex, glycogen, amylose, amylopectin, Sephadex G-10, 15, 25, 50, 75, 150 and 200) were tested for dextranase activity by incubating 2.5% (w/v) of each substrate with the enzyme at 55°C for 10 min. at pH 5 and the residual activity was determined according to **Sugiura *et al* (1973).**

RESULTS AND DISCUSSION

Mutants 12 and 18 were chosen for protoplast fusion in relation to both their dextranase productivity and antifungal response **(Table 1)**. Upon protoplast fusion between mutant 12 and 18, seven stable fusants were obtained and tested for their dextranase activity. As shown in **Table (2)** the seven fusants over-yielded the WT strain in dextranase activity by about 154% and 251% for fusant 3 and fusant 2, respectively. These results led to the conclusion that protoplast fusion between high enzyme productive strains of *P. funiculosum* is expected to give higher enzyme productive fusant strains. Our results are in agreement with those reported by other investigators **(Halos *et al* 1989; Talkhan, 2000)** who reported that some formed fusants surpassed their parental strains in enzymatic activity.

Ennzyme purification and characterization

Results from **Fig. (1)** showed that one UV peak with dextranase activity was produced from the first purification step on Q Sepharose FF column. This dextranase active peak was collected, concentrated and then loaded to Superose 12 prep. grade column and a dextranase active UV peak was produced with an elution volume of about 850 ml **(Fig. 2)**. The dextranase active peak from Superose 12 column was collected, concentrated and desalted and loaded to Mono Q FPLC column. Four dextranase active peaks were produced with different elution volumes **(Fig. 3)**. These peaks were collected separately for further studies. The different purification steps were estimated by SDS-PAGE electrophoresis (silver staining). **Fig. (4a)** showed the different purification steps including the culture filtrate (lane 2), gel filtration (lane 4) and Q sepharose FF (lane 5), but **Fig. (4b)** showed the purity of the last steps on Mono Q columns for the four components produced and it was found that all the pure components had the same molecular weight (of approx. 67kDa). pI was also measured with the use of broad and low pI protein kits and it was found that the pIs of the four components were around 3.0 **(Fig. 5a &b).**

Through the studying of the purification of dextranase enzyme from *Penicillium funiculosum*, two pure fractions (I&II) were produced. This enzyme was purified with the use of BioGel P-60, CM-and DEAE-cellulose **(Sugiura *et al* 1973)**. These two fractions had the same molecular weight (44,000Da) and had a very little difference in the pI (3.98 and 4.19 for I and II respectively). Also, two dextranase fractions were produced in the purification of dextranase from *Aspergillus cameus*. The enzyme was purified using a sequence of steps including ammonium sulfate precipitation, ion exchange on DEAE-cellulose and gel filtration on BioGel P-150 **(Hiraoka *et al* 1972)** and the fractions had the same molecular weight (71,000Da) with a very little difference in the pI (4.12 and 4.35 for I&II respectively). On the other hand, two dextranase pure fractions were produced on the purification of dextranase from *Penicillium notatum* 1 by DEAE-and CM-cellulose ion exchange and chromatofocusing using PBE 94 and the two fractions showed a little difference in the molecular weight (55.8 and 50.1kDa) and pI (4.9 and 4.75) **(Pleszczynska *et al* 1996).**

Table (3) summarizes all the purification steps of fusant 2 strain of *Penicillium funiculosum* .A pure dextranase components (I, II, III and IV) were produced with at the final purification step using Mono Q FPLC had a yield of 29.87, 7.88, 7.01 and 3.01 for dextranase components I, II, III and IV, respectively with a total of 47.86% of the original total activity. The purification folds were 9, 9, 7 and 3 for dextranase components I, II, III and IV with a total of 28 folds. **Lee and Fox (1985)** purified two dextranase enzymes (I&II) through two stage ion exchange chromatography, enzyme I showed a purification fold of 35.5 and 16% yield but dextranase II had purification fold of 19 and 4% yield.

Maximum dextranase activity was observed at pH 5 for all purified dextranase components (I, II, III and IV) under standard conditions by incubating the enzyme with 2.5% (w/v) buffered dextran (pH from 3-8) at 55°C **(Fig. 6)**. All of the four purified dextranase components showed a pH stability up to pH 10 when incubated alone in a buffer (pH from 3-11) at room temperature for 1 hours **(Fig. 7)**. It was found that both the optimum pH and stability were depending upon the microbial strain used. Dextranase enzyme from *Chaetomium gracile* (two fractions) showed an optimum pH of 5.5

Table 1. Enzyme production nand antinfungal sensitivity of the selected mutants

Mutants	UV exposure time (min.)	Enzyme production		Antifungal test			
		(U/ml)	%WT	Nystatin	Topsin	Griseofulvin	Cyclohexamide
12	4	292.53	208.71	+	-	-	+
18	10	269.21	192.07	-	+	-	-

WT = Wild type strain

Table 2. Dextranase activity of the fungal fusants resulted from protoplast fusion between mutants 12 and 18 along with parental strains of *Penicillium funiculosum* NRRL6014

Strain	Enzyme activity	
	U/ml	% to W.T.
W.T.	140.16	100.00
Mutant12	292.53	208.10
Mutant 18	269.21	192.07
Fusant 1	467.63	333.65
2	492.63	351.48
3	355.46	253.61
4	453.78	323.76
5	403.82	288.10
6	469.04	334.65
7	441.29	314.85

and the enzyme showed a pH stability in the pH range from 5.5 to 11 (**Hattori *et al* 1981**). But, pure dextranase enzyme from *Fusarium* sp. had a maximum activity at pH 6.5 and showed a pH stability from between pH 4.5 and 11.8 (**Shimizu *et al* 1998**). On the other hand, dextranase from *Aspergillus carneus* was found to have an optimum activity at pH 5.0 and showed a pH stability from pH 4.5 to 9.0 (**Hiraoka *et al* 1972**). Also dextranase from *Paecilomyces lilacinus* had an optimum dextranase activity at pH 4.5 (**Lee and Fox, 1985**), but dextranase from *Sporothrix schenckeii* showed an optimum pH of 5.0 and pH stability of 4.5-5.5 (**Arnold *et al* 1998**).

The results (**Fig. 8**) showed that the purified enzyme components have an optimum temperature of 55°C and the thermal stability was up to 35°C (**Fig. 9**), above which the activity was sharply decreased. Dextranase from *Fusarium moniliforme* had an optimum temperature of 55°C (**Simonson *et al* 1975**) and from *Chaetomium gracile* had an optimum dextranase activity at 55°C (**Hattori *et al* 1981**), and it was also 55°C for dextranase enzyme from *Paecilomyces lilacinus*, but the dextranase enzyme from *Fusrium sp.* showed an optimum activity at 35°C and had a thermal stability below 45°C (**Shimizu *et al* 1998**).

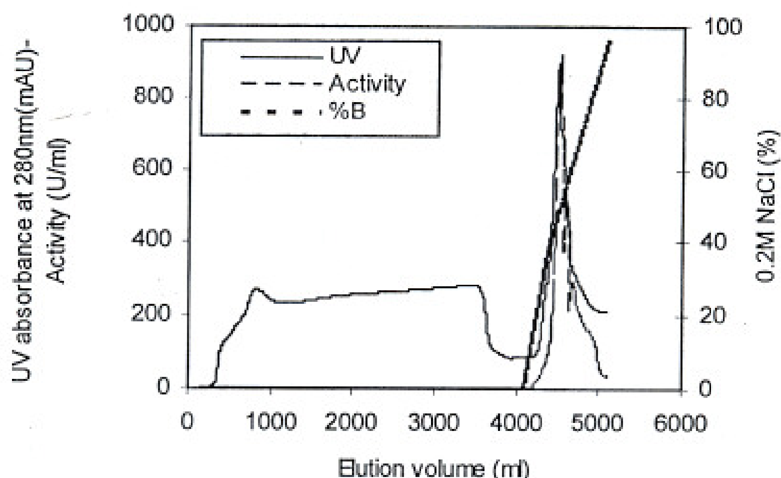

Fig. 1. Ion exchange chromatography on Q Sepharose (V_t 225 ml) fast flow. 400ml of the culture filtrate from fusant 2 was diluted to 3000ml with distilled water and then applied to the column. The column was equilibrated with 20mM of Bis-Tris (pH 4.8) and the elution was achieved by applying NaCl-gradient Of 0.2 M (100%) at a linear flow rate of 10 ml/min. The dotted line represents the dextranase activity in u/ml.

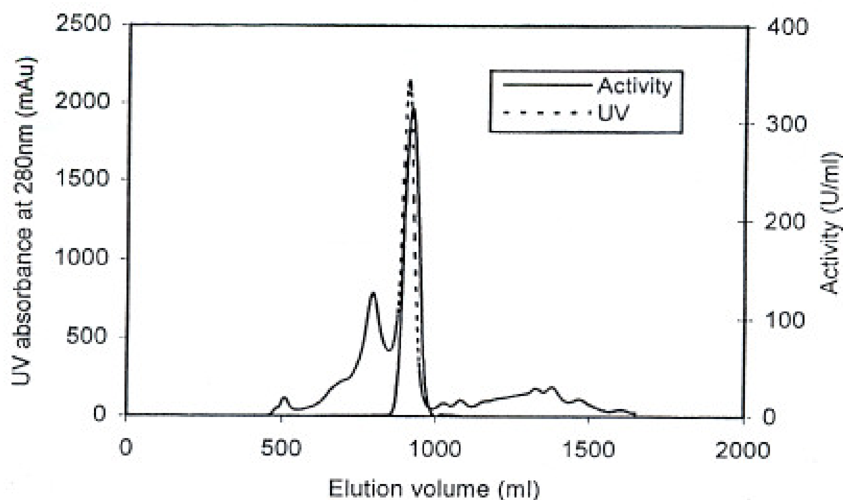

Fig. 2. Size-exclusion chromatography on Superose 12 prep grade 60/600. A concentrate (25ml) of the dextranase active material from Q Sepharose FF was loaded to a 6×60 cm Superose 12 prep. grade column (1700ml) equilibrated in 20 mM phosphate buffer pH 7.0 containing 0.2 M NaCl. The linear flow rate is 5 ml/min. The dotted line represents the dextranase activity in u/ml

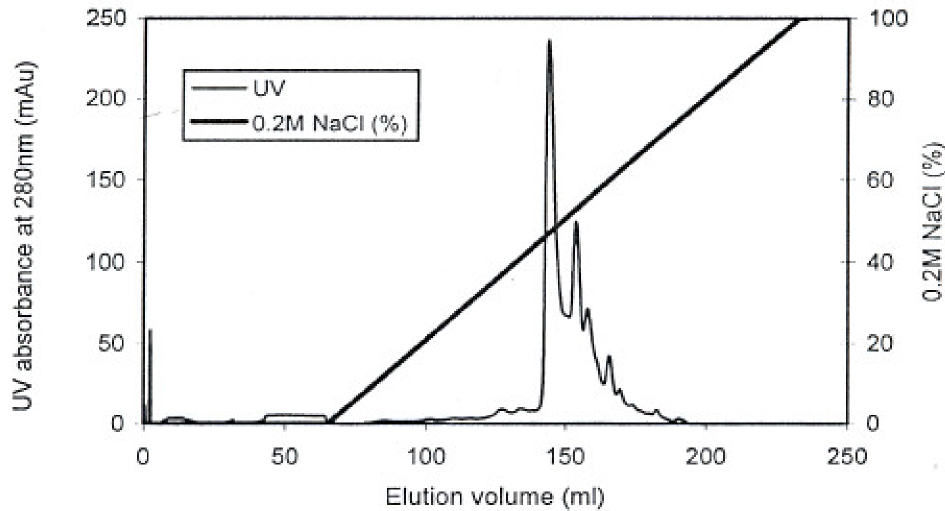

Fig. 3. Ion exchange chromatography on Mono Q FPLC. Sample 25ml of crude dextranase from supesose 12 after concentration and desalting was loaded to a 1×10 cm Mono Q FPLC column (10ml) equilibrated in 20mM Bis-Tris pH 4.8, containing 0.1M NaCl. The linear flow rate is 1ml/min. Four dextranase-active peaks were produced

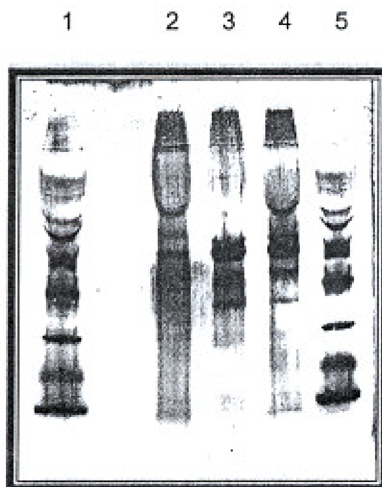

Fig. 4a. SDS-PAGE electrophoresis of different purification steps of *Penicillium funiculosum* NRRL 6014 fusant. Culture filtrate, ion exchange on Q sepharose FF and gel filtration on Superose 12 prep. grade 60/600 were analyzed by SDS/PAGE on Phast Gel gradient 8-25. Lane 1 and 5 represent the low molecular weight calibration protein, lane 2, 3, and 4 represent the culture filtrate, gel filtration step on superose 12 and Q ion exchange on Q sepharose respectively

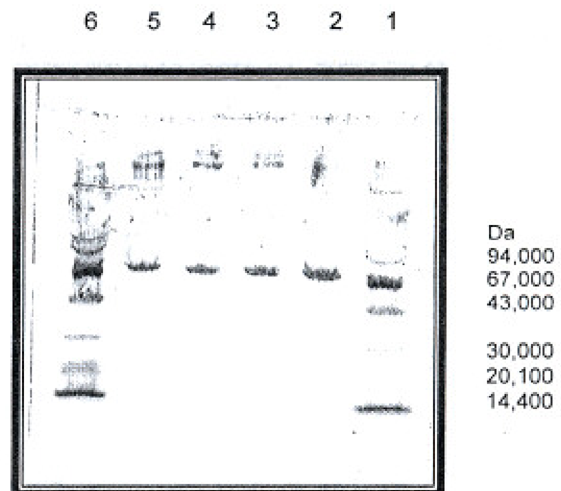

Fig. 4b. SDS-PAGE electrophoresis of purified dextranase fractions. The four separated peaks from Mono Q were analyzed by SDS/PAGE on Phast Gel Gradient 8-25. Lane 1 and 6 represent the low molecular mass calibration proteins and lane 2, 3, 4 and 5 represent the purified dextranase components I, II, III and IV respectively

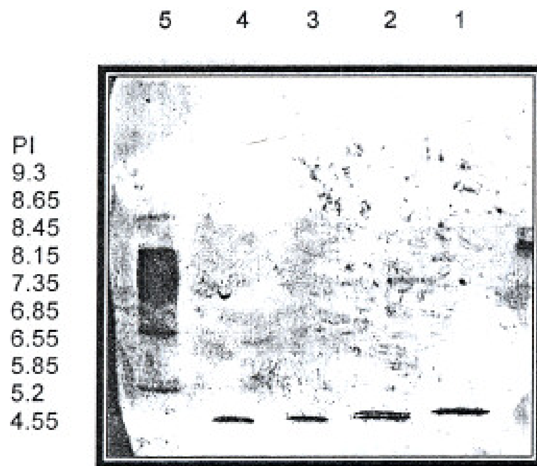

Fig. 5a. IEF electrophoresis of purified dextranase fractions. The four purified peaks from Mono Q FPLC were analyzed by IEF on Phast Gel IEF 3-9. Lane 1, 2, 3 and 4 represent the purified dextranase components I, II, III and IV respectively and lane 5 represent the broad pI calibration protein

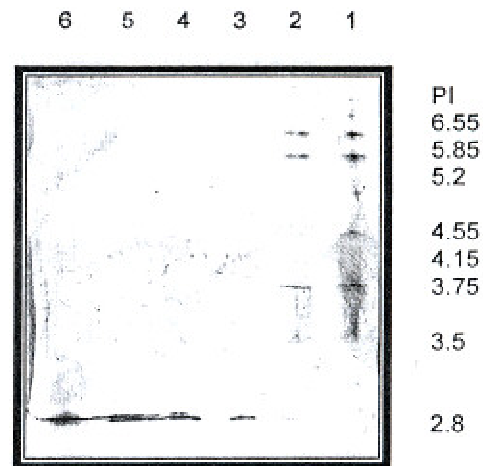

Fig. 5b. IEF electrophoresis of purified dextranase fractions. The four purified peaks from Mono Q FPLC were analyzed by IEF on Phast Gel IEF 2.5-6.5. Lane 3, 4, 5 and 6 represent the purified dextranase components I, II, III and IV respectively and lane 1and 2 represent the low pI calibration protein

Table 3. Purification of dextranase components from fusant 2 *Penicillium funiculosum* NRRL 6014

Purification step	Activity (U/ml)	Total activity (U)	Total protein mg)	Specific activity (U/mg protein)	Yield (%)	Purification (fold)
Culture filtrate	249.79	112403.70	63.00	1784.19	100	1
Q-Sepharose FF	2472.88	86550.85	11.69	7403.83	77.00	4
Superose 12prep grade60/600	3302.73	82568.15	8.95	9225.49	73.46	5
MonoQ FPLC						
I	4196.40	33571.20	2.16	15542.22	29.87	9
II	2951.20	8853.60	0.57	15532.63	7.88	9
III	1971.22	7884.88	0.6	13141.43	7.01	7
IV	675.81	3379.05	0.6	5632.50	3.01	3

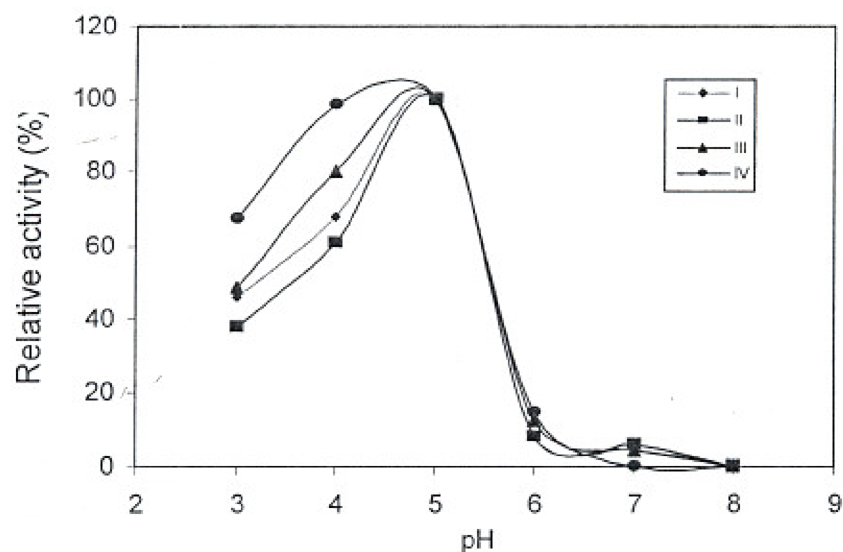

Fig. 6. Determination of the optimum pH of the purified dextranase components from *Penicillium funiculosum* NRRL 6014 (fusant 2)

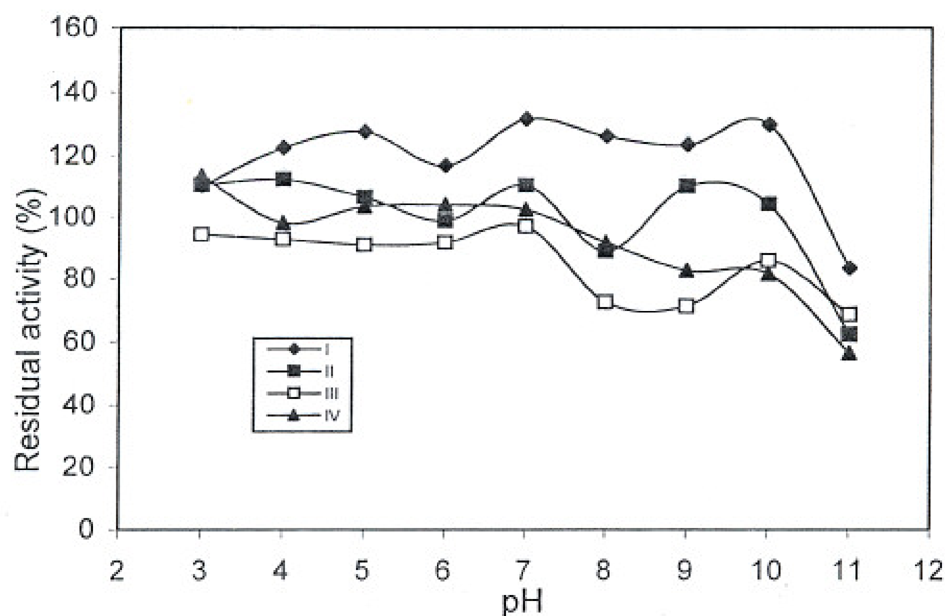

Fig. 7. Detection of the pH stability of the purified dextranase components from *Penicillium funiculosum* NRRL 6014 (Fusant 2) after 1h

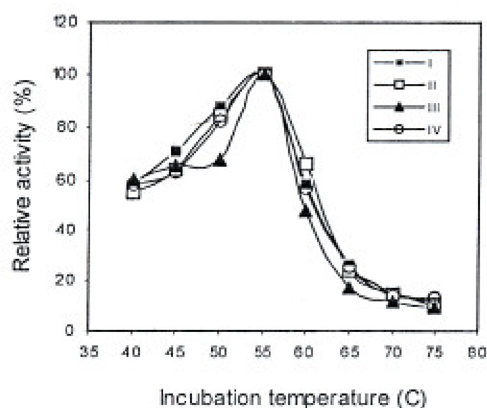

Fig. 8. Determination of the optimum incubation temperature of the purified dextranase components from *Penicillium funiculosum* NRRL 6014 (fusant 2)

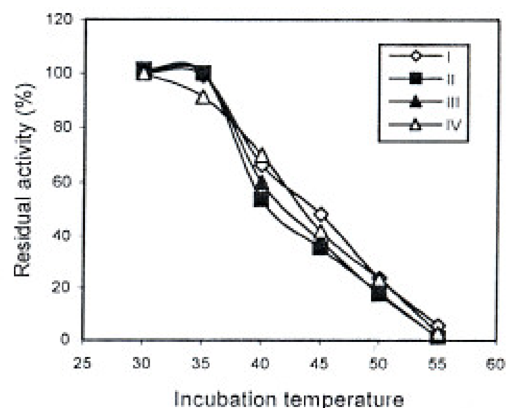

Fig. 9. Thermal stability of the purified dextranase components from *Penicillium funiculosum* NRRL 6014 (fusant 2) after 30 min

Table 4. The effect of different substrates on the purified dextranase enzyme from *Penicillium funiculosum* NRRL 6014 fusant

Substrate	Main linkage	Relative activity (%)			
		I	II	III	IV
Dextran T500	α-1,6	100.00	100.00	100.00	100.00
Amylose	α-1,4	0.00	0.00	0.00	0.00
Amylopectin	α-1,4	0.00	0.00	0.00	0.00
Glycogen	α-1,4	0.00	0.00	0.00	0.00
Starch	α-1,4	0.00	0.00	0.00	0.00
Cellulose	α-1,4	0.00	0.00	0.00	0.00
Xylan	α-1,4	0.00	0.00	0.00	0.00
Dextrin	α-1,6	0.00	0.00	0.00	0.00
DEAE-Sephadex	α-1,6	29.77	21.17	17.58	15.98
CM-Sephadex	α-1,6	0.00	0.00	0.00	0.00
Sephadex G-10	α-1,6	0.00	0.00	0.00	0.00
G-15	α-1,6	0.00	0.00	0.00	0.00
G-25	α-1,6	0.00	0.00	0.00	0.00
G-50	α-1,6	0.00	0.00	0.00	0.00
G-75	α-1,6	37.61	26.44	22.71	23.16
G-150	α-1,6	67.67	60.89	59.83	56.55
G-200	α-1,6	82.66	76.20	66.18	73.11

Table (4) illustrates the effect of different substrates on the activity of the purified dextranase components from *Penicillium funiculosum* NRRL 6014 Fusant 2 and it was found that the enzyme showed an activity towards α-1,6 glucosidic linkages in dextran and dextran derivatives (Sephadex G-75, 150 &200) but not with the other compounds and higher cross-linked dextrans (Sephadex G-10→50). Dextranase components (I, II, III and IV) didn't hydrolyze CM-containing α-1,6 linkage. It is concluded that this fact is due to an ionic or steric effect caused by carboxymethyl group of the substrate (**Hattori *et al* 1981**). Dextranase from *Penicillium funiculosum* hydrolysed Sephadex of G50, 75 and 200 and not hydrolysed Sephadex G25 (**Sugiura *et al* 1973**). Also, dextranase from *Penicillium aculeatum* showed a dextranase activity with Sephadex G50 and G200 and no activity was found for DEAE-cellulose and DEAE-sephadex (**Madhu and Prabhu, 1984**).

REFERENCES

Abdel-Aziz, M.S. (2005). Physiological and Genetical Studies on Dextranase Formation by Some Fungi. pp. 38-40. Ph.D. Thesis, Microbiology department, Faculty of Science, Ain Shams University, Cairo.

Arnold, W.N.; T.B.P. Nguyn and L.L. Mann (1998). Purification and characterization of the dextranase of *Sporothrix schenckii*. **Arch. Microbiol., 170: 91-98.**

Barfoed, S. and A. Mollgaard (1987). Dextranase solved dextran problems in DDS'beet sugar factory. **Zuker Industire (Berlin), 112: 391-395.**

Brown, C.F. and P.A. Inkerman (1992). Specific method for quantitative measurment of the total dextran content of raw sugar. **J. Agric. Food Chem., 40: 227-233.**

Caldwell, R.C.; H.T. Sandham; W.T. Man; S.B. Finn and A.J. Formicola (1971). The effect of a dextranase mouthwash on dental plaque in young adults and children. **J. Amer. Dent. Assoc., 82: 124-131.**

Day, D.F. and D. Kim (1992). A novel process for the production of dextrans of selected molecular size. **Aannals N. Y. Acad. Sci., 672: 573-576.**

Demain, A.L. and N.A. Solomon (1981). Industrial Microbiology. **Scientific American (1981 Sept.), 245: 67-75.**

El-Bondokly, A. and F. Talkhan (2007). Intrastrain crossing in *Trichoderma harzianum* via protoplast fusion to enhance chitinase productivity and biocontrol activity. **Arab. J. Biotechnol., 10: 233-240.**

Fitzgerald, R.J.; P.H. Keyesl; T.H. Stoudt and D.M. Spinell (1968). The effect of dextranase preparation on plaque and caries in hamsters, a preliminary report. **J. Amer. Dent. Assoc., 76: 301-304.**

Galvez-Mariscal, A. and A. Lopez-Munguia (1991). Production and characterization of a dextranase from *Paecilomyces lilacinus* strain. **Appl. Microbiol. Biotechnol., 36: 327-331.**

Halos, S.C.; R. Caday; J. Claudio and L.J. Pham (1989). Cellulose biodgradation studies: Application of rDNA and protoplast fusion techniques. **Proc. Int. Symp. Biotechnol. for Energy, Philippine, Dec.16-21, pp. 65-71.**

Hamada, S. and H.D. Slade (1980). Biology, immunology and carcinogenicity of *Stereptococcus mutans*. **Microbiol. Res., 44: 331-384.**

Hattori, A.; K. Ishibashi and S. Minato (1981). The purification and characterization of the dextranase from *Chaetomium gracile*. **Agric. Biol. Chem., 45(11): 2409-2416.**

Heyn, A.N.J. (1981). Molecular basis of auxin. Regulated extension growth and role of dextranase. **Proc. Natl. Acad. Sci., USA 78,** 6608/6612.

Hiraoka, N.; J. Fukumoto and D. Tsuru (1972). Studies on mold dextranases. III. Purification and some properties of *Aspergillus carneus* dextranase. **J. Biochem., 71: 57-64.**

Hoster, F.; R. Daniel and G. Gottschalk (2001). Isolation of a new *Thermoanaerobacterium thermosaccharolyticum* strain (FH1) producing a thermostable dextranase. **J. Gen. Appl. Microbiol., 47(4): 187-192.**

Igarashi, T.; H. Morisaki and N. Goto (2004). Molecular Characterization of Dextranase from *Streptococcus rattus*. **Microbiol. Immunol., 48(3): 155- 162.**

Koenig, D.W. and D.F. Day (1988). Production of dextranase by *Lipomyces starkeyi*. **Biotechnol. Lett., 10(2): 117-122.**

Lee, M. and P.F. Fox (1985). Purification and characterization of *Paecilomyces lilacinus* dextranase. **Annals N.Y. Acad. Sci., 542: 192-194.**

Lowry, O.H.; N.J. Rosenbrough; A.L. Farr and R.J. Randhall (1951). Protein measurment with the Folin phenol reagent. **J. Biol. Chem., 193: 265-275.**

Madhu, K. and A. Prabhu (1984). Studies on dextranase from *Penicillium aculeatum*. **Enzyme Microbial Technol., 6: 217-220.**

Murayama, Y.; H. Wada; J. Hayashi; T. Uchida; I. Yokomizo and S. Hamada (1973). Effect of dextranase from *Spicaria violaceae* IFO 6120 on the polysaccharides produced by oral Streptococci and on human dental plaque. **J. Dent. Res., 52: 658-667.**

Petronijevic, Z.; B. Cvetkovic; R. Nesic and S. Stankovic (1993). Influence of nutrient medium composition, initial pH and aeration on the production of dextranase in *Aspergillus ustus*. **Mikrobiologija, 30: 89-96.**

Pleszczynska, M.; J. Rogoliski; J. Szczodrak and J. Fiedurek (1996). Purification and some properties of an extracellular dextranase from *Penicillium notatum*. **Mycol. Res., 100(6): 681-686.**

Prabavathy, V.R.; N. Mathivanan; E. Sagadevan; K. Murugesan and D. Lalithakumari (2006). Intra-strain protoplast fusion enhances carboxymethylcellulase activity in *Trichoderma reesei*. **Enzyme Microbial Technol., 38: 719-723.**

Preobrazhenskaya, M.E.; A.L. Minakova and E.L. Rosenfeld (1974). Dextranase activity of pig spleen acid α-D-glucosidase. **Carbohydr. Res., 38: 267-277.**

Reymond, P. and M. Fevre (1986). Recombination following protoplast fusion of *Penicillium* strains used in the dairy industry. **Enzyme Microbial Technol., 8: 41-44.**

Shimizu, E.; T. Unno; M. Ohba and G. Okada (1998). Purification and characterization of an isomaltotriose-producing endodextranase from *Fusarium* sp. **Biosci. Biotechnol. Biochem., 67: 117-122.**

Simonson, L.G.; A.E. Liberta and A. Richardson (1975). Characterization of an extracellular dextranase from *Fusarium moniliforme*. **Appl. Microbiol. 30(6): 855-861.**

Sugiura, M.; A. Ito; T. Ogiso; K. Kato and H. Asano (1973). Studies on dextranase. Purification of dextranase from *Penicillium funiculosum* and its enzymatic properties. **Biochem. Biophys. Acta, 309: 357-362.**

Sun, J.; X. Cheng; Y. Zhang; Z. Yan and J. Zhang (1988). A strain of *Paecilomyces* producing high quality dextranase. **Annals N.Y. Acad. Sci., 542: 192-194.**

Sumner, J.B. (1924). A method for measuring of reducing sugars in urine. **J. Biol. Chem., 62: 287-290.**

Szczodrak, J.; M. Pleszczynska and J. Fiedurek (1994). *Penicillium notatum* 1 a new source of dextranase. **J. Indust. Microbiol. 13: 315-320.**

Talkhan, F.M. (2000). Mutations and protoplast fusion technique as a tool for improving α-amylase production in *Aspergillus foetidus*. **Annals Agric. Sci., Ain Shams Univ., Cairo, 45: 239-255.**

Wanda, S.Y. and R. Curtiss (1994). Purification and characterization of *Streptococcus sobrinus* dextranase produced in recombinant *Escherichia coli* and sequence analysis of the dextranase gene. **J. Bacteriol., 176: 839-3850.**

Webb, E. and I. Spencer-Martins (1983). Extracellular endodextranase from the yeast *Lipomyces starkeyi*. **Can. J. Microbiol., 29: 1029-1095.**

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الدمج الخلوى فى طافرات من فطر البنيسيليوم فنيكيولوزم لزيادة إنتاج إنزيم الدكسترانيز [٤]

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أختيرت طافرتـــان منتجتـــان للدكسترانيزعن طريـــق الأشعـــــة فـــــوق البنفـــسجية لفطـــر *Penicillium funiculosum* (NRRL 6014) لعمـــل دمج خلوى بينهما . هاتان الطافرتـــان همـــا ١٢و١٨ واللتان تم اختيارهما حسب نشاطهما الإنزيمى وكذلك لثباتهما . تم انتخاب ٧ مندمجة خلويـــة لهـــم نـــشاط إنزيمى يتفوق علـــى الأصـــل (الطافرتـــان و الأب الأصلى)، و كانت أحسن مندمجة خلوية مـــن حيـــث إنتاج إنزيم الدكسترانيز هـــى المندمجـــة ٢ ولـــذلك أختيرت لإجراء مزيد من الدراسة. باستخدام سلـــسلة متتالية من أعمدة الفصل الكروماتوجرافى تـــم تنقيـــة الإنزيم و حصلنا على أربعة مكونات إنزيمية نشطة، و بعمل SDS/PAGE وجد أن هذه المكونات لها وزن جزيئى يقارب ٦٧ كيلو دالتون و PI حوالى ٣ ، وأن النشاط الإنزيمى الأمثل كان عند ٥٥ درجـــة مئويـــة وأفضل درجة تركيز أيون هيدروجين هى ٥ ، كذلك وجد أن الرابطة ١-٦ فى الدكستران و بعض مشتقاته هى التى لها المقدرة على أن تعطى نشاط إنزيمى.

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