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Purification and Characterization of a Novel High Molecular Weight Dextransucrase from *Acetobacter tropicalis*

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Abstract: The purification and characterization of extracellular dextransucrase from *Acetobacter tropicalis* was carried out by using DEAE-Sepharose column chromatography. The enzyme was purified to 16-fold to homogeneity with 8 % recovery. The molecular mass of dextransucrase on SDS-PAGE and non-denaturing SDS-PAGE was found to be 180 and 360 kDa, respectively, suggesting that this enzyme exist in homodimer state in native form. This is a first report on purification of *A. tropicalis* dextransucrase with such a high molecular weight. Purified dextranuscrase was found to work best with 6 % (w/v) sucrose in acetate buffer (15 mM, pH 5.5) at 37°C temperature. The K_m and V_{max} values of purified enzyme were found to be 11.5 mM and 5000 U/mg, respectively. The presence of histidine, serine, tyrosine and lysine residues was detected at or near the active sites of enzyme when incubated with amino acid modifiers.

Key words: *Acetobacter tropicalis*, dextransucrase, dextran, purification, polyethylene glycol, amino acid modifier.

Introduction

The enzyme dextransucrase, also called as sucrose: 1, 6-α- D-glucan 6-á-glucosyltransferase (EC 2.4.1.5) catalyses the synthesis of dextran from sucrose. Dextran has been extensively exploited in pharmaceutical industry as, stabiliser in food industry, as blood volume expander and as a chromatographic matrix in fine chemical industries because of their nonionic hydrophilic nature and stability [2,26]. Dextrans are synthesized by dextransucrase from sucrose, produced mainly by bacteria belonging to genera *Leuconostoc, Streptococcus, Exigobacterium, Weissella* and *Lactobacillus* [14]. *Leconostoc mesenteroides* NRRL B-512F dextransucrase was the first enzyme used commercially to produce dextran [32] but, *Leuconostoc citreum* B/110-1-2 is another strain used commercially to produce dextran [37,38]. Very less information on purification of extracellular dextransucrase has been reported from *Leuconostoc* spp. [20]. Various methods such as precipitation by ethanol or polyethylene glycol, ammonium sulphate, phase partitioning ultrafiltration and chromatographic methods have been applied to purify this enzyme [20]. Dextransucrase purification is considered difficult as viscous dextran is always found as contaminant in the purified enzyme preparation. In this report *Acetobacter tropicalis* dextransucrase was purified by PEG precipitation followed by digestion of dextran with dextranase treatment, ammonium sulphate precipitation and DEAE-sepharose chromatography. Moreover, amino acids present at or near active site of dextransucrase were also determined.

Material and methods

Chemicals

All the chemicals used were of analytical grade and procured from Merck and Hi-media, India.

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The protein molecular weight markers were obtained from Merck Biosciences Bangalore, India.

Microorganism, culture medium, and production conditions

The strain of *Acetobacter tropicalis* was isolated from sugarcane field of Himachal Pradesh, India. The culture was maintained on nutrient agar slants (pH 7) containing 2.0 % (w/v) sucrose or in 20 % (v/v) glycerol stocks and sub culturing was done periodically. The production of dextransucrase was carried out by culturing 1 % (v/v) 12 h old preculture of *A. tropicalis* inoculated to the production medium (pH 7.0) containing (g/L) Sucrose 20, peptone 10, $MnSO_4.2H_2O$, 0.25, CH_3COONa 5.0 and incubated at 25°C for 8 h in laboratory fermenter at 450 rpm agitation and 0.5 vvm aeration [4]. The composition of seed and production medium were same.

Purification of dextransucrase

Chilled polyethylene glycol (PEG-200, 400, 1500, 4000 and 6000) were added to cell free supernatant at the final concentrations of 10- 40 % (v/v) and incubated at 4°C for 12 h. The dextransucrase precipitates were recovered by centrifugation at 15,000 x g at 4°C for 15 min. The PEG precipitated protein was dialyzed overnight against three changes of 15 mM acetate buffer (pH 5.5). Since the PEG-400 treated dextransucrase also contained precipitated dextran along with proteins, the removal of dextran became necessary for further purification of dextransucrase. The dialyzed enzyme was treated with 100 µL of commercial dextranase enzyme (5.0 U/mL,) with stirring for 1 h at 20°C. The dextranase treated enzyme was subjected to 40-60 % saturation of ammonium sulphate and precipitated proteins were recovered by centrifugation at 15,000 x g at 4°C for 15 min. The enzyme was dialyzed for 18 h against three subsequent changes of acetate buffer (15 mM, pH 5.5). The DEAE-Sepharose column was equilibrated with 100 mL acetate buffer and 3 mL of dialyzed enzyme (1175 U/mg, 0.13 mg/mL protein) was loaded on column and kept for binding at 4°C for 1 h. The column was washed at a flow rate of 0.5 mL/min with acetate buffer until the A_{280} of the effluent was less than 0.007 and remained constant. The dextransucrase protein was eluted from the column with NaCl gradient (0.5-0.1 M) and fractions of 1.5 mL were collected. The protein concentration and enzyme activity of each fraction were determined.

Estimation of molecular mass

The purity and subunit molecular weight was estimated by SDS-PAGE (8 %) according to Laemmli [19]. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 dissolved in a water:methanol:acetic acid (50:40:10) for 1 h, then destained in the same solvent. The activity of the purified enzyme was determined by activity staining (zymogram) of the dextransucrase enzyme fractions run on non-denaturing SDS-PAGE and discrete magenta coloured bands within the gel matrix confirm the dextransucrase activity [27,28]. The images of gels were recorded in a gel documentation system (Alpha Innotech Corporation, USA). The molecular weight analysis was done using Alpha Digi Doc RT and Alpha Ease FC software.

Biochemical characterization of dextransucrase of *A. tropicalis*

The purified dextransucarse obtained from *A. tropicalis* was further characterized. The enzyme activity was measured in various buffers (0.1 M) *viz*. Citrate buffer (pH 4.5-6.5), Borate buffer (pH 7.0-8.5), Sodium acetate Buffer (pH 4.0-5.5), Borax-NaOH buffer, (pH 9.0-10.0), Glycine-NaOH buffer (pH 8.5-10.5), Tris-HCl buffer (pH 7-9.0), Sodium phosphate buffer (pH 5.5-8.0), Potassium phosphate buffer, (pH 7.0-8.5), at 37°C for 30 min to find out optimum pH for exhibiting maximum dextransucrase activity.

In order to evaluate the optimum reaction temperature, the reaction mixture containing purified dextransucrase lyase was incubated at 25 to 50°C, for 30 min in a temperature controlled water bath. The substrate specificity of the enzyme was determined by adding the various substrates (sucrose, maltose, raffinose, mannitol, dextrose and lactose) at 2 % (w/v) concentration in the reaction mixture. The dextransucrase activity was measured at various sucrose concentration (2-20 %, w/v or 29-584 mM) and a

graph was plotted between 1/[S] vs 1/[V] and K_m and V_{max} values of the purified dextransucrase from *A. tropicalis* were calculated from the Line-weaver-Burk plot.

Determination of amino acids present at or near the active site responsible for the catalytic activity of dextransucrase of *A. tropicalis*

Amino acids present at or near active site was determined by incubating the purified dextransucrase of *A. tropicalis* with 100 µL of amino acid modifiers [Iodoacetamide (pH 6.0), Iodoacetamide (pH 8.0), diethylepyrocarbonate (DEPC), N-acetylimidazole, phenylglycoxal monohydrate, N-ethylmelimide (NEM)] at the concentration of 10 mM for 1 min. Sucrose (6 %, w/v) prepared in the acetate buffer (pH 5.5, 15 mM) was added to the enzyme treated with modifiers and reaction was carried out under standard assay condition.

Substrate protection of *A. tropicalis* dextransucrase against inactivation by amino acid modifiers

Protection of dextransucrase of *A. tropicalis* by sucrose against amino acid modifiers was also studied. Purified dextransucrase of *A. tropicalis* was incubated with sucrose (6 %, w/v) prepared in acetate buffer (pH 5.5, 15 mM) for 1 min. Thereafter, above mentioned amino acid modifiers (10 mM) were added into the reaction mixture. The reaction was carried out under standard assay conditions and dextransucrase activity was determined in each case.

Analytical methods

Enzyme assay

The assay mixture (2 mL) contained enzyme (10 µL) and sucrose (2 %, w/v) in sodium acetate buffer (25 mM, pH 5.5). The reaction mixture was incubated at 37°C for 30 min and amount of reducing sugar formed was analyzed by DNS (dinitrosalicylic acid) method [21]. Activity of dextransucrase from *A. tropicalis* was expressed in terms of International Units (IU) and has been defined as 1 µmol of D-fructose formed from sucrose per milliliter in one min under standard assay conditions.

Protein estimation

The protein content in the enzyme preparations was estimated according to the methods described by Bradford [3] using bovine serum albumin as standard.

Results

Purification of dextransucrase

The dextransucrase from *A. tropicalis* is extracellular in nature; hence, cell free supernatant was subjected to PEG precipitation. The PEGs of different molecula weight *viz.* 200, 400, 1500, 4000, 6000 etc. was used to precipitate dextransucrase of *A. tropicalis*. The precipitation with 25 % (v/v) PEG-400 gave the maximum specific activity of 876 U/mg with overall yield of 64 % (Table 1). Hence, 25 % (v/v) PEG-400 was selected on the basis of its suitability for purification of dextransucrase. The dextran always remained as contaminant in the PEG purified dextransucrase which makes the further purification difficult. In order to resolve this issue the PEG precipitated *A. tropicalis* dextransucrase was treated with dextranase enzyme and this treatment dissociated the aggregated forms of dextransucrase and gave 1036 U/mg specific activity with 63 % overall yield.

Further, the dextranase treated dextransucrase was subjected to cut of 40-60 % (w/v) saturation of ammonium sulphate, followed by dialysis and was loaded on DEAE-Sepahrose column. A 16-fold purification of dextransucrase was obtained by ion exchange chromatography with 8 % yield (Table 2). The profile of dextransucrase elution showed a distinct peak of enzyme (Fig. 1). The purified dextransucrase had a subunit molecular mass of 180 kDa as assessed by SDS-PAGE (Fig. 2) and total molecular weight of 360 kDa on native PAGE (Fig. 3.). The activity staining (zymogram) also authenticate this finding. This suggests that dextransucrase of *A. tropicalis* exist in homodimeric form.

Characterization of purified dextransucrase of *A. tropicalis*

Optimization of buffer pH and buffer molarity

The activity of purified dextransucarse of *A. tropicalis* was measured in various buffer of

Table 1. Precipitation of dextransucrase of *A. tropicalis* by different molecular weight PEGs

No.	PEG (%)	Volume (mL)	EA (IU)	Total Units	Yield (%)	Protein (mg/mL)	Total protein (mg)	SA (IU/mg)
	Crude	500	9.77	4885	-	0.07	35	140
1	Treatment with PEG of different molecular weights							
	35 % PEG-200	40	49	1960	40	0.080	3.20	613
	25 % PEG-400	40	78	3120	64	0.089	3.56	876
	20 % PEG-1500	40	28	1120	23	0.090	3.60	311
	15 % PEG-4000	40	8	164	21	0.045	1.24	132
	5 % PEG-6000	40	8	153	20	0.045	1.24	123
2	Repeated treatment with PEG-400							
	Step-1	40	78	3120	64	0.089	3.56	876
	Step-2	10	18	184	24	0.076	1.59	116
	Step-3	7	8	56	7	0.089	1.58	36

EA; enzyme activity
SA; specific activity
PEG; polyethylene glycol

Table 2. The summary of purification of dextransucrase from *A. tropicalis*

No.	Purification steps	Volume (mL)	EA (IU)	Protein (mg/mL)	Total EA(U)	Total protein (mg/mL)	SA (IU/mg)	Yield (%)	Fold purification
1	Cell free supernatant	500	9.77	0.07	4885	35	140	100	1
2	PEG precipitation	40	78	0.089	3120	3.56	876	64	6.3
3	Dialyzed enzyme	40	77.7	0.088	3108	3.50	888	63	6.3
4	Dextranase treatment	40	77.7	0.087	3108	3.00	1036	63	7.4
5	$(NH_3)_2SO_4$ precipitation	6	142	0.13	852	0.79	1078	17	7.7
6	Dialyzed enzyme	6	141	0.13	848	0.72	1175	17	8.1
7	DEAE-Sepharose	9	44.4	0.02	400	0.18	2222	8	16

EA- Enzyme activity
SA- Specific activity

different pH (4.0 to 10.0). The sodium acetate buffer (pH 5.5) was found most suitable for the dextransucrase activity (data not shown). The molarity of acetate buffer was further optimized and results revealed that 15 mM acetate buffer of pH 5.5 was found to be most appropriate for maximum (3535 U/mg) dextransucrase activity (Fig 4.).

Optimization of reaction temperature

The purified dextransucrase of *A. tropicalis* showed maximum specific activity of enzyme (4306 U/mg) at 37°C and subsequent increase in incubation temperature results in sharp decrease in activity of enzyme possibly due to thermal denaturation (Fig. 5).

Substrate specificity

The purified dextransucrase enzyme from *A. tropicalis* showed the specificity towards its natural substrate sucrose only and no activity was found with other substrates used. The optimum

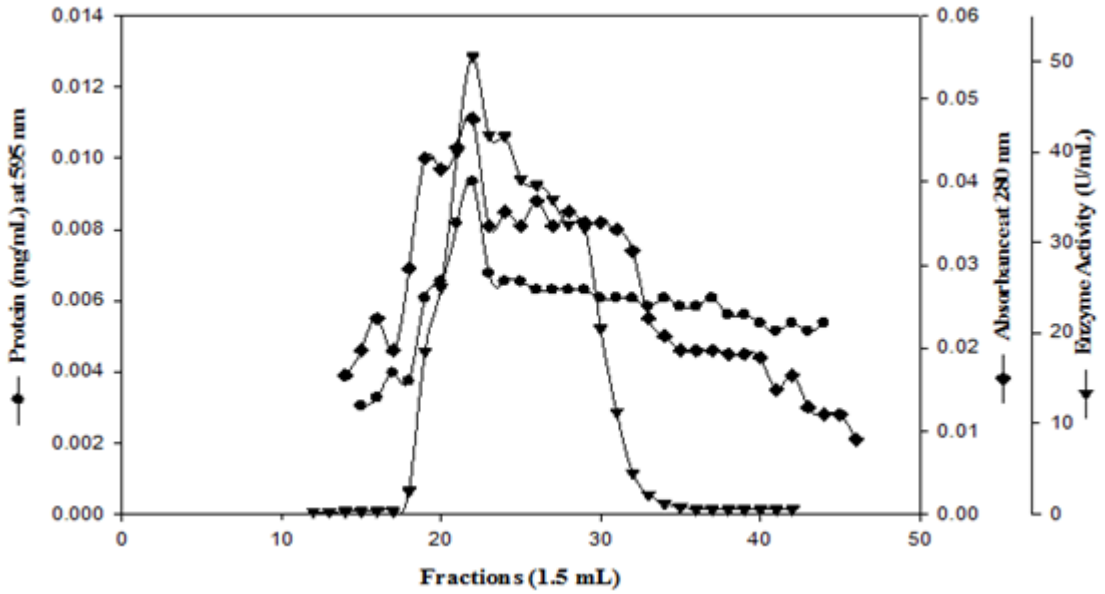

Fig. 1. The elution profile of dextransucrase of *A. tropicalis* during DEAE Sepahrose chromatography

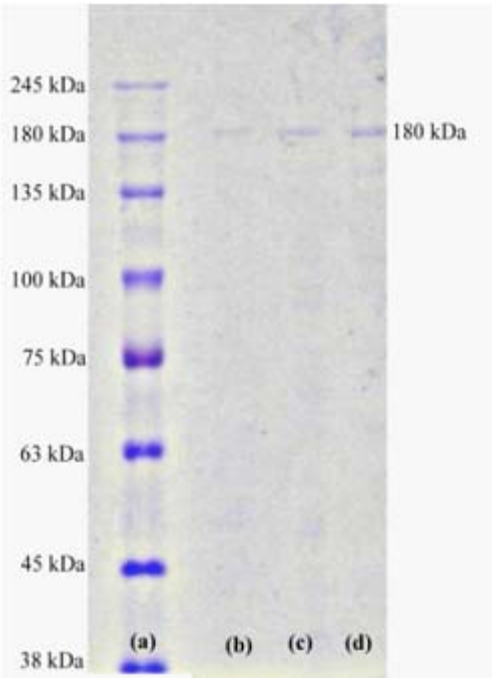

Fig. 2. SDS-PAGE of dextransucrase of *A. tropicalis* (a) SDS-PAGE protein molecular weight marker (b, c and d) Purified dextransucrase

sucrose concentration for dextransucrase of *A. tropicalis* was determined by using varying concentrations (2-20 % w/v or 29-584 mM) of sucrose prepared in acetate buffer. The maximum activity of purified dextransucrase was found to be 5112 U/mg with 6% (w/v) sucrose (Fig 6).

Determination of K_m and V_{max} of purified dextransucarse of *A. tropicalis*

The Lineweaver-Burk plot showed the K_m and V_{max} values of 11.5 mM and 5000 U/mg, respectively, for sucrose by purified dextransucarse *A. trpicalis* (Fig. 7).

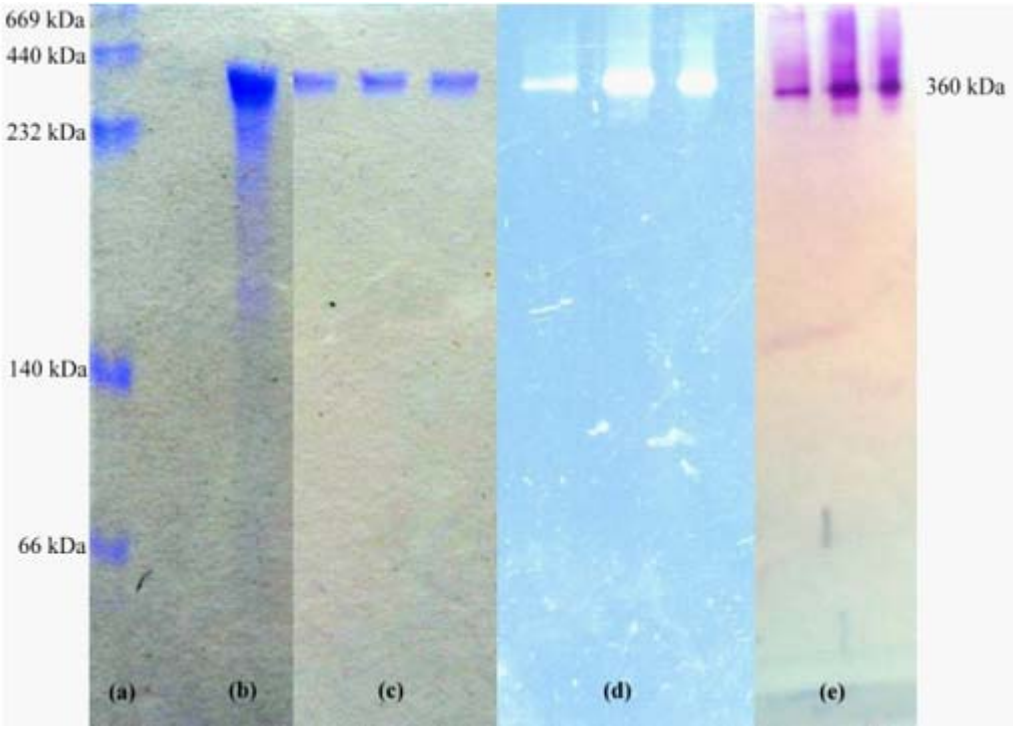

Fig. 3. Non-denaturing SDS-PAGE of purified dextransucrase from *A. tropicalis* (a) Native-PAGE protein molecular weight marker (b) Dialysed enzyme (c) Purified fractions (d) White bands of dextran synthesized by purified dextransucrase (e) periodic acid Schiff staining of the dextran determining the activity of the purified dextransucrase

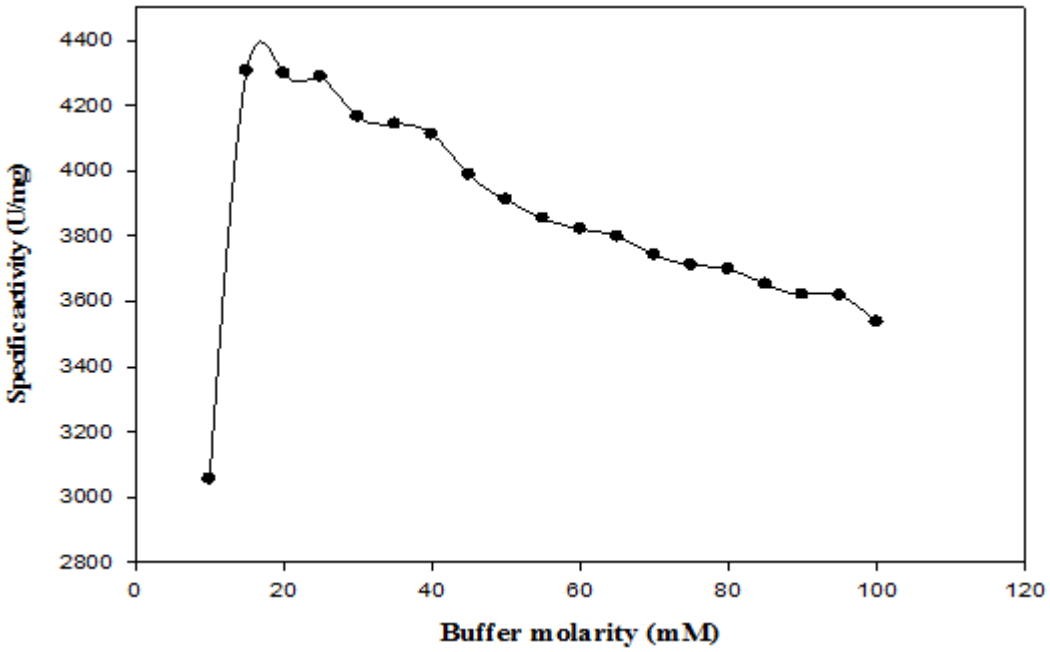

Fig. 4. Optimization of acetate buffer molarity for activity of *A. tropicalis* dextransucrase

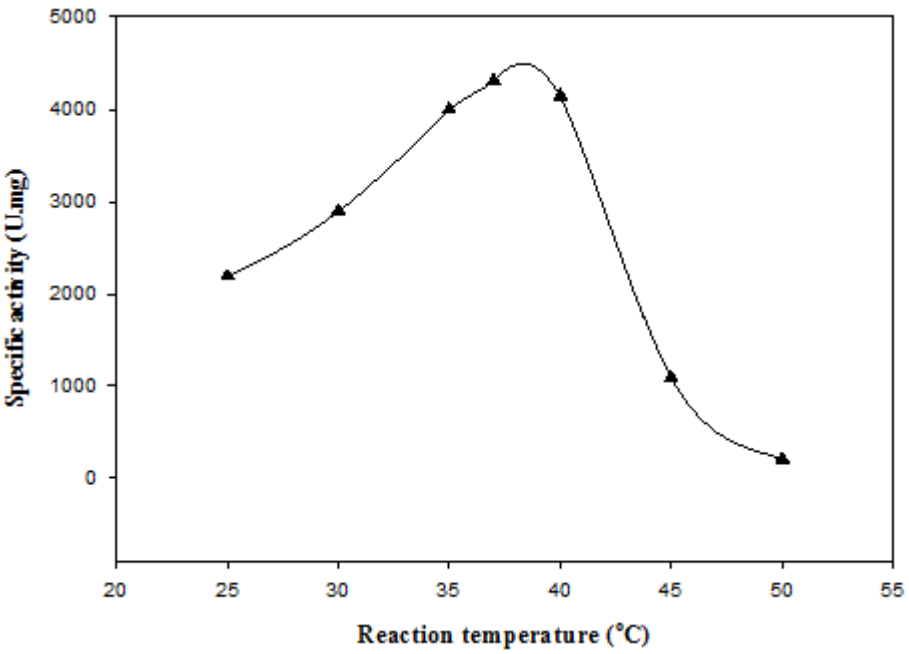

Fig. 5. Effect of incubation temperature on dextransucrase activity of *A. tropicalis*

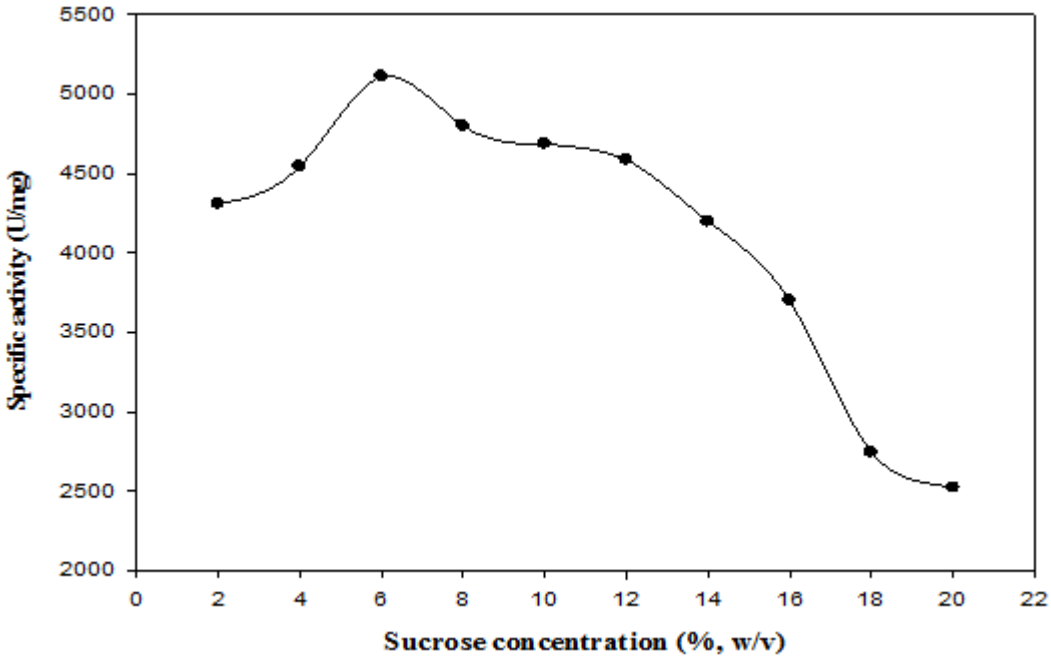

Fig. 6. Effect of sucrose concentration on specific activity of purified *A. tropicalis* dextransucarse

Determination of amino acids present at or near the active site responsible for the catalytic activity of dextransucrase of *A. tropicalis*

Amino acids present at or near active site of the dextransucrase of *A. tropicalis* were determined by incubating the enzyme with various amino acid modifiers. It was noticed that all the amino acid modifiers reduced the enzyme activity drastically suggesting that these affecting amino acids might be present at or near active site of the dextransucrase of *A. tropicalis* (Table 3). Since, iodoacetamide (pH 6.0) is modifier of histidine and phenylglycoxal monohydrate is a modifier of histidine, lysine, cystein and serine, the

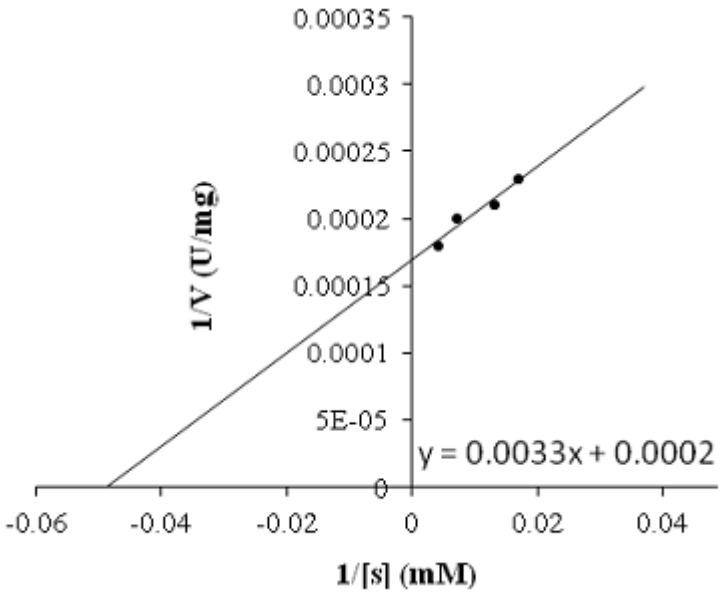

Fig. 7. The Lineweaver-Burk plot of purified dextransucarse from *A. tropicalis*

Table 3. Determination of amino acid present at catalytic site by amino acid modifiers and substrate protection of *A. tropicalis* dextransucrase against inactivation by these inhibitors

No.	Amino Acids Modifiers	Amino Acids Modifiers	Amino acids determination		Substrate protection	
			SA (IU/mg)	RA (%)	SA (IU/mg)	RA (%)
1	Iodoacetamide (pH 6.0)	Histidine	190	7	1710	60
2	Iodoacetamide (pH 8.0)	Cystine	370	13	2083	73
3	Phenylglycoxal monohydrate	Serine, lysine, cystein, histidine	288	10	2005	70
4	N- acetylimidazole	Tyrosine, cystine	350	12	1522	52
5	Diethylepyrocarbonate	Tyrosine	210	7	1243	43
6	N-Ethylmelimide	Arginine	260	9	1617	56
	Control		2872		2872	

SA: Specific activity
RA: residual activity

loss of activity of *A. tropicalis* dextransucrase by them might be due to the presence of these residues at or near enzyme active site. The loss of the activity of *A. tropicalis* dextransucrase by N-acetylimidazole and DEPCe indicate that tyrosine might be involved in enzume catalytic activity (Table 3). The residual activity of enzyme by modifying effect of NEM has been found to be 9% which also confirm the presence of arginine at active site of dextransucrase of *A. tropicalis*.

Substrate protection of dextransucrase of *A. tropicalis* against inactivation by amino acid modifiers

The protection of purified *A. tropicalis* dextransucrase against inactivation of these reagents was achieved when the enzyme was incubated with substrate sucrose. The results showed that the sucrose pretreated dextransucrase of *A. tropicalis* was able to retain most of its activity even in the presence of amino acid

modifiers (Table 3).

Discussion

The extracellular dextransucrase of *A. tropicalis* was purified to homogeneity by subjecting the cell free supernatant to PEG precipitation, dextranase treatment, ammonium sulphate precipitation, dialysis and DEAE-Sepahrose column chromatography. Since the cultivation medium for dextransucrase production by *A. tropicalis* contained 2 % (w/v) sucrose, produced enzyme also converts sucrose in to dextran which subsequently complex the enzyme purification. It has been reported that for the PEG precipitation, dextransucrase should be present in the aggregated form with dextran [25]. Out of PEGs of different molecular weight as used, only PEG-400 (25 %, v/v) was found to be most appropriate for the precipitation of *A. tropicalis* dextransucrase. The PEG-400 at 25 % (v/v) has also been reported previously for the precipitation of dextransucrase of *P. pentosaceus* with 8.5 % yield [25]. However, the precipitation of *L. mesenteroides* NRRL B-640 dextransucrase with 40 % (v/v) PEG-400 resulted in very low yield (2.8 %) of enzyme [29]. The other PEGs didn't contribute effectively in the precipitation of dextransucrase. This loss in enzyme activity may be due to the separation of dextran during fractionation. The dextran always remained as contaminant in the PEG precipitated dextransucrase which is subsequently responsible for the highly viscous nature of the enzyme preparation and makes the further purification difficult. In order to resolve this issue, the PEG precipitated *A. tropicalis* dextransucrase was treated with dextranase enzyme to dissociated the aggregated forms of dextransucrase. Dextranase treatment removes dextran from the enzyme and makes a clear solution, which was subsequently subjected to ammonium sulphate precipitation, dialysis and DEAE-Sepharose column chromatography. The overall yield of *A. tropicalis* dextransucrase obtained was 8 % with 16-fold purification.

The PEG-1500 precipitated dextransucrase of *L. mesenteroides* NRRL B-640 was purified to 61-fold on Sephacryl S-200HR gel-filtration column with yield of 1.25 % [9]. The dextranase treated crude enzyme of *L. mesenteroides* B-512FM was purified on Bio-Gel A-5m column and purified enzyme showed a specific activity of 84 U/mg [7]. Further, Sepharose 6B column has also been used for effective purification of dextranase treated dextransucrase of *L. mesenteroides* by Kobayashi and Matsuda [18] and purified enzyme exhibited specific activity of 72.1 U/mg.

The dextransucrase was reported to exist in either single or multiple forms having molecular mass in the range of 64-245 kDa [8,9,16,18,22,30,40]. The molecular mass of *A. tropicalis* dextransucrase on SDS-PAGE and non-denaturing SDS-PAGE was found to be 180 and 360 kDa, respectively, suggesting that this enzyme exist in homodimer state in native form. Dextransucrase from *L. mesenteroides* NRRL B-640 purified with PEG-400 showed multiple protein bands on SDS-PAGE with a prominent band corresponding to the size of 180 kDa [27]. However, the same enzyme showed a single band on non-denaturing native PAGE confirming the multimeric nature of this enzyme [29]. The dextransucrase of *P. pentosaceus* was found to exist in single form of approximately 180 kDa [25]. Iliev and Vasileva [13] carried out SDS-PAGE analysis of dextransucrases from *L. mesenteroides* Lm 22 and *L. mesenteroides* Lm 28 and both the strains showed only one major band at 180 kDa. However, *L. mesenteroides* NRRL B-1299 produces three types of dextransucrases with molecular weight of 173, 184 and 220 kDa [17]. The *L. citrium* B/110-1-2 was known to produce two cell-associated dextransucrases with molecular weights of 160 and 240 kDa and a soluble dextransucrase of 160-180 kDa [38]. Recently, native SDS-PAGE analysis of purified dextransucrase of *Weissella confusa* Cab3 showed a single homogenous band of 178 kDa [34]. The enzyme produced by *A. tropicalis* seems to be of very high molecular weight and dextransucarse with such a large molecular weight has not been reported in the literature so far.

The purified dextransucarse of *A. tropicalis* was characterized for its ability to perform optimally under diverse physico-chemical conditions. The various physico-chemical parameters *viz.* incubation pH and temperature substrate specificity, kinetic properties were

studied for purified dextransucarse of *A. tropicalis*. The properties of dextransucrase vary from source to source and the pH plays a decisive role in maintaining the ionic state of the enzyme that ultimately influences the activity. Sodium acetate buffer (15 mM) of pH 5.5 was found to be most suitable for *A. tropicalis* dextransucrase activity. The dextransucrase of *L. paramesenteroides* was found to be active at pH 4.5-5.5 [12]. *L. mesenteroides* NRRL B-512(F) dextransucrases was reported to work best in 20 mM sodium acetate buffer of pH 5.4 [5]. Recently, it has been reported that purified dextransucrase of *W. cibaria* JAG8 was stable in 10-500 mM acetate buffer (pH 5.4), whereas, *S. mutans* dextransucrase was most active in sodium meleate buffer (50 m mM) of pH 6.8 [11,31]. The change in dextransucarse activity with alteration of pH might be due to the alteration in the ionization of amino acids resulting in the disruption of ionic bond which are responsible for maintaining 3-D shape of the protein. All enzymes work within a temperature range specific to the organism. Hence it becomes necessary to know the temperature at which reaction proceeds with maximum velocity. The purified dextransucrase of *A. tropicalis* exhibited maximum activity 37°C. However, various researchers have found 30°C as the optimum temperature for the dextransucrase of *L. mesenteroides* NRRL B-512(F), *L. mesenteroides* NRRL B-1299 and *P. pentosaceus*, whereas, *L. mesenteroides* B-512(F)M dextransucrase showed the optimum incubation temperature of [5,25,35].

All enzymes show a characteristic specificity for the reaction they perform and the substrates they could utilize. The dextransucrase of *A. tropicalis* was found to be an inducible enzyme [4] and showed maximum specificity towards its natural substrate sucrose. The purified enzyme exhibited maximum activity 6 % (w/v) sucrose prepared in acetate buffer (pH 5.5, 15 mM). The dextransucarse of *A. trpicalis* showed typical Michaelis-Menten kinetics at low substrate concentration. The K_m and V_{max} values of purified enzyme for sucrose were found to be 11.5 mM and 5000 U/mg, respectively. A mutant (DSRN1) was developed by mutation in DSRB742 gene of *L. mesenteroides* and dextransucrase of this mutant exhibit the K_m of 18 mM which was far less compared to the K_m (88 mM) value of the enzyme obtained from native gene [6,23]. The K_m and V_{max} values of the dextransucrase from *W. cibaria* were reported to be 13 mM and 27.5 U/mg, respectively for substrate sucrose [31].

A variety of amino acid modifiers were used to see the nature of amino acids present at or near active site of the dextransucrase of *A. tropicalis* as these modifiers reduce the enzyme activity by replacing the existing amino acids with others. The present study suggests the possibility of the presence of histidine, cystine, serine, lysine, cystein, tyrosine and arginine residues at or near the active site of *A. tropicalis* dextransucrase. According to previous study, besides catalytic triad (Asp229/Asp286, Glu257/Glu328, and Asp328/Asp393) in the secondary structure of glucansucrase, the active site contains two histidine residues important for activity and stabilization of the transition state of dextransucrase [33]. Its mutation in *L. mesenteroides* NRRL B-512 (F) DSRS and in *S. mutans* GS-5 GTFB results in very low residual enzyme activities [23,36]. Moreover, histidine residues *B. circulans* 251 and in *N. polysaccharea* were reported to involved in transition state stabilization [24,33]. The presence of serine has also been confirmed at the active site of *L. reuteri* 121 dextransucrase and its double mutation showed a very strong change in enzyme activity [15]. Moreover, the statistical and kinetic analysis of the inactivation of enzyme by DEPC showed that two histidine residues are essential for the enzymatic activity [20]. The inactivation of *L. mesenteroides* NRRL B-512(F) dextransucrase by o-phthalaldehyde also confirms the essential presence of lysine residue for the activity of dextransucrase [10]. The catalytic core of dextransucrases commonly starts with two to four conserved tyrosine residues. Mutation of one of four of these tyrosine residues, at positions 169 to 172 of *S. mutans* GS-5, may affect the overall activity [36]. The loss of the activity of *A. tropicalis* dextransucrase by NEM and DEPC indicate that tyrosine might also be present at or near catalytic region of this enzyme.

As mentioned earlier dextransucrase activity

was strongly inhibited by DEPC, iodoactamide and NEM. The protection of purified *A. tropicalis* dextransucarse against inactivation of these reagents was achieved when the enzyme was incubated with substrate sucrose. This might be due to the fact that sucrose occupied most of the active sites on the enzyme before the action of amino acid modifiers and preserve the enzyme activity.

These results also imply the stable conformation of the enzyme in the presence of its natural substrate and consequent denial of the accessibility of fragile active site to the modifiers that may be due to the occupation of the active sites by the substrate. The necessity of carrying out the enzyme conjugation in the presence of the substrates to avoid activity loss by Ashihara *et. al.*, [1] reconfirm the protective role played by the substrates against thermal or other type of enzyme inactivation. It has been proved that labile cellulases were resistant to proteolytic attack in the presence of their appropriate substrates. Starch protected amylase of *Bacillus* sp. has shown 2.5 times more activity than unprotected enzyme [39].

Conclusion

The extracellular dextransucrase from *A. tropicalis* was purified to homogeneity and subsequently characterized. The dextransucrase catalyzes the conversion of sucrose to dextran and this property makes it a good biocatalyst for the production of industrially relevant molecules. Moreover, the purification of proteins is essential first step in understanding its structural and functional properties and allow bio-organic chemists to modify specific residues in order to make them better biocatalyst.

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Conflict of interest

The authors confirm that the contents of this article have no conflicts of interest.

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