# Purified dextransucrase from *Pediococcus pentosaceus* CRAG3 as food additive

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The extracellular crude dextransucrase (0.67 U/mg) from *P. pentosaceus* CRAG3 (GenBank accession number JX679020) after PEG-1500 fractionation gave specific activity, 20.0 U/mg which by gel filtration resulted in 46.0 U/mg. The purified dextransucrase displayed molecular size of approximately, 224 kDa. The optimum assay conditions for dextransucrase activity were 5% sucrose in 20 mM sodium acetate buffer (*p*H 5.4) and 30 $^{\circ}$C. The dextransucrase was stable up to 40 $^{\circ}$C and at pH range of 5.4-7.0. The metal ions such as $Co^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ stimulated the dextransucrase activity by 56, 44, 14 and 12%, respectively. It was most stable at -20 $^{\circ}$C with half-life of 307 days. Amongst various additives used, glycerol and Tween 80 provided significant stability to the enzyme with half-life 15.5 and 85.5 h, respectively as compared to control (6.9 h). The solidification of sucrose supplemented milk by purified dextransucrase due to dextran synthesis displayed its application as additive for improving the texture of dairy products.

**Keywords:** Dextransucrase, Gel filtration, Milk, *Pediococcus pentosaceus*, Polyethylene glycol

Glucansucrases (GSs) are large size extracellular enzymes that catalyse the synthesis of different types of α-glucans such as dextran, mutan, alternan and reuteran using sucrose as a substrate. Depending upon the nature of synthesized product they are categorize as dextransucrase (EC 2.4.1.5), alternansucrase (EC 2.4.1.140), mutansucrase (EC 2.4.1.5) and reuteransucrase (EC 2.4.1.5)[1]. Glucansucrases have been identified in lactic acid bacteria (LAB). The dextran production by *Pediococcus* genus have not been much studied[2-4]. The high molecular weight of dextransucrase is due to its aggregated form in presence of dextran[5]. Since the dextransucrase production is induced by sucrose, there is continuous production of dextran in culture medium which makes it viscous and thereby the purification of enzyme becomes unwieldy. The purification of dextransucrase can be done by ultra-filtration, salt and PEG precipitation, chromatography and phase-partitioning, alone or in combination[5]. However the simplest, effective and one step purification method amongst them is fractionation by polyethylene glycol (PEG)[6-8]. It involves the precipitation of high molecular weight proteins which occur in aggregated forms[9]. Due to their large molecular weight and aggregate forming

tendency in solution of dextransucrase, non-ionic hydrophilic polymer PEG is used for purification[7,8,10,11].

Dextransucrase exists in single or multiple forms having molecular weight in the range 64,000-245,000 Da[7,8,11-15]. *Leuconostoc mesenteroides* NRRL B-512F is a widely studied for dextransucrase[7,16]. In one study it has been reported to produce two main forms of dextransucrase of molecular weight nearly 177 and 158 kDa as determined by SDS-PAGE[17]. The dextransucrase from *Pediococcus pentosaceus*[11] isolated from sugarcane field soil, *Weissella confusa* Cab3[14] and *Weissella cibaria* JAG8[15] were reported to be 180, 178 and 177 kDa, respectively. The effects of different metal ions on dextransucrase from *P. pentosaceus*[11], *W. cibaria* JAG8[15,] and *Leuconostoc dextranicum* NRRL B-1146[18] showed that $Ca^{2+}$ and $Mg^{2+}$ enhanced the activity of the enzyme. The addition of $CaCl_2$ with purified dextransucrase from *L. mesenteroides* NRRL B-512F increased its activity[19]. The dextransucrase from lactic acid bacteria produce dextran which serve in food industries to improve the textural properties of various food products such as fermented dairy products and baked goods[20,21]. The present study describes the purification of dextransucrase from *Pediococcus pentosaceus* CRAG3 using fractionation and gel filtration. The purified enzyme has been identified and confirmed as dextransucrase by Periodic Acid Schiff's (PAS) staining procedure using sucrose and

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raffinose. The biochemical properties of purified dextransucrase have been analysed. The application of dextransucrase in improving of texture of dairy products is also analysed.

## Materials and Methods

*Isolation and culturing of microorganism*—The bacterium *Pediococcus pentosaceus* CRAG3 (Genbank Accession Number JX679020) was isolated from fermented cucumber[22]. It was propagated in modified MRS agar medium[23] as a stab incubated at 30 °C, stored at 4 °C and subcultured every 15 days.

*Dextransucrase assay and protein concentration determination*—The dextransucrase assay was carried out in 1 mL reaction mixture containing 5% (w/v) sucrose in 20 mM sodium acetate buffer (*p*H 5.4) and 20 μL of enzyme sample. The enzyme reaction was performed at 30 °C for 15 min. An aliquot (100 μL) was taken from the reaction mixture and the reducing sugar was estimated by the method of Nelson[24] and Somogyi[25]. One unit (U) of dextransucrase activity is defined as the amount of enzyme that releases 1 μmol of reducing sugar (fructose) per min at 30 °C and *p*H 5.4. Protein concentration in supernatant was estimated by the method of Lowry *et al.*[26] using Bovine serum albumin (BSA) as standard in the range of 25-500 μg/mL.

*Production and purification of dextransucrase*—The enzyme production medium[27] was used for dextransucrase production. Fermentation was carried out with 1% (v/v) inoculum of overnight grown culture of *P. pentosaceus* CRAG3 in 50 mL enzyme production medium[27] and incubated at 25 °C and 180 rpm for 6 h. The cell pellet was removed by centrifuging at 8,000×*g* at 4 °C for 10 min and the cell free supernatant was used for two stage purification of enzyme by applying fractionation with polyethylene glycol (PEG) followed by gel filtration.

In first stage, purification of dextransucrase was carried out by using different concentrations of polyethylene glycols (PEGs) of different molecular weights (PEG-400 and PEG-1500) as described earlier[8]. The samples of purified dextransucrase were analysed for enzyme activity and protein concentration. The dextransucrase sample displaying highest activity was further purified by gel filtration.

The glass column (1.5×50 cm) (Bio-Rad Laboratories, USA) packed with Sephacryl S-300HR matrix (Sigma, St. Louis, MO, USA) of bed volume 80 mL. 2 mL dialyzed enzyme (20 U/mg, 0.3 mg/mL)

was loaded on column connected to chromatography system (Biologic LP, Bio-Rad Labs, USA). Fractions (50) each of 3 mL were eluted using 20 mM sodium acetate buffer (*p*H 5.4) at a flow rate of 0.5 mL/min and those having high absorbance at 280 nm were analysed for enzyme activity and protein concentration. The fractions with considerable higher enzyme activity were pooled, lyophilized and run on SDS-PAGE to analyse the purity and size of the enzyme. *In situ* activity of dextransucrase was done by periodic acid Schiff's base (PAS) protocol as described later.

*Electrophoretic analysis of purified dextransucrase*—The electrophoretic analysis of purified dextransucrase from *P. pentosaceus* CRAG3 was carried out by denaturing SDS-PAGE using 7% gel following the method of Laemmli[28]. The dextransucrase sample purified by 10% (v/v) PEG-1500 was also run on native-PAGE to check its purity. After run, the protein bands were stained with 0.25% (w/v) Coomassie Brilliant Blue (CBB R-250) for 30 min and destained by repeated washing using a solution containing (v/v) 20% methanol and 10% acetic acid. Molecular weight marker (10-220 kDa) from Invitrogen, India, was loaded along with the protein samples as a standard. The molecular mass of protein band was determined by Hedrick's plot[29].

*In situ* activity of dextransucrase was determined by running the gel under non-denaturing SDS-PAGE using the method of Holt *et al.*[30] with some modifications. The dextransucrase activity was detected by incubating the gels separately in 5% sucrose or 2% raffinose for 14-16 h, followed by staining of polysaccharide according to a periodic acid-Schiff's procedure[31].

*Optimization of assay conditions for dextransucrase activity*—The assay conditions of dextransucrase activity were optimized in terms of sucrose concentration, *p*H, ionic strength of buffer and temperature of reaction. In order to determine the optimum substrate concentration, the sucrose concentration was varied from 0.1-15% (w/v). The reaction mixture (1 mL) containing 20 mM sodium acetate buffer (*p*H 5.4), 20 μL purified dextransucrase (20 U/mg) and varying concentrations of sucrose was used and enzyme activity and protein concentration were determined as described above. Similarly, the other parameters such as *p*H (4.0-7.0), ionic strength (10-100 mM) and temperature (10-50 °C) were varied and optimized. The kinetic parameters ($K_m$ and $V_{max}$)

were determined using optimized $p$H, ionic strength and temperature and varying substrate concentrations.

*Effects of metal ions on dextransucrase activity*—The effects of various metal ions such as $Mg^{2+}$, $Ca^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$ and $Mn^{2+}$ on dextransucrase activity were studied. The salt solutions were prepared in 20 mM sodium acetate buffer ($p$H 5.4) to make their final concentration between 0-10 mM. The reaction was carried out in 1 mL mixture containing 5% (w/v) sucrose in 20 mM sodium acetate buffer ($p$H 5.4), 20 µL of enzyme (20.0 U/mg, 0.30 mg/mL) and the salt and the enzyme activity was determined as described above.

*Thermostability and pH stability studies of dextransucrase*—The thermostability of purified dextransucrase (20.0 U/mg, 0.3 mg/mL) was determined by incubating it at various temperatures (10-60 °C) for 1 h. The residual enzyme activity was determined as described above. The effect of $p$H on dextransucrase was studied by incubating it at different $p$H (3.5-7.0). The lyophilised dextransucrase (1 mg) was dissolved in 1 mL of 20 mM sodium acetate buffer with varying $p$H (3.5-7.0). The aliquots (20 µL) were taken at regular time interval to determine the residual enzyme activity.

*Effects of storage temperatures on dextransucrase activity*—The storage stability of dextransucrase was studied by incubating the dextransucrase (20 U/mg, 0.3 mg/mL) at different temperatures (0, 4 and -20 °C). The aliquots (20 µL) were taken at regular time interval to determine the residual enzyme activity. The half-life ($t_{1/2}$) of dextransucrase was measured at different temperatures with respect to time assuming that the decay followed first order kinetics following the method of Naidu *et al*[32].

*Effects of additives on dextransucrase stability*—The effects of various additives such as dextran (500 kDa), PEG-8000, glutaraldehyde, glycerol and Tween 80 on the stability of dextransucrase at 30 °C were determined. The aqueous solutions (1 mL) of additives containing dextransucrase (20.0 U/mg, 0.3 mg/mL) in 20 mM sodium acetate buffer ($p$H 5.4) with final concentrations of 2 µg/mL (w/v) dextran (500 kDa), 10 µg/mL (w/v) PEG-8000, 0.1% (v/v) glutaraldehyde, 0.5% (v/v) glycerol and 0.1% (v/v) Tween 80 were incubated at 30 °C for 15 min. The residual activity and half-life ($t_{1/2}$) of dextransucrase was determined as described above.

*Application of dextransucrase on solidification of sucrose-supplemented milk*—The dextransucrase from

*P. pentosaceus* CRAG3 was evaluated for its application on sucrose-supplemented semi-skimmed milk as per the method described by Bejar *et al*[33]. To the semi-skimmed milk (20 mL), sucrose was added at final concentration of 2 and 5% (w/v). The partially purified dextransucrase (20 U/mg, 0.3 mg/mL, 4 mL) from *P. pentosaceus* CRAG3 (to make final concentration of 5 U/mL) was added to each of the sucrose-supplemented milk samples. The mixtures were incubated at 30 °C for 20 h. The solidified milk was then visually analysed for the production of dextran and compared with the control (milk without enzyme).

**Results and Discussion**

*Production and purification of dextransucrase*—Purification by fractionation with polyethylene glycol: The cell free supernatant containing dextransucrase (0.67 U/mg, 5.2 mg/mL) was subjected to fractionation with different concentrations of PEG-400 and PEG-1500. With PEG-400 dextransucrase showed maximum specific activity of 13.0 U/mg 20 fold purification with 5% yield at 33% (v/v) concentration (Table 1). The purification of dextransucrase with other concentrations of PEG-400 showed lower specific activities. Purification of dextransucrase with PEG-1500 was performed in a concentration range of 10-25%. The maximum specific activity of 20.0 U/mg with 30 fold purification and 15.6% overall yield was obtained at 10% (v/v) PEG-1500 (Table 1). The increase in concentration above 10% (w/v) of PEG-1500 did not favour the purification of dextransucrase with higher enzyme activity. Similar results were reported on dextransucrase purification from other lactic acid bacteria such as *L. mesenteroides* NRRL B-512F[7], *L. mesenteroides* NRRL B-640[8] and *P. pentosaceus*[11]. Dextransucrase from *P. pentosaceus* isolated from sugarcane field soil purified by 10% (v/v) PEG-1500 gave specific activity of 25.9 U/mg with 45 fold purification[11].

Electrophoretic analysis of PEG purified dextransucrase: The electrophoretic analysis of dextransucrase purified with 10% (w/v) PEG-1500, on SDS-PAGE gels under denaturing conditions showed two bands (Fig. 1a, Lane 1). The denatured dextransucrase gave bands of approximately, 224 and 188 kDa molecular size as determined by Hedrick's plot[29]. This might be due to the denaturation of dextransucrase run under denaturing conditions as also reported earlier[34]. However, the same enzyme

Table 1—Purification of dextransucrase by fractionation with PEG and gel filtration

| Sample | Volume (mL) | Enzyme activity (U/mL) | Overall yield (%) | Protein (mg/mL) | Sp. Activity (U/mg) | Purification (fold) |
|---|---|---|---|---|---|---|
| Cell free supernatant | 30 | 3.50 | - | 5.20 | 0.67 | - |
| PEG-400 | | | | | | |
| 20% (v/v) | 2.0 | 6.50 | 12.38 | 0.80 | 8.09 | 12.0 |
| 25% (v/v) | 2.0 | 1.85 | 3.60 | 0.22 | 8.41 | 13.0 |
| 33% (v/v) | 1.7 | 5.00 | 8.10 | 0.38 | 13.2 | 19.0 |
| 40% (v/v) | 1.0 | 5.30 | 5.04 | 0.41 | 12.9 | 20.0 |
| PEG-1500 | | | | | | |
| 10% (v/v) | 3.0 | 5.46 | 15.60 | 0.27 | 20.21 | 30.0 |
| 15% (v/v) | 3.9 | 4.80 | 17.82 | 0.38 | 12.66 | 19.0 |
| 20% (v/v) | 2.0 | 1.20 | 2.30 | 0.16 | 7.52 | 11.0 |
| 25% (v/v) | 2.0 | 1.11 | 2.10 | 0.15 | 7.40 | 11.0 |
| Sephacryl S300 HR | 9.0 | 1.52 | 13.03 | 0.033 | 46.0 | 69.0 |
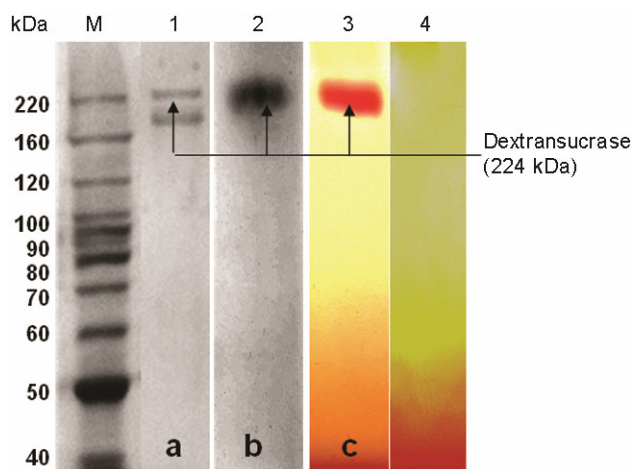


Fig. 1—Polyacrylamide gel (7%) electrophoresis of dextransucrase and its confirmation by PAS staining method. (a) Denaturing SDS-PAGE: Lane: (M) Protein molecular mass marker: 40-220 kDa; (1) 10% (v/v) PEG-1500 fraction stained with CBB R250; (b) Native-PAGE: (2) 10% (v/v) PEG-1500 fraction stained with CBB R250; (c) Non-denaturing SDS-PAGE: 10% (v/v) PEG-1500 fraction stained with PAS staining using (3) Sucrose as substrate; (4) Raffinose as substrate.

sample showed predominantly a single, intact and homogeneous protein band on native-PAGE (Fig. 1b, Lane 2). This showed that dextransucrase remains in single molecular form in native state after purification and dialysis but shows two bands only under denaturing conditions. After PAS staining, on incubating the gel with 5% sucrose for 16 h, single magenta colour band appeared which corresponded well with the 224 kDa protein band observed in non-denaturing SDS-PAGE. This was due to the formation of polysaccharide on polyacrylamide gel confirming the presence of dextransucrase (Fig. 1c, Lane 3).

Magenta colour band on sucrose-incubated gel showed the native and active form of the purified dextransucrase. The absence of band on gel incubated with 2% raffinose ruled out the presence of fructansucrase (Fig. 1c, Lane 4).

Purification of dextransucrase by gel filtration: The dextransucrase purified by 10% PEG-1500 having specific activity of 20.0 U/mg was further purified using gel filtration. The purification profile showed a single peak of specific activity and protein (absorbance, $A_{280}$) between fraction numbers 10 and 15 (Fig. 2a). The fractions with high specific activities (fraction number 11-13) were pooled. The pooled enzyme fractions showed specific activity of 46 U/mg with 13% overall yield and 69 fold purification. The dextransucrase from *L. mesenteroides* NRRL B-640 purified with 10% PEG-1500 gave 23.0 U/mg specific activity with 40 fold purification while further purification by gel filtration showed a specific activity of 35.0 U/mg with 61 fold purification[8].

Electrophoretic analysis of dextransucrase purified by gel filtration: The pooled dextransucrase (46 U/mg) from *P. pentosaceus* CRAG3 was run on denaturing SDS-PAGE using 7% gel. After gel filtration a prominent band of 224 kDa and a light band of 188 kDa (Fig. 2b, Lane 1) were obtained. The presence of single 224 kDa magenta colour band after PAS staining, on gel incubating with 5% sucrose in non-denaturing SDS-PAGE, confirmed the presence of dextransucrase (Fig. 2b, Lane 2). However, no activity band was observed on gel incubated with 2% raffinose which confirmed the absence of fructansucrase (Fig. 2b, Lane 3). Dextransucrase from *L. mesenteroides* NRRL B-640[8], *P. pentosaceus*[11], *W. confusa* Cab3[14],

*W. cibaria* JAG8[15] and *L. dextranicum* NRRL B-1146[18] showed similar molecular size of approximately, 180, 180, 178, 177 and 205 kDa, respectively.

*Optimization of assay conditions for dextransucrase activity*—The effects of *p*H, ionic strength of buffer and temperature on dextransucrase activity showed that 20 mM sodium acetate buffer, *p*H 5.4 and 30 °C gave maximum enzyme activity (20.0 U/mg). The rate of reaction increased with
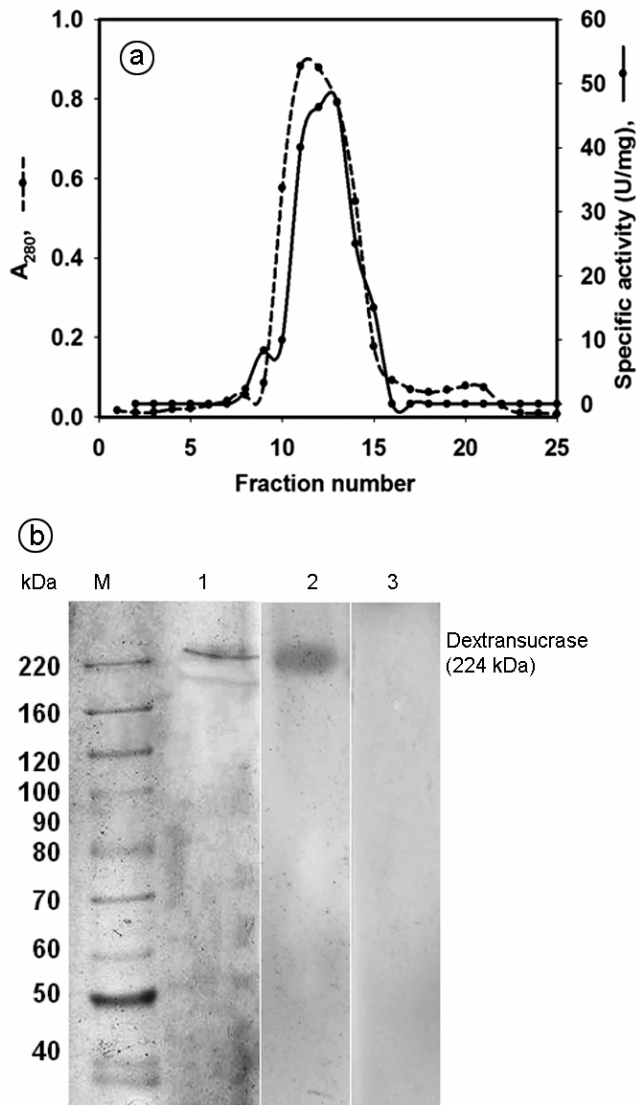


Fig. 2—Purification of dextransucrase from *Pediococcus pentosaceus* CRAG3 by gel filtration using Sephacryl S300HR column (a). SDS-PAGE gel (7%) displaying purified dextransucrase by gel filtration (b). Lane: (M) Protein molecular mass marker (10-220 kDa); Sephacryl S-300HR pooled fraction: Stained with CBB R250 (1), Stained with PAS staining using sucrose as substrate (2) and Stained with PAS staining using raffinose as substrate (3).

increase in the concentration of sucrose up to 146 mM (5%, w/v) but decreased thereafter, which might be due to substrate inhibition. This showed that dextransucrase from *P. pentosaceus* CRAG3 did not follow classical Michaelis-Menten kinetics. Similar pattern was observed with dextransucrase from *P. pentosaceus*[11] and *L. mesenteroides* NRRL B-512F[19]. However, dextransucrase from *L. dextranicum* NRRL B-1146 exhibited Michaelis-Menten kinetics[18]. The kinetic parameters for dextransucrase from *P. pentosaceus* CRAG3 using sucrose were $K_m$ = 15.4±1.4 mM and $V_{max}$ 20.9±1.2 µmol/mg/min.

*Effects of metal ions on dextransucrase activity*— The effects of different metal ions on dextransucrase activity were studied. Forman and Kennedy[35] reported that the salts affect water structure of enzymes thereby affecting their solubility and activity. $Mg^{2+}$, $Ca^{2+}$, $Co^{2+}$ and $Zn^{2+}$ stimulated the activity while $Ni^{2+}$ and $Mn^{2+}$ diminished the enzyme activity as compared to control (Table S1). The $Co^{2+}$ (4 mM) and $Ca^{2+}$ (2 mM) ions were most effective cations which stimulated the dextransucrase activity by 56 and 44%, respectively (Table S1). The dextransucrase activity was stimulated by 14% in presence of 2 mM $Mg^{2+}$ ion (Table S1). $Mg^{2+}$ and $Ca^{2+}$ ions enhance the catalytic activity of enzyme by stabilizing the three-dimensional protein structure[19]. Miller and Robyt[17] reported that $Ca^{2+}$ ion is associated with catalytic sites of dextransucrase. Similar results of increasing dextransucrase activity in presence of $Ca^{2+}$ ion were reported in *P. pentosaceus* (6 mM, 150% enhancement)[40] and *L. mesenteroides* NRRL B-640 (4 mM, 108% enhancement)[36]. The $Zn^{2+}$ at its final concentration of 1 mM resulted in 11% stimulation in dextransucrase activity (Table S1). On further increasing the concentration above 1 mM the enzyme activity decreased gradually. The ions such as $Mn^{2+}$ and $Ni^{2+}$ negatively affected the dextransucrase activity. On addition of 2 mM $MnSO_4$ and 1 mM $NiSO_4$ the enzyme activity decreased by 18 and 12%, respectively (Table S1). On further increasing the concentration of $MnSO_4$ and $NiSO_4$ to 8 mM the enzyme activity was lost by 89 and 90%, respectively.

*Thermostability and pH studies of P. pentosaceus CRAG3 dextransucrase*—The thermostability studies of dextransucrase from *P. pentosaceus* CRAG3 at different temperatures displayed its mesophilic nature. The enzyme was stable up to 40 °C when incubated for 1 h (Fig. S1). Above 40 °C the enzyme activity was rapidly lost. It proved to be better thermostable enzyme than dextransucrase from other lactic acid

bacteria such as *W. cibaria* JAG8[16] and *L. mesenteroides* NRRL B-640[36] which lost activity above 35 and 30 °C, respectively. The effect of pH on stability of dextransucrase from *P. pentosaceus* CRAG3 is shown in Fig. 3 and expressed as residual activity. The dextransucrase was more stable towards acidic to neutral pH range (5.4-7.0) when incubated at 30 °C. On comparing the assays carried out at six different pH values, it is clear that although the enzyme presented good stability at *p*H range of 5.4-7.0 during all the evaluated time period, the assay carried out at *pH* 5.4 presented the highest enzyme activity. The residual activity of dextransucrase at pH 5.4 was 82% after 3 h (Fig. 3). However, the enzyme activity reduced to 21 and 12% at pH 4.0 and 3.5, respectively (Fig. 3). Similar results were observed in fusion enzyme of endodextranase and dextransucrase by Kim *et al*[37]. The dextransucrase from *L. mesenteroides* was stable between *p*H 5.0-6.5[38] while that from *W. cibaria* JAG8[16] and *L. mesenteroides* B-742[39] were stable at 5.4 and 6.5, respectively.

*Effects of storage temperatures and additives on dextransucrase activity*—The residual activity profile of dextransucrase from *P. pentosaceus* CRAG3 at -20, 4 and 30 °C is shown in Fig. S2-A. The dextransucrase lost only 5 and 11% activity on incubating at -20 and 4 °C, respectively after 21 days which showed that -20 °C is suitable temperature for storing dextransucrase (Fig. S2-A). The residual

activity of dextransucrase at 30 °C was 35% after 24 h (Fig. S2-A). Similar results were observed in previously reported *P. pentosaceus* and its mutant where the residual activity was 26 and 41%, respectively[40]. The half-life ($t_{1/2}$) of dextransucrase alone and with additives was calculated assuming that its decay followed first order kinetics (Table 2). On storing the dextransucrase at -20 and 4 °C the $t_{1/2}$ increased to 306.9 days and 128.6 days, respectively (Table 2). Thus, -20 °C was selected for storage of dextransucrase. The dextransucrase from *W. confusa* Cab3 showed maximum storage stability at 4 °C[14].

Effect of various additives on dextransucrase activity is shown in Fig. S2-B. The residual activity of dextransucrase at 30 °C in presence of Tween 80 after 12 h was 90% as compared to control (46%), which showed that it was most effective stabiliser for dextransucrase (Fig. S2-B). In presence of glycerol the residual activity was 83% after 9 h of incubation at 30 °C. PEG-8000 moderately stabilized the enzyme since the residual activity of dextransucrase in its presence after 12 h was 39% (Fig. S2-B). The stability of dextransucrase in aqueous solution is enhanced by adding co-solvents such as Tween 80 and glycerol which are known to reduce the aggregation of protein and thereby increase the stability of enzyme[41,42]. Glycerol is also known to convert the protein from its native state to more stabilised compact state[42]. However, the other additives did not stabilize the enzyme. The residual activity of dextransucrase in presence of dextran (500 kDa) and gluteraldehyde after 12 h was only 27, and 19%, respectively (Fig. S2-B). The residual activity of *P. pentosaceus* and its mutant in presence of Tween 80, PEG-8000, dextran (100 kDa), glycerol and without any stabilizer at 30
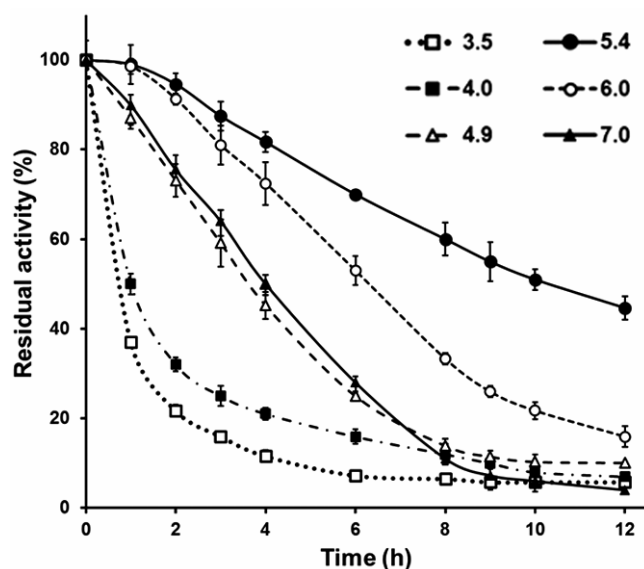


Fig. 3—The enzyme (20 U/mg, 0.30 mg/mL) in 20 mM sodium acetate buffer (pH 5.4) was pre-incubated in same buffer of varying pH. The aliquots were assayed for dextransucrase activity at 30 °C at different time intervals.

Table 2—Effect of additives on *Pediococcus pentosaceus* CRAG3 dextransucrase.

| Dextransucrase with additive | Half-life ($t_{1/2}$) | | |
|---|---|---|---|
| | 30 °C | 4 °C | -20 °C |
| Dextransucrase | 06.90 h | 128.6 d | 306.9 d |
| Dextransucrase + Tween 80 (0.1%) | 85.48 h | Nd | Nd |
| Dextransucrase + Glycerol (0.5%) | 15.53 h | Nd | Nd |
| Dextransucrase + Dextran (2 µg/mL) | 06.32 h | Nd | Nd |
| Dextransucrase + Gluteraldehyde (0.1%) | 05.08 h | Nd | Nd |
| Dextransucrase + PEG-8000 (10 µg/mL) | 08.83 h | Nd | Nd |

*Nd= not determined*

°C was 66, 24, 26, 19 and 26% and 68, 28, 45, 38 and 41%, respectively[40]. Similar results were observed with dextransucrase of *L. mesenteroides* NRRL B-640 where residual activity at 30 °C was 92, 44, 38, 36, 32 and 6% with Tween 80, glycerol, PEG-8000, dextran (500 kDa), control and glutaraldehyde, respectively at 20 h[36]. Amongst various additives used Tween 80 proved to be better stabiliser giving stabilization of dextransucrase with $t_{1/2}$ of 85.48 h at 30 °C as compared to control having $t_{1/2}$ of 6.9 h (Table 2). The presence of glycerol and PEG-8000 along with dextransucrase resulted in enhanced half-life to 15.53 and 8.83 h as compared to control (6.9 h). However, as dextran and gluteraldehyde negatively affected the stability of dextransucrase with $t_{1/2}$ of 6.32 and 5.08 h, respectively.

*Application of dextransucrase on the solidification of sucrose-supplemented milk*—Varying degrees of solidification were achieved on addition of sucrose at concentrations 2 and 5% (w/v) as displayed in Fig. S3. The solidification of sucrose supplemented milk due to addition of dextransucrase was accredited to the formation of dextran. The application of dextransucrase from *Weissella* sp. TN610 in solidification of milk was observed by Bejar *et al*[33]. Similar results have also been observed in dextran from *W. hellenica* SKKimchi3[37] where the bacterial strain (and not the dextransucrase enzyme as the present study) was cultivated in skim milk supplemented with 10% (w/v) sucrose to produce *in vivo* dextran. This property served application of dextransucrase from *P. pentosaceus* CRAG3 as a food additive to improve the texture of sucrose-supplemented milk based products. The dextran synthesised in milk interacts with milk proteins and result in modification in texture of milk. The changes in milk texture are influenced by branching and nature of linkages in dextran, its charge and molecular mass, proteins present in the milk and on dextran/milk protein ratio[43]. These properties suggest the importance of dextransucrase in food industry to enhance the rheological properties of dairy and bakery products[21,44].

## Conclusions

The crude dextransucrase (0.67 U/mg) from *P. pentosaceus* CRAG3 after purification with 10% (w/v) PEG-1500 gave 20.0 U/mg specific activity. This was further purified by gel filtration resulting in specific activity of 46.0 U/mg. The purified dextransucrase (224 kDa) showed $K_m$ of 15.42 mM and $V_{max}$ of 20.9 µmol/mg/min. The stimulation of dextransucrase was observed in presence of $Co^{2+}$, $Ca^{2+}$, $Mg^{2+}$ and $Zn^{2+}$ ions displaying the importance of these metal ions for its catalytic efficiency. The dextransucrase was stable up to 40 °C showing its superior property over other previously reported dextransucrases which were stable up to 30 °C. It was most stable at *p*H range of 5.4-7.0 and temperature -20 °C with half-life of 307 days, hence -20 °C was chosen to store dextransucrase. The additives such as Tween 80 and glycerol enhanced the stability of dextransucrase with half-life ($t_{1/2}$) of 85.5 and 15.53 h, respectively as compared to control (6.9 h). The formation of dextran by purified dextransucrase in sucrose supplemented milk signified its importance in improving the texture of dairy and bakery products. Therefore, this study opens a new horizon to explore dextransucrase from a novel isolate *P. pentosaceus* CRAG3 with higher activity and stability which can serve as a potential candidate for food applications.

## Supplementary data

Supplementary data (Table S1 and Figure S1-3) associated with the article may be obtained from the correspondent author on request.

## Acknowledgement

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