

Characterization of dextransucrase from *Leuconostoc mesenteroides* T3, water kefir grains isolate

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Abstract

The production of dextransucrase (DS) by *Leuconostoc mesenteroides* T3, novel isolate from water kefir grain, was studied and optimized. Bacterial supernatant reached activity of 3.1 U/ml when the culture was grown at 23 °C and under static culture condition using classical Tsuchiya medium for DS production. The increase of sucrose concentration to 7% led to an increase of DS activity by 52% compared to the control. Medium with 2% beef extract and 1% yeast extract resulted in 4.52 U/ml, which was 47% higher than in the control (with 2% yeast extract). Finally, the increase of K₂HPO₄ concentration from 2 to 3% resulted in the increased enzyme activity by 28%. Enzyme purified by polyethylene glycol 400 fractionation displayed maximum activity at 30 °C and pH 5.4. Zymogram analysis confirmed the presence of DS of approximately 180 kDa. The addition of divalent cations Ca²⁺, Mg²⁺, Fe²⁺ and Co²⁺ led to a minor increase of DS activity, while the addition of Mn²⁺ was the most prominent with 73% increase. These findings classify dextransucrase from *Leuconostoc mesenteroides* T3 as promising candidate for production of dextran, which has numerous applications in various industries.

Keywords: lactic acid bacteria, *Leuconostoc mesenteroides*, dextransucrase, dextran.

Available online at the Journal website: <http://www.ache.org.rs/HI/>

Lactic acid bacteria (LAB) are one of the main classes of microorganisms that are known to produce several industrially important biomolecules among which exopolysaccharides (EPS) have the widest range of uses [1]. Fermentation processes for EPS production are quite expensive due to purification costs, giving an advantage to the use of enzymes. Current challenges in the LAB enzymes production include both strain improvement and enhancement of enzyme production.

Dextransucrase (DS) is an extracellular enzyme that belongs to the class of glucosyltransferases [2] and it is mainly produced by microorganisms belonging to the families *Lactobacillaceae* and *Streptococcaceae*, especially by the genera *Lactobacillus*, *Leuconostoc* and *Streptococcus* [3]. Enzyme DS catalyzes the transfer reaction of glucosyl residues from sucrose to dextran polymer chain and releases fructose [4,5]. In the presence of appropriate acceptor (glucose, maltose, isomaltose, etc.) this enzyme can also produce oligosaccharides [6].

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Paper received: 21 April, 2016

Paper accepted: 12 December, 2016

<https://doi.org/10.2298/HEMIND160421046M>

SCIENTIFIC PAPER

UDC 63(497.113):632.954:66.061:543

Hem. Ind. 71 (4) 351–360 (2017)

The growing importance of DS stems from the wide application of dextran [7–10]. Dextran is a high-molecular-mass homopolysaccharide made of glucose. The glucose molecules in the main chain of dextran are linked by linear alpha 1-6 glycosidic bonds and by alpha 1-2, alpha 1-3 and/or alpha 1-4 branching linkages [11]. The proportion of branching and the chain length influenced the rheological properties of dextran [12].

Depending on its solubility and molecular mass, dextran has had successful industrial application so far, such as in medical, pharmaceutical, food, textile and chemical industries [7–10]. Dextrans of low molecular weight have been used as a blood plasma substitute for the last 60 years while the high molecular weight dextrans can be used as viscosifying, stabilizing, gelling, sweetening and emulsifying agents in food production [9,13,14]. There are also approved patents related to the use of dextran in the preparation of bakery products [15,16].

In the present study, *Leuconostoc mesenteroides* strain T3, water kefir grains' isolate described earlier [17] has been used for the production of DS. The growth conditions and culture medium composition for the highest DS production from the aforementioned strain were determined. The DS was purified by polyethylene glycol fractionation and characterized in terms of temperature, pH value and the influence of ions.

EXPERIMENTAL

Microorganism and culture conditions for dextransucrase production

The microorganism used in this study is *Leuconostoc mesenteroides* T3 natural isolate from water kefir grain, identified as *L. mesenteroides* based on the almost full-length 16S rRNA gene sequence (KT924430), described earlier [17].

Production of dextransucrase from *L. mesenteroides* T3

For DS production, the organism was grown in 100 ml enzyme production medium described by Tsuchiya *et al.* [18], with 4% of sucrose. The medium was composed of sucrose, 4%; yeast extract, 2%; K₂HPO₄, 2%; MgSO₄·7H₂O, 0.02%; MnSO₄·4H₂O, 0.001%; FeSO₄·7H₂O, 0.001%; CaCl₂·2H₂O, 0.001%; NaCl, 0.001%, while the pH was adjusted to 6.9. The culture was incubated at 23 °C for 16 h under static condition. The culture broth was then centrifuged at 7000 rpm at 4 °C for 10 min, and the cell free supernatant was analyzed for enzyme activity and protein concentration as described below.

Enzyme assay and protein estimation

The enzyme assay was carried out in 450 µl reaction mixture containing 10% sucrose in 20 mM sodium acetate buffer (pH 5.4) and 50 µl cell free supernatant at 30 °C for 15 min. After that time the reaction was stopped by adding 500 µl of DNS. The enzyme activity was determined by measuring the concentration of released reducing sugars by DNS method [19], using fructose as a standard. The absorbance was measured at 540 nm using spectrophotometer (Ultrospec 3300 pro, Amersham Biosciences). One unit of DS activity was defined as the amount of enzyme releasing 1 µmol of reducing sugars per min.

The protein concentration of the cell free supernatant was determined using the method of Lowry *et al.* [20], using bovine serum albumin as a standard.

Screening for factors affecting dextransucrase production

Growth conditions (temperature and aeration) and culture medium composition (sucrose, nitrogen source, K₂HPO₄ and Tween 80) for enzyme production were screened and optimized.

Effects of growth conditions on dextransucrase production

The influence of temperature and shaking on production of DS by *L. mesenteroides* T3 was tested. The temperature was varied from 17 to 37 °C under static condition, using 100 ml Tsuchiya medium [18]. The effect of shaking condition on enzyme production was analyzed at 90, 120, 150 and 180 rpm at fixed temperature of 23 °C. Every 4 h, during the period of 24 h,

1ml of culture was taken out from flask and centrifuged at 6000 rpm and 4 °C for 10 min. The DS activity in cell free supernatant was determined in triplicate.

Effects of culture medium composition on dextransucrase production

The effects of various concentrations of medium components, *i.e.*, sucrose, nitrogen source (yeast extract, meat extract and peptone) and K₂HPO₄, on DS production were studied by changing the concentration of one component, and keeping other components constant in Tsuchiya medium [18]. Concentration of sucrose was varied from 1 to 10% with step of 1%, nitrogen sources were varied from 0.5–4.0 % with step of 0.5%, and K₂HPO₄ was varied from 1–6% with step of 1%. The isolate was grown in different media at 23 °C under static condition. Broth samples of 1 ml were periodically withdrawn and analyzed for enzyme activity.

Dextransucrase purification

DS was precipitated from cell free supernatant by addition of 25% PEG 400 (Zorka Pharma, Serbia) [21]. After precipitation at 4 °C for 24 h, the mixture was centrifuged at 10000 rpm for 15 min at 4 °C. The pellet was dissolved in 20 mM sodium acetate buffer (pH 5.4). These fractions were subjected to dialysis using 10 kDa cutoff membrane (Thermo scientific, USA). The DS fractions were analyzed for enzyme activity and protein estimation.

SDS-PAGE analysis and activity staining of dextransucrase

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under denaturing condition according to the method of Laemmli [22] using 7.5% polyacrylamide running gel. The enzyme was purified by 25% PEG 400 fractionation. The protein samples were prepared in 0.0625 M Tris-HCl buffer (pH 6.8) containing 2% sodium dodecylsulfate, 10% glycerol, 5% β-mercaptoethanol and 0.02% bromophenol blue and boiled at 100 °C for 3 min. The electrophoresis was carried out using Tris-Glycine buffer (pH 8.3) at room temperature with a current of 12 mA throughout stacking gel and of 25 mA throughout running gel (Cleaver Scientific Ltd., OmniPAGE Mini System). Proteins were stained using Coomassie brilliant blue G-250.

The *in situ* detection of DS activity in gel was carried out following the method of Holt *et al.* [23] with modification described by Purama and Goyal [24]. The protein samples for non-denaturing SDS-PAGE were prepared in the same manner as described above, with exclusion of β-mercaptoethanol component and boiling step. The gel was washed in 20 mM sodium acetate buffer solution (pH 5.4) with 0.3 mM CaCl₂ and 0.1% Tween 80 at 30 °C for 30 min to remove SDS. Subsequent incubation in 20 mM sodium acetate buffer

(pH 5.4) containing sucrose (10%) at 30 °C for 48 h enabled dextran formation within gel on locations having DS activity. The sucrose treated gel was washed first with 75% ethanol for 40 min at room temperature and with a periodic acid solution (periodic acid, 1%, and acetic acid, 3%) for 45 min at 30 °C and finally with a solution containing 0.2% sodium metabisulphite and 5 vol.% acetic acid for 5 min at room temperature. The cleaned gel was stained with Schiff's reagent (15 mL) (0.5%, Fuchsin basic, 1%, sodium bisulphate and 0.1 M HCl) for 10 min to form magenta bands within the gel matrix, an indication for DS activity.

Optimum temperature and thermal stability of dextransucrase

The optimum temperature of DS was studied by adding 50 µl of the purified enzyme (16.7 U/mg, 0.47 mg/ml) to 450 µl enzyme reaction mixture containing 10% sucrose in 20 mM sodium acetate buffer (pH 5.4). The reaction mixture was incubated at different temperatures varying from 20 to 50 °C for 15 min. The enzyme activity was determined in 50 µl sample using DNS method, as previously described. The thermal stability of the enzyme was determined by incubating 0.5 ml of the purified enzyme with 0.5 ml 20 mM sodium acetate buffer (pH 5.4, 16.7 U/mg, 0.47 mg/ml) at different temperatures ranging from 20 to 60 °C for 30 min. The residual enzyme activity was determined as described earlier.

Optimum pH and pH stability of dextransucrase

The optimum pH of DS was determined by incubating 50 µl (16.7 U/mg, 0.47 mg/ml) of the purified enzyme in 450 µl reaction mixture containing 10% concentration of sucrose in 20 mM sodium acetate buffer of different pH, ranging from 3.5 to 6.5. The reaction mixture was incubated at 30 °C for 15 min and enzyme activity was determined using previously described method. In order to determine the pH stability, 100 µl of purified enzyme was incubated for 30 min at 30 °C at different pH ranging from 3.0 to 5.0 in 100 mM sodium acetate buffer and pH 6.0 to 8.0 in 100 mM sodium phosphate buffer, and then the aliquots of 50 µl were assayed for residual enzyme activity.

Determination of kinetic parameters of dextransucrase

The kinetic constants were evaluated by measuring the initial reaction rate at different sucrose concentrations ranging from 1.5 to 450 mM. The data were used to create a Lineweaver–Burk plot, and K_m and V_{max} were analyzed from the plot.

Effect of ions and EDTA on dextransucrase activity

The effect of $CaCl_2$, $MgCl_2$, $CoCl_2$, $MnSO_4$, $FeSO_4$, $FeCl_3$ and EDTA addition (in concentrations between 0

and 10 mM) on the activity of the purified DS (16.7 U/mg, 0.47 mg/ml) was assessed. The assays were carried out at 30 °C for 20 min in 0.5 ml reaction mixtures containing salt or EDTA and sucrose (10%) in 20 mM sodium acetate buffer (pH 5.4), and 50 µl enzyme. After specified time the reaction was stopped, the enzyme activity was measured as described earlier, and expressed as residual activity in percentages.

Statistical analysis

All the experiments were carried out in triplicate and the presented data are mean values of three independent experiments with \pm standard deviation. Mean values of experiments were compared using analysis of variance. One-way ANOVA followed by Tukey test was applied to evaluate the effect of the investigated parameters. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of growth conditions on dextransucrase production

The production of DS is highly influenced by growth of bacterial cells and optimal condition for their growth. Considering that, the incubation temperature had prominent influence on DS production. In this set of experiments we tested temperature in the range from 17 to 37 °C under static condition. The maximum activity of 3.10 ± 0.09 U/ml was obtained at 23 °C (Fig. 1). The DS activity obtained at this temperature was significantly higher ($P < 0.05$) compared to the activities obtained at other tested temperatures. Incubation at 37 °C gave the lowest measured activity of 0.40 ± 0.12

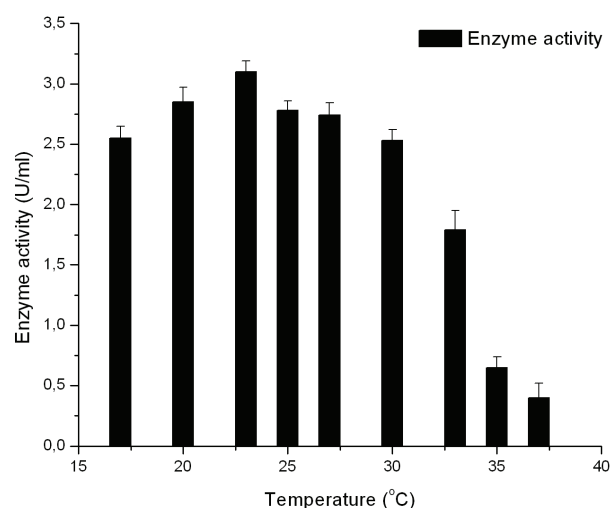


Figure 1. Dextransucrase activity of the cell free supernatant obtained by incubation of *Lc. mesenteroides* T3 at various temperatures and under static condition in enzyme production medium with 4% sucrose. The mean value of three independent experiments \pm standard deviation is presented.

U/ml. It was 87% lower than at 23 °C.

This is in accordance with other authors' results [25,26] and can be explained by deactivation of the enzyme at higher temperatures. The lowest incubation temperature of 17 °C reduced enzyme activity by 18% compared to 23 °C, which most likely reflects the slower metabolism. This was reported in our previous results for growth rate of *L. mesenteroides* T3 [17]. The aeration of production medium, by shaking at orbital shaker in range from 90 to 180 rpm at 23 °C, gave lower enzyme activity. It was observed that shaking at 90 rpm at 23 °C led to 12% less activity of DS (2.70 ± 0.12 U/ml) compared to the same temperature under static condition (3.1 ± 0.09 U/ml). Higher rpm produced even lower results. These results indicate the microaerophilic nature of the bacterium.

Effects of culture medium composition on dextransucrase production

Effect of sucrose concentration

DS from *Leuconostoc* sp. are known to be inducible enzymes [27]. Sucrose is the only known substrate that is able to induce this enzyme production [28]. The sucrose concentration in term of influence on DS production, ranging from 1 to 10% was studied. The original production medium (Tsuchiya *et al.* 1952) that contains 4% sucrose was used as a control. The maximum enzyme activity of 4.71 ± 0.13 U/ml was observed at 7% sucrose (Fig. 2). The DS activity obtained at this sucrose concentration was significantly higher ($P < 0.05$) compared to the activities obtained at other sucrose concentrations. The further increase of sucrose concentration led to decreasing of enzyme production. At 10% sucrose the enzyme activity was lower by almost 70% compared to the optimal concentration of 7% (Fig. 2).

This may be due to the binding of DS on dextran formed by released enzyme from excess sucrose. The similar results can also be found for other dextran-producing strains such as NRRL B-640 in research of Purama and Goyal [29]. The lower concentration of sucrose (5%) in the growth medium was the optimal for DS production by *Weissella confusa* Cab3 [30] and *Lactobacillus plantarum* DM5 [31].

Effect of nitrogen source

The effects of yeast extract, beef extract and peptone in various concentrations on DS production were studied. The concentration of nitrogen sources were varied from 0.5–4.0%. The results (Fig. 3) showed that the DS activity depend on source as well as on the concentration of nitrogen. The beef extract as a sole nitrogen source in a concentration of 2%, demonstrated the highest positive impact on DS, exhibiting an activity of 3.81 ± 0.04 U/ml, which was 15% higher than in the control medium containing 2.0% yeast extract.

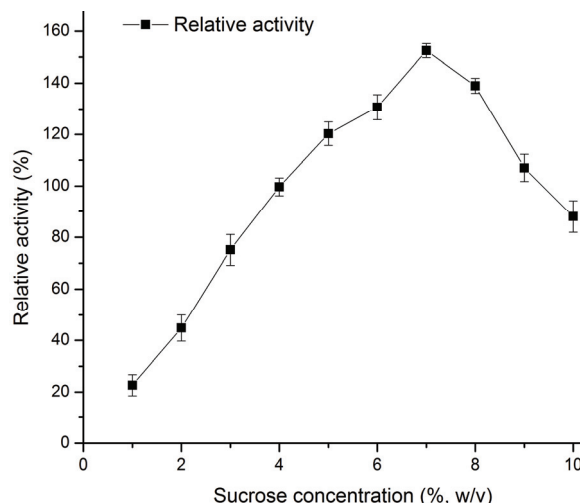


Figure 2. Dextransucrase activity of the cell free supernatant obtained by incubation of *Lc. mesenteroides* T3 with various concentrations of sucrose in enzyme production medium at 23 °C. The mean value of three independent experiments \pm standard deviation is presented.

Lower activity (3.64 ± 0.02 U/ml) was observed in medium with 1% of yeast extract, but statistically significantly lower was in medium with 2% of peptone as sole nitrogen source (2.28 ± 0.06 U/ml). The DS activity obtained with beef extract compared to the activity obtained with yeast extract as sole nitrogen source was not significantly higher ($P < 0.05$).

With the increase of the nitrogen concentrations beyond 2.0%, a slight decrease in the enzyme activity was observed regardless of nitrogen source used individually. However, the combination of some nitrogen sources with increased total concentration stimulates heightened DS activity.

Given that the 2% beef extract was the most effective nitrogen source, we tested its combinations with yeast extract and peptone, in concentrations in which they were the best as the sole nitrogen sources. Combining 2% peptone with 2% of beef extract enhanced activity for 31% compared with the control medium. Medium with 2% of beef extract and 1% of yeast extract exhibited activity of 4.52 ± 0.10 U/ml, which is 47 and 19% higher than in the control (2% of yeast extract) and in medium with 2% of beef extract respectively. This combination of nitrogen sources gave significantly higher ($P < 0.05$) result compared to any measured activity obtained from sole nitrogen sources (Supplementary file 1). Similar results were observed from other DS producing strains such as *Lc. mesenteroides* NRRL B-640 [29], *L. mesenteroides* PCSIR-3 [32] and *Lb. plantarum* DM5 [31], for which the combination of two nitrogen sources was better than either one sole source.

Effect of K_2HPO_4 and Tween 80

Phosphate is currently used in order to neutralize the effect of lactic acid produced by *L. mesenteroides*

T3 during the fermentation process. The effect of various concentrations (1 to 6%) of K_2HPO_4 in the fermentation medium on DS production was studied. Our results show that the maximum enzyme activity of 4.02 ± 0.08 U/ml was obtained at 3% K_2HPO_4 , which was significantly ($P < 0.05$) higher (for 28%) compared to control medium having 2% K_2HPO_4 (Fig. 4). However, the higher concentration (above 4%) of K_2HPO_4 did not support the enzyme activity. In fact, the concentration of 6% of K_2HPO_4 leads to decreasing of DS activity 54%.

Goyal and co-workers [33] found that 2.5% K_2HPO_4 was the optimal for DS production from *L. mesenteroides* NRRL B-512F. In addition, the effect of the Tween 80, known as surfactant that can enhance the production of some enzymes [34] on DS production was also studied. However, it was showed that the addition of Tween 80 in concentrations ranging from 0.1 to 1 vol.% in the test medium had no effect on DS production, which is in contradiction with results reported by other authors [31,33].

Dextranucrase purification

Extracellular DS obtained from cell free supernatant with specific activity of 0.56 U/mg and protein concentration of 5.7 mg/ml was precipitated by 25% PEG 400. After the first step of enzyme purification specific activity was 11.90 U/mg indicating 21-fold purification in a single step. This procedure of purification was then repeated, obtaining the enzyme with specific activity of 16.70 U/mg showing 30-fold purification. This enzyme was further characterized.

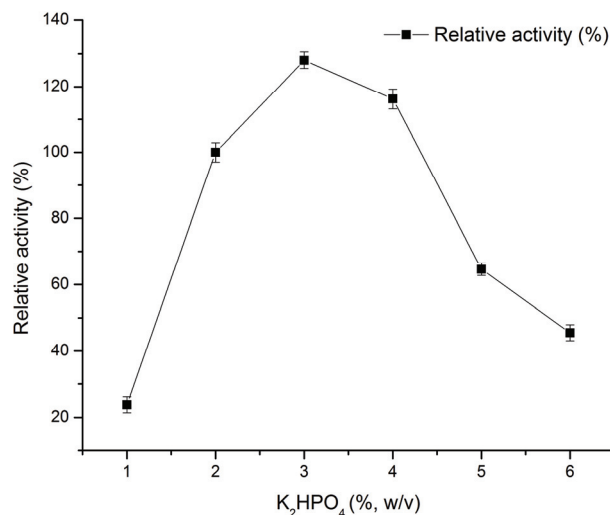


Figure 4. The dextranucrase activity of *L. mesenteroides* T3 cell free supernatant obtained after growing the bacterium in presence of various concentration of K_2HPO_4 in enzyme production media at 23 °C. The mean value of three independent experiments with \pm standard deviation is presented.

SDS-PAGE analysis and activity staining of dextranucrase

The molecular weight of the purified enzyme (25% PEG 400) was determined by 7.5% SDS polyacrylamide gel under denaturing condition and showed one isoform of approximately 180 kDa with Coomassie brilliant blue G-250 staining (Fig. 5, lanes 1 and 2). The purified enzyme was also run on SDS-PAGE gels under non-denaturing condition for the *in situ* activity detection of DS by PAS staining. The PAS staining of the gel showed

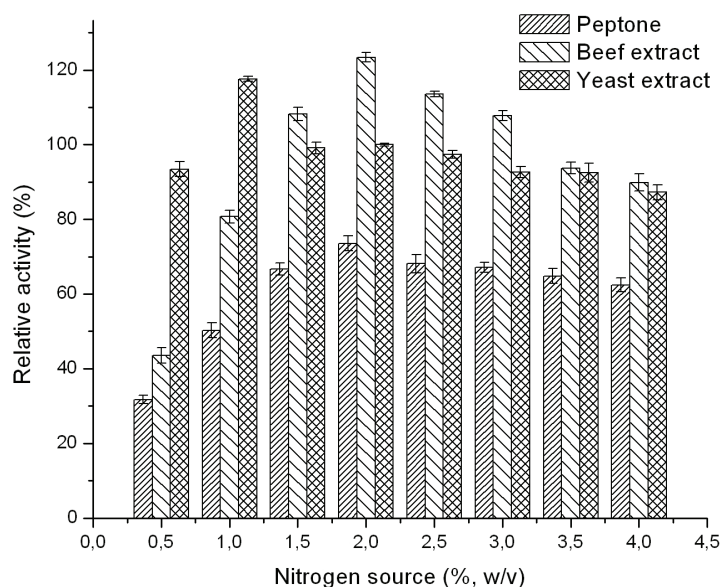


Figure 3. The dextranucrase activity of *L. mesenteroides* T3 cell free supernatant obtained after growing the bacterium in presence of various nitrogen sources in enzyme production media at 23 °C. The mean value of three independent experiments \pm standard deviation is presented.

one activity band of molecular weights of approximately 180 kDa when incubated in 10% sucrose solution (Fig. 5, lane Z). It has been reported that the molecular weight of extracellular glucansucrase was in the range of 120–200 kDa [22].

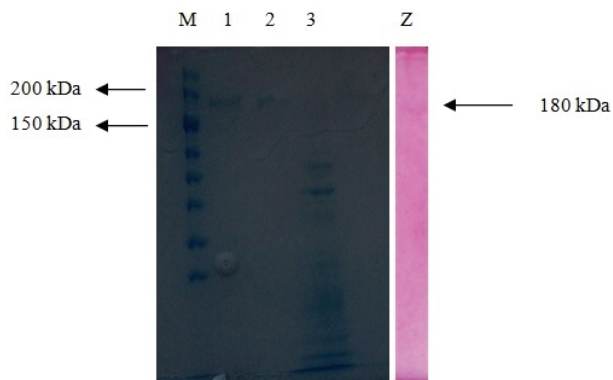


Figure 5. SDS-PAGE and zymogram of the dextransucrase activity of the purified enzyme. Lane M: protein molecular mass marker (60 to 250 kDa) from Thermo Scientific; lane 1: purified dextransucrase after two precipitation steps with 25% PEG 400 fractionation under denaturing condition; lane 2: purified dextransucrase after one precipitation steps with 25% PEG 400 fractionation under denaturing condition; lane 3: crude bacterial supernatant; Z: zymogram of the dextransucrase activity of the purified enzyme by 25% PEG 400 fractionation (PAS staining) of glucan formed using sucrose as substrate.

Optimum temperature and thermal stability of dextransucrase

The purified DS showed maximum activity within the temperature range of 30–33 °C with a specific acti-

vity of ~16.70 U/mg at pH 5.4 in 20 mM sodium acetate buffer (Fig. 6).

This result is supported by the findings that optimum temperature for DS activity from *L. mesenteroides* B-512F [35], *L. dextranicum* NRRL B-1146 [36] and *Pediococcus pentosaceus* [14] ranges between 30–35 °C. The results for DS thermal stability showed that enzyme was stable at temperatures lower than 30 °C, whereas with the increase of temperature above 35 °C the stability was rapidly lost, indicating the denaturation of the enzyme.

Optimum pH and pH stability of dextransucrase

The maximum DS activity was observed at pH 5.4 with a specific activity of 16.70 U/mg, although it was not significantly different from activity at pH 5.2 which was 16.0 U/mg (Fig. 7). A 4% reduction in the activity was observed at pH 5.2, and 6% at pH 5.6. Higher loss of the enzyme activity of 35 and 83% occurred at pH 6.4 and pH 4.0, respectively (Fig. 7).

The optimum pH for DS production from our strain is in correlation with other reports for glucansucrases from various lactic acid bacteria *viz.* *L. mesenteroides* NRRL B-640 [29], *W. confusa* Cab3 [30] and *Lb. plantarum* DM5 [31]. The enzyme was stable in pH (4.5–6.0) range similar to other glucansucrases from *L. dextranicum* NRRL B-1146 [36] and *P. pentosaceus* SPA [14].

Determination of kinetic parameters of dextransucrase

Kinetic constants K_m and V_{max} were obtained by measuring the initial reaction rate in a wide range of sucrose concentrations (Supplementary material). The

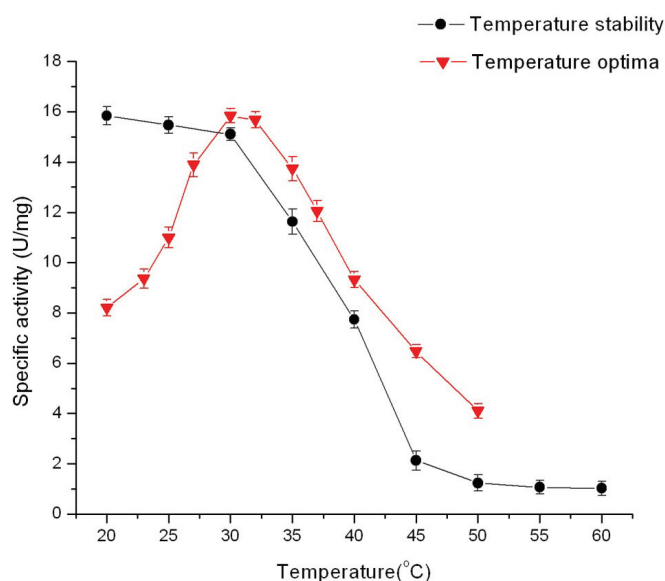


Figure 6. Effect of temperature on activity (—▼—) and stability (—●—) of dextransucrase. The enzyme activity measurements for temperature optima were performed in temperature (20–50 °C) range. For stability studies, the enzyme was incubated at different temperatures (20–60 °C) for 30 min. The assay was performed in optimum conditions and the data are mean values of three independent experiments with \pm standard deviation.

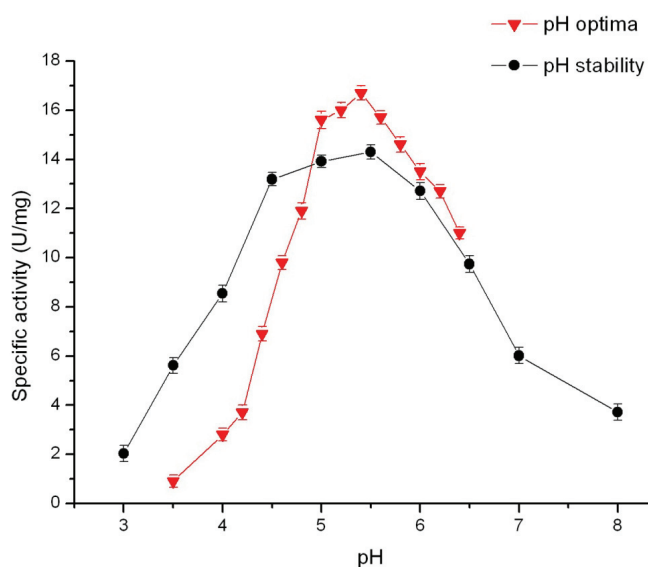


Figure 7. Effect of pH on activity (—▼—) and stability (—●—) of dextransucrase. The enzyme activity measurements for pH optimum were performed in pH (3.5–6.5) range. For stability studies, the enzyme was incubated at different pH (3.0–8.0) for 30 min. The assay was performed in optimum conditions and the data presented are mean values of three independent experiments with \pm standard deviation.

enzyme exhibited Michaelis–Menten type kinetic behavior. The K_m value was 0.014 M which is similar to that reported on other *Leuconostoc* DSs (0.026 and 0.015 M) [37,38].

Effect of salts and EDTA on dextransucrase activity

The metal ions, Ca^{2+} , Mg^{2+} , Fe^{2+} and Co^{2+} , in low concentrations caused a marginal increase of DS activity, whereas the addition of Mn^{2+} had a significant effect (Table 1). The addition of 1 mM CaCl_2 , MgCl_2 , FeSO_4 and CoCl_2 to the DS caused an improvement of enzyme activity by 6, 8, 14 and 12%, respectively. The slight enhancement of DS activity achieved with the addition of divalent ions has been observed in other studies [31,36,39]. This effect has been attributed to the stabilization effect of the divalent ions on the tertiary enzyme structure [36,40].

Table 1. Effect of salts on the activity of purified dextransucrase from *L. mesenteroides* T3

Salt	Residual activity ^a , %	
	Maximum	Minimum
CaCl_2	106.2 \pm 2.1 (1 mM)	67.2 \pm 1.4 (9 mM)
MgCl_2	108.1 \pm 1.1 (1 mM)	85.3 \pm 1.6 (10 mM)
CoCl_2	112.1 \pm 1.1 (1 mM)	67.4 \pm 1.6 (10 mM)
MnSO_4	172.9 \pm 2.1 (4 mM)	125.7 \pm 1.2 (10 mM)
FeSO_4	112.3 \pm 1.3 (1 mM)	38.9 \pm 1.2 (9 mM)
FeCl_3	98.0 \pm 1.2 (1 mM)	7.9 \pm 1.0 (5 mM)
EDTA	83.8 \pm 0.9 (1 mM)	46.2 \pm 0.8 (9 mM)

^aThe mean values of three independent experiments are presented with \pm standard error

The addition of Mn^{2+} in a wide range of concentrations had a significant activation effect on the studied DS. The highest measured enzyme activity was 73% higher than the activity of the enzyme without ions (control), and was achieved by the addition of 4 mM MnSO_4 . Further addition of MnSO_4 had a less prominent positive effect, but even in the concentration of 10 mM the effect of Mn^{2+} was not inhibitory. These results contradict the results of many authors in which Mn^{2+} shows a negative effect on enzyme activity [31,36,41]. There is only one report about slightly positive impact of 1 mM Mn^{2+} on DS from *L. mesenteroides* CGMCC 1.544 [42]. Majumder and Goyal [43] showed that Mn^{2+} addition in the culture medium had a positive effect on DS production, but there was no literature data that this ion had positive effect on enzyme activity. This reproducibly detected effect shows an unusual ion specificity of the DS from our strain. Being manganese, the trace elements makes DSs from *L. mesenteroides* T3 suitable candidate for use in the food industry.

Among all tested cations only Fe^{3+} was inhibitory in all tested concentrations, while the concentrations higher than 5 mM caused a complete loss of enzyme activity. Kobayashi and Matsuda [41] and Majumder *et al.* [43] observed the same negative effect of Fe^{3+} on DS activity.

EDTA at all concentrations displayed a denaturing effect on DS, and 54% of enzyme inactivation was observed with 9 mM EDTA (Table 1). Similar result was also found in the case of glucansucrase from *Lactobacillus plantarum* DM5 [31] for effects of EDTA.

CONCLUSION

This study shows a thorough technological characterization of conditions for production of DS using a novel strain *L. mesenteroides* T3. The production of enzyme could be enhanced by combination of 2% beef extract and 1% yeast extract as nitrogen source. The optimal production was observed without shaking and at 23 °C. The enzyme was enriched (purified) by double precipitation with 25% PEG 400. Active subunits of the enzyme were identified in SDS PAGE experiment and showed that it was composed of one subunit of size 180 kDa. The purified DS exhibited maximal activity at 30 °C and pH 5.4, and was stable in acidic pH and at low temperatures. The enzyme could be stabilized with addition of any divalent cation but the addition of Mn²⁺ had a significant effect on the enzyme activity, which makes it the unique enzyme in its class. The data obtained from the biochemical characterization of DS can highly contribute to the technological process of dextran production and its utilization in different branches of industry, particularly food industry.

Supplementary material

Supplementary data are available from corresponding author on request.

Acknowledgements

The financial support for this investigation given by Ministry of Science, Education and Technological Development of the Republic of Serbia under the project TR 31035 is gratefully acknowledged.

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IZVOD

KARAKTERIZACIJA DEKSTRANSAHARAZE IZ BAKTERIJE *Leuconostoc mesenteroides* T3, PRIRODNOG IZOLATA IZ ZRNA VODENOG KEFIRA

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(Naučni rad)

Dekstransaharaza (DS) je ekstracelularni enzim koji pripada klasi glukozil-transferaza i uglavnom ga proizvode mikroorganizmi iz porodice *Lactobacillaceae* i *Streptococcaceae*, posebno vrste rodova *Lactobacillus*, *Leuconostoc* i *Streptococcus*. U radu je ispitana produkcija DS od strane bakterije *Leuconostoc mesenteroides* T3, izolovane iz zrna vodenog kefira. Optimizovana je hranljiva podloga za produkciju enzima i enzim je okarakterisan. Aktivnost sirovog enzima u bakterijskom supernatantu nakon gajenja kulture u klasičnoj Čučijinoj podlozi na 23 °C pod statičkim uslovima je bila 3.1 U/ml. Ova podloga je korišćena kao kontrola sa kojom su poređeni svi rezultati dobijeni u eksperimentima optimizacije. Povećanjem koncentracije saharoze na 7% enzimski aktivnost je povećana 52%. U podlozi sa 2% mesnog i 1% kvašćevog ekstrakta izmerena je aktivnost od 4.52 U/ml, što je 47% više u poređenju sa kontrolom (koja sadrži 2% kvašćevog ekstrakta). Povećanjem koncentracije K₂HPO₄ sa 2 na 3% dovelo je do porasta DS aktivnosti za 28%. Enzim je prečišćen taloženjem pomoću polietilen glikola 400 i utvrđeno je da pokazuje najveću aktivnost na temperaturi 30 °C i na pH vrednosti 5.4. Zimogramom je potvrđeno prisustvo DS od oko 180 kDa. Dodatak dvovalentnih katjona, Ca²⁺, Mg²⁺, Fe²⁺ i Co²⁺, dovelo je do manjeg povećanja DS aktivnosti, dok je veći pozitivan uticaj (od čak 73%) imao jedino dodatak Mn²⁺. Ovi nalazi klasifikuju deksransaharazu iz bakterije *Leuconostoc mesenteroides* T3 kao obećavajućeg kandidata za proizvodnju dekstrana, koji ima brojne primene u različitim industrijama.

Ključne reči: Bakterije mlečne kiseline • *Leuconostoc mesenteroides* • Dekstransaharaza • Dekstran