Production and properties of dextransucrase by free and immobilized cells of *Leuconostoc paramesenteroides*

Ahmed F. Abdel Fattah[a], Amal M. Hashem[a], Mona A. El-Refai[a] and Hassan M. Gebreel[b]

[a]Department of Natural and Microbial Products Chemistry, National Research Center, Dokki and [b]Faculty of Sciences, Department of Microbiology, Ain Shams University, Cairo, Egypt

Correspondence to Amal M. Hashem, MD, Department of Natural and Microbial Products Chemistry, National Research Center, Tahrir Street, Dokki, 12311, Cairo, Egypt
Tel: +20 106 255 9809; fax: +20 233 370 931; e-mail: amal_mhashem@yahoo.com

Received 2 October 2011
Accepted 5 February 2012

Egyptian Pharmaceutical Journal 2012, 11:42–48

Six bacterial strains were tested for the production of dextransucrase. The highest dextransucrase activities were produced by local strain *Leuconostoc paramesenteroides* after 24 h in static culture conditions. Dextransucrase was induced using sucrose (10 g%) as a carbon source and corn steep liquor (3.5 ml%) as a nitrogen source. Fe^{2+} favoured enzyme production. Cells of *L. paramesenteroides* were immobilized using three supports, calcium alginate, agar and κ-carrageenan. Calcium alginate beads were able to produce dextransucrase for four runs with 100% activity. The optimum temperature for enzyme reaction ranged from 30 to 65°C at pH 5.4. In the absence of substrate, the enzyme retained 100% activity for 30 min at 40°C and 69% activity for 10 min at 60°C.

Keywords:
cell immobilization, dextransucrase properties, *Leuconostoc*

Egypt Pharm J 11:42–48
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1687-4315

Introduction

Dextrans are a class of bacterial polysaccharides whose molecular structure is composed exclusively of monomeric α-D-glucopyranosyl units, linked mainly by (α,1-6) glucosidic bonds [1].

Dextransucrase (sucrose: 1,6-α-D-glucan 6-α-D-glucosyltransferase) (enzyme code 2.4.1.5) that polymerizes the glucosyl moiety of sucrose to form dextran, polymer an α-(1→6)-linked glucan with α-linked branches [2,3]. It is a member of a family of glucosyltransferases and fructosyltransferases, also called glucansucrases and fructansucrases, synthesized by the species *Leuconostoc* and *Streptococcus* [2].

Dextransucrase is an industrially important enzyme because of its synthesis of dextran, which has many important industrial and medical uses. It is also important because of its theoretical and practical aspects in understanding the mechanism of glucan synthesis and its ability to synthesize a wide variety of oligosaccharides by glucosyl transfer reactions to acceptors [4].

Many different species of the genera *Leuconostoc*, *Lactobacillus* and *Streptococcus* have been known to synthesize extracellular dextransucrase under appropriate growth conditions; the dextran product (and hence the enzyme) is strain specific. For example, when grown on sucrose, *Leuconostoc mesenteroides* releases the enzyme dextransucrase, which polymerizes the remaining sucrose to dextran with molecular weights of several million [5]:

$$n C_{12}H_{22}O_{10} \xrightarrow{\text{dextransucrase}} (C_6H_{12}O_6)_n + n(C_6H_{12}O_6)$$

sucrose → dextran + fructose

The present work was undertaken to investigate, by culturing, the pattern of dextransucrase activities in extracellular bacterial enzyme preparations under different conditions. Some properties of the enzyme produced were also studied.

Subjects and methods

Microorganisms: *L. mesenteroides* NRRL 1400, *Bacillus megaterium* NRRL 14308, *Lactobacillus acidophilus* NRRL 4495 and *Aeromonas hydrophila* NRRL 1358 were obtained from the US Department of Agriculture NRRL (Peoria, Illinois, USA).

Bacillus spp. was obtained from Culture Collections of the National Research Centre (Dokki, Cairo, Egypt). *Leuconostoc paramesenteroides* was isolated from sugarcane and was identified at the Microanalytical Centre at the Faculty of Sciences, Cairo University.

Media and culture conditions

Leuconostoc was maintained and preserved on different media according to the methods described by Jeanes [6] by three serial transfers on medium A, and then transferred to medium B.

Medium A: Deep liver broth contains 10 ml of liver extract, 0.5 g of Difco yeast extract, 1 g of Difco tryptone, 0.2 g of dipotassium hydrogen phosphate and 0.5 g of glucose, per 100 ml of distilled water. The pH was adjusted to 7.4. A few liver particles were placed in each 16 × 150 mm rimless test tube and sterilized at 121°C for 15 min.

Medium B: It had the same composition as the maintenance medium A, except that glucose was replaced

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with sucrose (10%). Agar (2%) was added while liver particles were omitted.

Production medium

The basal medium for inoculum and enzyme production contained a final concentration of the following in g/l: sucrose, 100; magnesium sulphate heptahydrate, 0.2; yeast extract, 2.5; and dipotassium hydrogen phosphate, 5. The phosphate was sterilized separately and added aseptically to the cold sterile solution of the other ingredients. The initial pH of the sterile mixture was about 7.0 [6].

Fermentation

Cultivation was carried out in 250 ml Erlenmeyer flasks, each containing 100 ml of the production medium, and sterilized for 15 min at 121°C (1.5 atm).

The stock culture was used to prepare preinoculum by inoculating a 250 ml Erlenmeyer flask containing 100 ml of medium. The flask was incubated at 25°C on a rotary shaker (120 rpm) for 24 h. Ten percent inoculum of the resulting growth was used to inoculate fresh production medium flasks and incubated under a static and shaking condition for 24, 48 and 72 h at 25°C. When fermentation was completed, the liquor for the whole fermentation period was divided into two parts. One part of the viscous culture fluid was diluted with an equal volume of distilled water, treated with two volumes of ethanol and centrifuged in a cooling centrifuge at 6000 rpm for 10 min to precipitate the bacterial cells. The centrifugate solution was further treated with ethanol to precipitate dextran as described by Jeanes [6]. The second part of the fermentation liquor was diluted with an equal volume of distilled water and centrifuged in a cooling centrifuge at 6000 rpm for 10 min to remove the bacterial cells, and dextransucrase and protein were assayed in the supernatant.

Analyses

Enzyme assay was carried out according to the method described by Girard and Legoy [7] and dextransucrase activity was determined by measuring the initial rate of fructose biosynthesis using the dinitrosalicylic acid method.

Determination of total sugars

Carbohydrate contents in sugar beet molasses and sugarcane molasses were determined using the phenol sulphuric acid method described by Dubois *et al.* [8]. Sugar beet molasses and sugarcane molasses were obtained from Egyptian Company for Sugar and Distillation (Hawamdia, Egypt).

Determination of protein

Protein concentration was estimated according to the method described by Lowery *et al.* [9].

Optimization of culture conditions

The effect of addition of various carbohydrates (sucrose, maltose, glucose, lactose, sugarcane molasses, and sugar beet molasses), organic and inorganic nitrogen (peptone, corn steep liquor, urea, Baker's yeast, soya bean and sodium nitrate) and some additives ($CaCl_2$, $ZnCl_2$, $FeSO_4 \cdot 7H_2O$, NaCl, $MnSO_4 \cdot H_2O$ and wheat bran) were evaluated for enzyme yield.

Cultivation was carried out at 25°C under static conditions and the incubation periods ranged from 24 to 72 h. The experiments were conducted in triplicate, and the results were the average of three independent trials.

Immobilization of whole bacterial cells

Cells were encapsulated in beads of the immobilization matrix. These beads were easily transferred to new medium after the first fermentation was completed, and so the same inocula were used continuously. Immobilization of cells was carried out on calcium alginate, agar, κ-carrageenan, glass wool, 100% wool and glass beads.

Calcium alginate beads

The fermentative inoculum (24 h) was prepared. Bacterial cells were entrapped in 1, 2 and 3% calcium alginate gel beads as described by Woodward [10]. Briefly, sodium alginate was prepared by dissolving 1, 2 or 3 g in 50 ml of distilled water and then autoclaving at 108°C for 10 min; 50 ml of bacterial suspension was added to the sterile alginate solution to obtain the final concentration; 20 ml of the alginate bacterial cell mixture was drawn using a sterile syringe and allowed to drop through the needle into a 250 ml Erlenmeyer flask containing 100 ml of sterile 2% $CaCl_2$ as a cross-linking agent to obtain spherical beads of calcium alginate gel entrapping the bacterial cells. The beads were left in the calcium chloride solution for 1 h and then washed several times with sterile saline solution (0.85% NaCl). The beads obtained from 20 ml alginate were added to 100 ml sterile medium in a 250 ml Erlenmeyer flask to give the final concentration of 10% and incubated at 25°C in a rotary shaker at 70 rpm. At the end of the fermentation period (24, 48, 72 h) the culture was decanted and the supernatant was investigated for dextransucrase activity. The beads were washed several times with sterile saline solution and transferred to a new sterile medium under sterile conditions.

Agar beads

Various concentrations of agar were prepared to give final agar concentrations of 1.5–3.0% after the addition of the inoculum [11]. Thereafter, 0.15, 0.2, 0.25 and 0.3 g of agar were each dissolved in 5 ml of distilled water, sterilized and cooled to ~40°C, and 10 ml bacterial inoculum was added to each flask. When it had solidified it was cut into equal-sized pieces and 100 ml of sterile medium was added to a 250 ml Erlenmeyer flask and incubated for 24, 48 and 72 h at 25°C in a rotary shaker at 70 rpm. At the end of the fermentation period, the culture was decanted and the supernatant was assessed for dextransucrase activity. The beads were washed several times with sterile saline solution and then transferred to a new sterile medium under sterile conditions until the dextransucrase activity had decreased.

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κ-carrageenan

The wet cells obtained from 10 ml culture of *L. paramesenteroides* were mixed with 10 ml of 3% κ-carrageenan at 45°C [12]. The mixture was quickly cooled at 4°C and cut into $2 \times 2 \times 2\,cm^3$ fragments. The beads obtained from 10 ml gel were added to 100 ml medium. At the end of the fermentation period, the culture was decanted and the supernatant was assayed for dextransucrase activity.

Immobilization by adsorption

Different solid supports were tested for their ability to immobilize bacterial cells. These supports were glass wool, 100% wool and glass beads. The wet cells obtained from 10 ml culture of *L. paramesenteroides* were suspended in 10 ml distilled water and added to 1 g of each of the tested sterile supports.

Each adsorption process was carried out in 250 ml Erlenmeyer flasks. The mixture in each flask was left for 1 h and then decanted; 100 ml of sterile culture medium was added to the loaded support and incubated for 24 h at 25°C in a rotary shaker at 70 rpm/min.

At the end of the fermentation period, the culture was decanted and the supernatant was investigated for dextransucrase activity.

Repeated batch experiments

This was carried out in 250 ml Erlenmeyer flasks each containing 100 ml of fermentation medium. Immobilized cells were added to each flask. Fermentation was conducted at 25°C for 24 h under shaking conditions (70 rpm).

Results and discussion

The results showed that *L. paramesenteroides* produced the highest dextransucrase activity (42 U/ml) after 24 h in static culture. In contrast, *L. mesenteroides* NRRL 1400 produced 42 U/ml in static and shaken culture after 24 h (Table 1).

The level of the protein content of the supernatant was not a criterion for the productivity of the dextransucrase activity. *L. paramesenteroides* was selected in the succeeding studies as the most promising dextransucrase producer.

Table 1 Survey of some bacterial cultures for the biosynthesis of dextransucrase using submerged and static techniques at different incubation periods

		Protein content of culture filtrate (mg/ml)		Dextransucrase activity (U/ml)	
Bacterial cultures	Incubation period (h)	Static	Submerged	Static	Submerged
Leuconostoc paramesenteroides	24	2.5	2.6	42.0	24
	48	2.2	2.0	29.0	34
	72	2.0	2.0	20.3	23.8
Leuconostoc mesenteroides NRRL 1400	24	5.0	2.5	42.0	42.0
	48	2.2	2.3	30.0	29.0
	72	2.2	1.9	30.0	20.0
Bacillus megaterium NRRL 14308	24	0.84	1.2	15.2	40.0
	48	1.1	1.1	2.0	12.6
	72	1.0	1.1	NC	12.6
Aeromonas hydrophila NRRL 1358	24	1.0	0.78	6.3	6.3
	48	1.0	0.97	3.2	12.6
	72	0.86	0.97	NC	12.6
Bacillus spp.	24	0.74	1.06	3.2	3.2
	48	0.92	1.88	3.2	12.6
	72	0.8	0.88	NC	12.6
Lactobacillus acidophilus NRRL 4495	24	0.84	0.84	NC	NC
	48	0.72	0.84	NC	NC
	72	0.66	0.84	NC	NC

Initial pH = 7.0.
Temperature = 25°C.
NC, no activity.

Table 2 Effect of different carbon sources on the biosynthesis of dextransucrase by *Leuconostoc paramesenteroides*

Carbon source	Protein content of culture filtrate (mg/ml)	Dextransucrase activity of culture filtrate (U/ml)	Conversion of glucose to dextran (%)
Sucrose (control)	2.5	42	50
Maltose	1.7	11.9	10
Glucose	1.6	26.6	10
Lactose	1.85	8.4	1.5
Sugarcane molasses	3.75	NC	No dextran
Sugar beet molasses	3.4	NC	No dextran

Incubation period 24 h, on equivalent carbon base.
Temperature = 25°C.
Initial pH = 7.0.
NC, no activity.

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Sucrose (100 g/l) was replaced in the basal medium with equivalent quantity of various carbon sources (100 g/l as carbon source) and tests were carried out to determine the productivity of these sources (Table 2). Dextransucrase was produced with high activity (41.2 U/ml) when sucrose was used as carbon source compared with other tested carbon sources.

Other authors have indicated the constitutive nature of dextransucrase [5,13–18]. However, it was reported by Padmanabhan and Kim [5] that dextransucrase was highly induced by 2% sucrose.

The concentration of sucrose in the culture medium had a significant effect on the production of dextransucrase, and concentration of 10–12% gave the highest enzyme yield (Fig. 1). Higher sucrose concentration (20%) inhibited the enzyme yield to 48.5%, which was because of the increase in the viscosity of the culture medium. Similar results were obtained by Lazic *et al.* [13] for production of dextransucrase by *L. mesenteroides* when medium containing 20% sucrose was used.

Kitaoka and Robyt [19] found that dextransucrase concentration was the highest in the culture medium containing 2.5% sucrose or 1.8% glucose.

Cortezi *et al.* [17] reported that *L. mesenteroides* FTO45B produced dextransucrase when grown in medium containing 4% sucrose. Also, Purama and Goyal [18] reported that dextransucrase was produced in medium containing 3% sucrose.

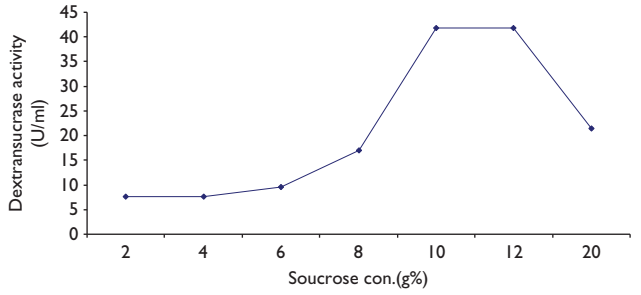
To study the effect of the nitrogen source on dextransucrase production, sucrose (100 g/l) was used as the carbon source.

Yeast extract from the original culture medium was eliminated and equivalent N-basis organic (peptone, corn steep liquor, urea, baker's yeast, soya bean) and inorganic (sodium nitrate) nitrogen sources were used.

Results in Table 3 indicated that corn steep liquor was the best nitrogen source and gave 42 U/ml dextransucrase activity compared with other organic and inorganic nitrogen sources tested. The effect of different concentrations of corn steep liquor was also studied, ranging from 3 to 5.5% (Fig. 2). The results indicated that 3.5% corn steep liquor yielded the highest dextransucrase activity (42 U/ml); increasing the concentration up to 5.5% decreased enzyme activity by 22.7% compared with control (3.5%) of corn steep (control) gave the highest enzyme activity. The results are in accordance with those of Rodrigues *et al.* [16] who found that *L. mesenteroides* 512 F grows in medium containing yeast extract or corn steep as nitrogen source.

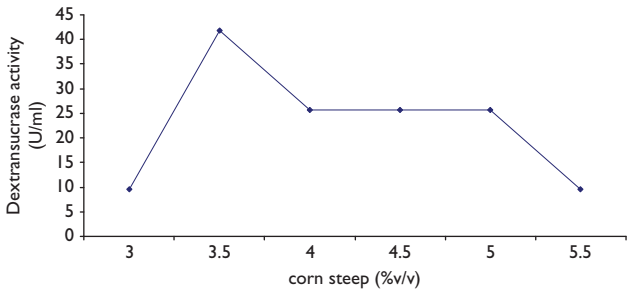
Dols *et al.* [15] reported that *L. mesenteroides* NRRL B-1299 grown in medium containing 2% yeast extract was capable of producing dextransucrase enzyme. In contrast,

Figure 1



Effect of different concentrations of sucrose on the biosynthesis of dextransucrase by *Leuconostoc paramesenteroides.*

Figure 2



Effect of different concentrations of corn steep liquor on the biosynthesis of dextransucrase by *Leuconostoc paramesenteroides.*

Table 3 Effect of different nitrogen sources on the production of dextransucrase by *Leuconostoc paramesenteroides*

Nitrogen source	Protein content of culture filtrate (mg/ml)	Dextransucrase activity of culture filtrate (U/ml)	Conversion of glucose to dextran (%)
Yeast extract[a] (control)	2.5	41.2	50
Peptone	3.5	34.9	26.4
Corn steep liquor	2.15	41.8	50
Urea	0.75	NC	No dextran
Baker's yeast	2.0	36.6	37
Soya bean	1.17	39	40
Sodium nitrate	1.0	16	22

Temperature = 25°C.
Incubation period = 24 h.
Sucrose concentration = 10%.
NC, no activity.
[a]Control = 0.25% yeast extract.

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Table 4 Effect of the addition of different additives to the culture medium on the biosynthesis of dextransucrase by *Leuconostoc paramesenteroides*

Additive concentration (g%)	Protein content of culture filtrate (mg/ml)	Dextranscurase activity of culture filtrate (U/ml)	Conversion of glucose to dextran (%)
None (control)	2.15	41.8	50
$CaCl_2$ (0.01)	2.35	16.8	16
$ZnCl_2$ (0.01)	2.10	25.6	28
$FeSO_4 \cdot 7H_2O$ (0.01)	2.90	46.8	60
NaCl (0.01)	1.85	27.9	29
$MnSO_4 \cdot H_2O$ (0.01)	2.35	16.8	16
Wheat bran (1.0)	2.85	20.3	26

Temperature = 25°C.
Incubation period = 24 h.
Sucrose concentration = 10%.
Nitrogen source: corn steep liquor (3.5%).

Table 5 Production of dextransucrase by immobilized cells of *Leuconostoc paramesenteroides* on calcium alginate beads

Calcium alginate (g%)	Period of incubation (h)	Protein content (mg/ml)	Dextransucrase activity (U/ml)
Control (free cells)	24	3	45.7
2	24	2.8	28
	48	2.5	14.6
	72	2.5	4.8
2.5	24	2.50	20
	48	2.75	15.8
	72	2.70	14.2
3	24	2.5	14.2
	48	2.7	14.2
	72	2.5	12.8
Repeated batch of 2% calcium alginate after 24 h			
		Protein content (mg/ml)	Dextransucrase activity (U/ml)
24	1	2.8	28
	2	2.8	28
	3	2.8	28
	4	3	8

Table 6 Production of dextransucrase by immobilized *Leuconostoc paramesenteroides* on agar beads

Agar concentration (g%)	Incubation period (h)	Protein content (mg/ml)	Dextransucrase activity (U/ml)
Control (free cells)	24	3	45.7
0.15	24	3	17.2
	48	5.4	11.4
	72	5.4	6.8
0.2	24	4.0	10.0
	48	4.5	10.4
	72	4.7	6.8
0.25	24	2.8	5.1
	48	2.8	3.4
	72	4.5	3.4
0.3	24	2.8	5.1
	48	2.8	3.4
	72	3.4	3.4
Repeated batch of 0.15% agar after 24 h			
		Protein content (mg/ml)	Dextransucrase activity (U/ml)
24	1	3	16
	2	3	5.2

Table 7 Production of dextransucrase by immobilized *Leuconostoc paramesenteroides* on glass wool

Concentration of carrier [glass Wool (% w/v)]	Incubation period (h)	Protein content (mg/ml)	Dextransucrase activity (U/ml)
Control (free cells)	24	3	45.8
1	24	2.8	20.6
	48	3.0	27.0
	72	3.3	14.3
Repeated batch after 24 h of 1 g wool			
		Protein content (mg/ml)	Dextransucrase activity (U/ml)
24	1	4	20.6
	2	4	20.6

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Purama and Goyal [18] reported that *L. mesenteroides* produced dextransucrase when grown in medium containing 1.8% yeast extract and 0.5% beef extract.

The effect of addition of wheat bran and some metal ions at different concentrations to the culture medium on dextransucrase productivity is shown in Table 4. $Fe(So_4)_7 \cdot H_2O$ at 0.001% concentration markedly increased dextransucrase activity by 12% to reach 46.8 U/ml compared with control.

The obtained results agreed with those reported for other *L. mesenteroides* species [17,20,21].

The results in Table 5 indicate that the bacterial cells immobilized on 2% calcium alginate gave the highest enzyme yield (28 U/ml) after 24-h incubation. The immobilized cells achieved considerable enzyme productivity up to the fourth cycle (Table 5). At each cycle the enzyme productivity was achieved for 24 h.

Agar concentrations were used for beads of 1.5, 2, 2.5 and 3% concentration, and the density of cells was kept constant. At all concentrations the highest dextransucrase yield obtained after 24 h (Table 6) using 1.5% agar beads afforded the highest dextransucrase yield (17.2 U/ml). Repeated batch fermentation was carried out for two runs only, but after three runs the activity decreased rapidly. This may have been because of leakage of enzyme from the agar. On immobilization of cells of *L. paramesenteroides* by glass wool, dextransucrase activity was optimum after 24 h, which was equal to that of free cells (45.8 U/ml); the immobilized cells lost 53% of its productivity in the third cycle (Table 7).

When κ-carrageenan, natural wool, synthetic sponge and glass beads were used, the immobilization did not succeed in producing as highly active dextransucrase as when agar or calcium alginate was used.

Table 8 Effect of temperature on the activity of free dextransucrase produced by *Leuconostoc paramesenteroides*

Temperature of the reaction (°C)	Specific activity of the free dextransucrase (U/mg protein)
20	11.0
25	15.2
30 (control)	15.2
35	15.2
40	15.2
45	15.2
50	15.2
55	15.2
60	15.2
65	15.2
70	9.6
75	6.0

PH = 5.4.
Time of the reaction = 10 min.

Table 9 Effect of different pH values of the reaction on the activity of free *Leuconostoc paramesenteroides*

pH value	Specific activity of the free dextransucrase (U/mg protein)
4	8.0
4.5	15.2
5	15.2
5.4 (control)	15.2
5.5	15.2
6	10.2
6.5	10.2
7	4.6

Temperature = 30°C.
Time of the reaction = 10 min.

Properties

Effect of temperature on the activity of dextransucrase produced by *Leuconostoc paramesenteroides*

The enzyme reaction was carried out at various temperatures. As shown in Table 8 the optimum temperature for the enzyme activity was 25–65°C and activity decreased to 63.2% at 70°C.

There are substantial commercial advantages in carrying out enzymatic reactions at higher temperatures [22]. Enzymatic digestion at increased temperatures (> 60–65°C) may reduce microbial contamination of the material being processed. In addition, higher temperatures increase the rate of substrate digestion and increase the solubility of the polymeric substrates, such as carbohydrates, rendering them more amenable to enzymatic attack [23].

Effect of pH on the activity

Under assay conditions, enzymes from *L. paramesenteroides* showed maximal activity at pH 5.4 (Table 9).

Effect of temperature on stability

Thermal stability of the enzyme solution was investigated by incubating the enzyme in the absence of its substrate

Table 10 Thermal stability of free dextransucrase prepared from *Leuconostoc paramesenteroides*

	Time of exposure (min)							
	5	10	15	20	25	30	45	60
Temperature (°C)	Specific activity (U/mg protein)							
40	15.2	15.2	15.2	15.2	15.2	15.2	7.6	NC
45	15.2	15.2	15.2	15.2	15.2	4.2	NC	NC
50	15.2	15.2	5.5	NC	NC	NC	NC	NC
55	15.2	12.5	3.5	NC	NC	NC	NC	NC
60	15.2	10.5	1.6	NC	NC	NC	NC	NC
70	1.5	1.5	NC	NC	NC	NC	NC	NC

NC, no activity.

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at various temperatures between 40 and 70°C in a water bath at different time periods up to 60 min, and the residual activities were determined under standard conditions (pH 5.4, 30°C). No activity loss occurred when the enzyme was heated for 25 min at 40 and 45°C. At 50 and 60°C the enzyme was still stable for 5 and 10 min, respectively; at 70°C the enzyme solution had lost its activity completely (Table 10). Data presented in Tables 1–10 have been calculated using the static culture technique.

Conclusion

These results suggest that *L. paramesenteroides* is a possible industrial source of dextransucrase because of its ability to produce high dextransucrase activity and high thermostability, which were observed in the culture filtrates. More comparative studies are in progress for further confirmation.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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