Effect of Nutrients by One Variable At A Time (OVAT) Approach on the Dextransucrase Production from Leuconostoc mesenteroides NRRL B-640

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Citation

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Abstract

The medium composition including macro and micronutrients were optimized for maximizing the yield of dextransucrase from Leuconostoc mesenteroides NRRL B-640 using 'one-variable-at-a-time' approach. Interestingly, the increase in dextransucrase activity was significant (3-fold), from 5 U/ml to 15 U/ml with sucrose increase from 2% to 7%. By doubling yeast extract from 2% to 4%, resulted 10% higher enzyme production. The K_2HPO_4 increase from 2 to 3% gave 15% higher enzyme activity. Other nitrogenous sources like peptone and beef extract separately, enhanced the enzyme activity by 15%. Tween 80 enhanced dextransucrase production by 12%. Effect of micronutrients displayed 10% increased dextransucrase production by individual addition of $MgSO_4$, $MnSO_4$ or NaCl. The present results show that nutrient requirements are not only species specific, also strain specific. This strain required higher K_2HPO_4 and sucrose for higher enzyme production. Leuconostoc mesenteroides NRRL B-640 gave higher enzyme production as compared to most studied strains.

INTRODUCTION

Leuconostoc mesenteroides synthesizes the extracellular dextransucrase that is used for synthesis of dextran which has numerous applications in pharmaceutical, food and fine chemical industries [13,15]. Oligosaccharides synthesized from dextransucrase are used as neutraceuticals, stabilizers and prebiotics [13]. The culture conditions and maintenance medium compositions of several strains of L. mesenteroides have been optimized for production of dextransucrase [7,5,9,10,23]. Many of these have been studied for nutrient effect for maximizing the dextransucrase production [1,2,4,6,8,11,17,18,21,23,24]. The effects of certain nutrients on dextransucrase production by L. mesenteroides were reported [8]. It was reported that 2% sucrose, corn steep liquor and yeast extract as good sources of nitrogen for growth of the culture and higher enzyme production [8]. The yield of enzyme is also affected by the type of yeast extract used. It was shown that different commercial grades of yeast extracts had different effect on the final cell concentration and the enzyme yield [1]. Peptone and beef extract separately in addition to yeast extract resulted in enhanced enzyme activity [8]. An optimum concentration of 4% yeast extract was reported for dextransucrase production form L. mesenteroides NRRL B-1299 [6]. The influence of nitrogen/carbon ratio on dextransucrase production by L. mesenteroides NRRL B-512F was studied and a slow rate of enzyme synthesis and lower fermentation time was observed by the addition of nitrogen source, which was contradictory to the findings of other reports [11].

There are several reports on sucrose effect on dextransucrase production by various strains of Leuconostoc spp [2,6,8,11,18,21,24]. However, dextransucrase production by wild-type L. mesenteroides grown on glucose or maltose instead of sucrose has also been reported [21]. Behravan et al. (2003) used sugar-beet molasses as a sucrose source and wheat bran as substitute for yeast extract. The enzyme production was dependent on the concentration of K_2HPO_4 and it increased with the increase in K_2HPO_4 concentrations [8]. A maximum activity of 2.2 UmL^{-1} from L. mesenteroides IBT-PQ was reported using sucrose 3%, yeast extract 2% and K_2HPO_4 2.5% [4]. The effect of medium composition on enzyme activity from a newly isolated strain of L. mesenteroides (PCSIR-3) was studied and higher enzyme activity was found in the medium containing 3 times K_2HPO_4 [24]. An increase in K_2HPO_4 from 0.1 to 0.3M showed increased biomass and enzyme production by L. mesenteroides NRRL B-512F grown in shake flask culture [17]. The low cost carbon and nitrogen sources like sugar-beet molasses, corn steep liquor and wheat bran extract for large-scale preparation of dextransucrase from L. mesenteroides by fermentation have been reported [2].

Various reports on effect of micronutrients on dextransucrase production from L. mesenteroides are available [6,8,16,24]. The micronutrients such as $MgCl_2$, $MgSO_4$ and NaF were shown to have significant effect on dextransucrase production from L. mesenteroides NRRL B-512F [8]. It was reported that Mn^{2+} ions were essential for dextransucrase production from L. mesenteroides NRRL B-1299 [6]. The presence of magnesium, manganese and calcium salts in the medium not only increased the enzyme activity but also the yield of the dextran [24]. There is no report available on Leuconostoc mesenteroides NRRL B-640 for dextransucrase production. The aim of present study was to optimize the medium composition both macro and micronutrients for maximizing the yield of dextransucrase from L. mesenteroides NRRL B-640 by one variable at a time approach.

MATERIALS AND METHODS

MICROORGANISM

L. mesenteroides NRRL B-640 was procured from Agricultural Research Service (ARS-Culture collection), USDA, Peoria, USA. Ingredients required for maintenance and enzyme production media were from Hi-Media Pvt. Ltd., India. All the chemicals required for reducing sugar estimation, protein estimation and buffer preparation were of high purity grade.

MAINTENANCE AND INOCULUM PREPARATION OF

Cultures were maintained in modified MRS agar stab that was prepared by substituting sucrose with glucose as a carbon source [7]. A loop of culture from an agar stab was transferred to 5 ml of sterile medium described by Tsuchiya et al. (1952). The cultures were grown at 25°C with 200 rpm for 12-16h. 1% of the culture inoculum was used for the enzyme production from L. mesenteroides NRRL B-640.

PRODUCTION OF DEXTRANSUCRASE

The enzyme was produced using the media described by Tsuchiya et al. (1952) and the enzyme production media contained in (%, w/v) sucrose, 2; yeast extract, 2; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.02; $MnSO_4.4H_2O$, 0.001; $FeSO_4.7H_2O$, 0.001; $CaCl_2.2H_2O$, 0.001; NaCl, 0.001 and the pH was adjusted to 6.9. Unless stated otherwise, all fermentations were carried out in triplicate sets of 100 ml enzyme production medium (EPM) in 250 ml Erlenmeyer flask at 25°C under shaking condition incubated at 200 rpm. The samples (5 ml) were withdrawn at indicated time intervals and centrifuged at 10,000 rpm for 10 min at 4°C to separate the cells. The supernatant (cell free extract) was analyzed for enzyme activity and protein concentration.

ENZYME ACTIVITY ASSAY

The assay of dextransucrase was carried out in 1 ml of a reaction mixture in 20 mM sodium acetate buffer, pH 5.4, containing 292 mM sucrose and using the cell free extract (10-20 µl) as the enzyme source. The reaction mixture was incubated at 30°C for 15 min. The enzyme activity was measured by estimating the liberated reducing sugar by the Nelson-Somogyi procedure [14,22]. Aliquots (0.2 ml), from the reaction mixture were analyzed for reducing sugar concentration. The absorbance was measured at 500 nm using a UV-visible spectrophotometer (Varian, Cary 100) against a blank using D-fructose as a standard. One unit (U) of dextransucrase activity is defined as the amount of enzyme that liberates 1 µmol of reducing sugar per min at 30°C in 20 mM sodium acetate buffer, pH 5.4. All assays were performed in duplicate sets.

EFFECT OF SUCROSE ON DEXTRANSUCRASE PRODUCTION

The effect of sucrose concentration on dextransucrase production was studied by varying its concentration from 1 to10% in the enzyme production medium by keeping the concentration of other components constant. The medium containing the 2% sucrose was considered as control.

EFFECT OF YEAST EXTRACT AND KHPO ON DEXTRANSUCRASE PRODUCTION

The effect of yeast extract was studied in combination with the phosphate concentration. The yeast extract concentration was varied from 1.5% to 4%, where the control was 2% yeast extract. The effect of phosphate on the dextransucrase production was studied by varying the concentration from 1.5% to 3%, where the control was 2% phosphate.

EFFECT OF PEPTONE, BEEF EXTRACT AND TWEEN 80 ON DEXTRANSUCRASE PRODUCTION

The effects of peptone and beef extract on dextransucrase production were studied separately in addition to the presence of yeast extract. The effect of peptone was studied by varying the concentration form 0.1% to 1.5%, whereas the beef extract was varied from 0.5% to 2% taking the medium as described by Tsuchiya et al. (1952) as control which contained no peptone or beef extract. The effect of Tween 80 on enzyme production was studied by varying its concentration from 0.1 to 0.5% (v/v) in the medium. The media containing no Tween 80 served as a control.

EFFECT OF MGSO, MNSO, NACL AND CACL ON DEXTRANSUCRASE PRODUCTION

The effects of $MgSO_4$ and $MnSO_4$ on dextransucrase production were studied separately by varying the concentrations from 0.02 to 0.06% and 0.001 to 0.005%, respectively. The medium described by Tsuchiya et al. (1952) containing 0.02 % $MgSO_4$ and 0.001 % $MnSO_4$ were taken as controls. The effect of NaCl and $CaCl_2$ on enzyme production were studied separately by varying the concentration of both the salts from 0.001 to 0.005%, taking the medium described by Tsuchiya et al. (1952) containing 0.001% of each salt in the production medium as controls.

RESULTS

EFFECT OF SUCROSE

There was a steep rise (5 fold increase) in the dextransucrase production and activity from L. mesenteroides NRRL B-640 from 2.9 to 15 U/ml when the sucrose concentration increased from 1% to 7% in the enzyme production media. The dextransucrase production attained saturation above 7% sucrose. The increase in dextransucrase activity was 3-fold, from 4.8 U/ml to 15 U/ml with an increase in sucrose concentration from 2% (control) to 7% in the medium. The maximum enzyme activity of 17 U/ml was observed at 10% sucrose concentration (Fig. 1). As the sucrose concentration increased there was an increase in viscosity of the broth due to the subsequent formation of exopolysaccharide from the available and residual sucrose by the released enzyme, in the medium. Surprisingly, the handling of this viscous cell free extract was not as difficult as reported for L. mesenteroides NRRL B-512F [8,12] for enzyme activity determinations. This difference might be due to higher solubility characteristics of the dextran produced by L. mesenteroides NRRL B-640.

EFFECT OF YEAST EXTRACT AND KHPO

An increase in yeast extract concentration from 1.5% to 4% caused an increase in dextransucrase activity at all concentrations of K_2HPO_4 used (Fig. 2). The increase in activity was approximately 10% at all K_2HPO_4 concentrations used. The increase in K_2HPO_4 concentration form 1.5% to 3% resulted in significant increase (approx. 20%) in dextransucrase activity at all yeast extract concentrations (Fig. 2). The maximum activity of 5.9 U/ml was achieved at a combination of 4% yeast extract and 3% K_2HPO_4. However, for large scale production of enzyme it would be economical to use a combination of 2% yeast extract and 3% K_2HPO_4 that gave 5.6 U/ml which is 15% higher than the control 4.8 U/ml containing 2% of K_2HPO_4 and 2% of yeast extract that will also save 2% of yeast extract.

Figure 1

Figure 1: Effect of sucrose concentration on dextransucrase production from NRRL B-640. The maximum enzyme activity obtained at each sucrose concentration was plotted.

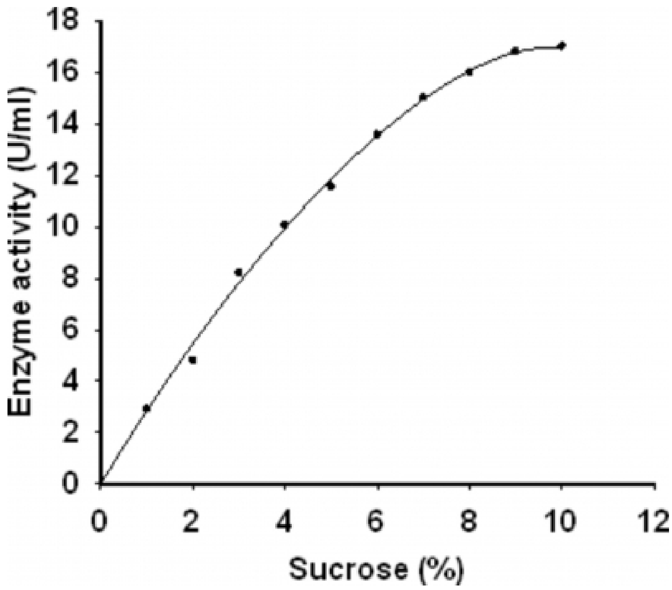
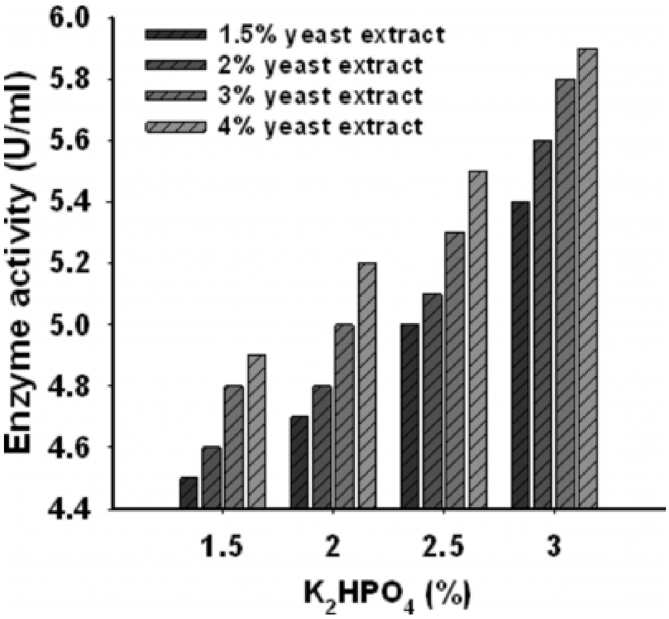


Figure 2

Figure 2: Effect of yeast extract and KHPO on dextransucrase production from NRRL B-640. The maximum enzyme activity obtained at various combinations of yeast extract and KHPO were plotted.

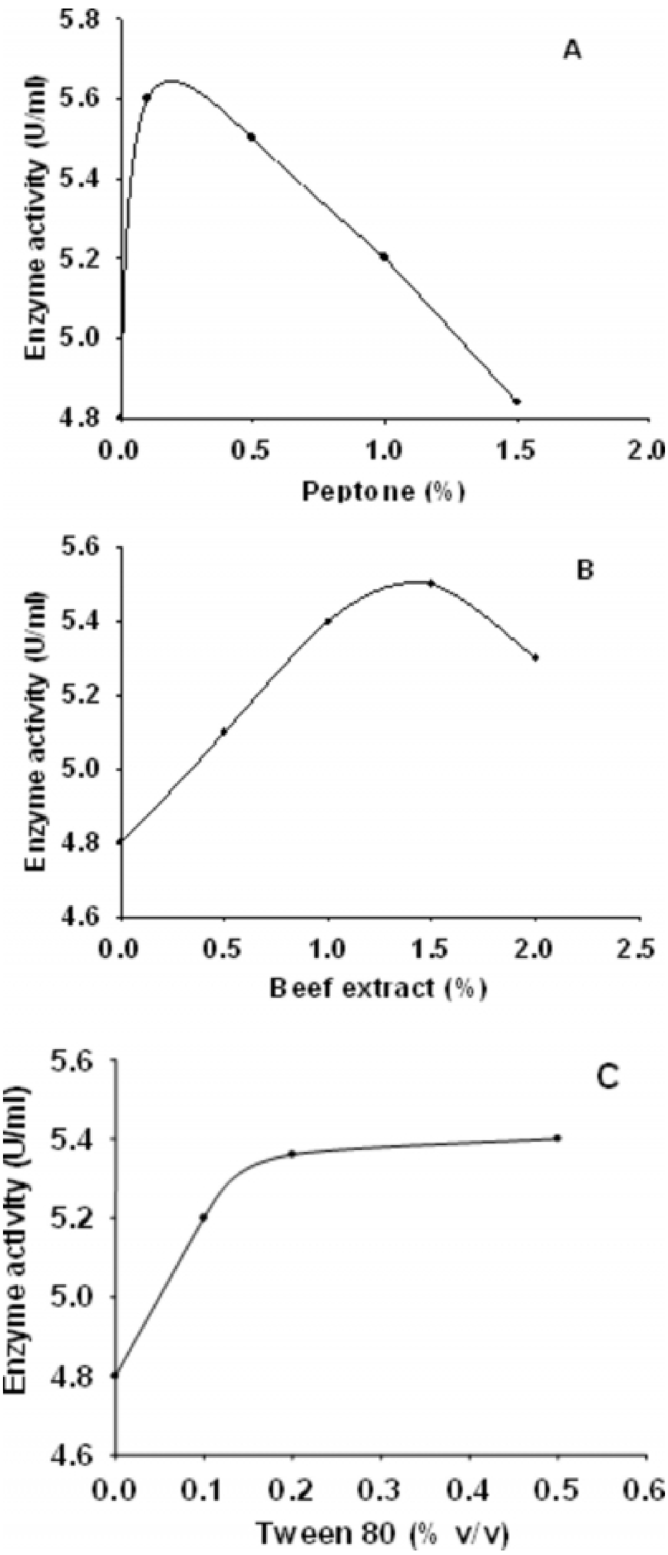


EFFECT OF PEPTONE, BEEF EXTRACT AND TWEEN 80

The effect of peptone on dextransucrase production was studied by varying the concentration from 0.1% to 1.5%. The addition of only 0.1% peptone to control medium showed 17% increase in dextransucrase activity (Fig. 3A). Further increase in peptone concentration beyond 0.5%, did not favor the enzyme production, rather a decrease in enzyme production was observed. This might be due to effect of certain trace elements present in the peptone. Effect of beef extract on dextransucrase production was studied by varying its concentration from 0.5% to 2%. With an increase in the beef extract from 0.5% to 1.5% an increase in enzyme production was observed (Fig. 3B). The addition of 1.5% beef extract gave 15% increase in enzyme production over control medium. Further increase in beef extract concentration beyond 1.5% decreased the enzyme production. Addition of Tween 80 to the medium stimulated the production of dextransucrase. The production of dextransucrase increased with increase in concentration of Tween 80 (Fig. 3C). 0.1% Tween 80 gave a 10% increase in enzyme activity that was saturated at higher concentrations, 0.5% (Fig. 3C). This result is similar to the earlier reports [19,25]. They showed that addition of Tween 80 to the enzyme production medium altered the fatty acid composition of the membrane thus enhancing the secretion of the dextransucrase and its activity [19,25].

Figure 3

Figure 3: Effect of (A) peptone and (B) beef extract (C) Tween 80 on dextransucrase production.



EFFECT OF MGSO, MNSO, NACL AND CACL

The effect of $MgSO_4$ on dextransucrase was studied by increasing the concentration in the production medium from 0.02% to 0.05%. Dextransucrase production enhanced with the increase in $MgSO_4$ from 0.02 % (4.8 U/ml) to 0.04% (5.3 U/ml) (Fig. 4A) showing a 10% increase in the enzyme production (Table 1). The magnesium ions are reported to play role in the signal transduction by enhancing the enzyme production and its release in to the medium [26]. Leuconostoc Spp. are known to be micro-aerophilic microorganisms [6,23]. The $MnSO_4$ was shown to decrease the oxygen toxicity of the L. mesenteroides cells [3]. The effect of the $MnSO_4$ on the dextransucrase production was studied by varying the concentration from 0.001% to 0.005% and a 13% increase was observed in the enzyme production at 0.005% $MnSO_4$ concentration (Fig. 4B). The effect of sodium ions were studied by increasing the concentration from 0.001% to 0.005% in the production medium. An increase up to 0.003% increased the enzyme production (5.4 U/ml) from 4.8 U/ml at 0.001% NaCl concentration and further increase rather reduced the enzyme production. A 12% increase in the enzyme production was observed at 0.003% medium as compared to control (Fig. 4C). It was surprising that the calcium ions displayed negative effect on the enzyme production. The effect of $CaCl_2$ was studied by increasing the concentration from 0.001% to 0.005% production medium (Fig. 4D). There was a 15% decrease in enzyme activity with the increase in 5 fold $CaCl_2$ concentration as compared to the control medium.

Figure 4

Figure 4: Effect of (A) MgSO, (B) MnSO, (C) NaCl and (D) CaCl on dextransucrase production.

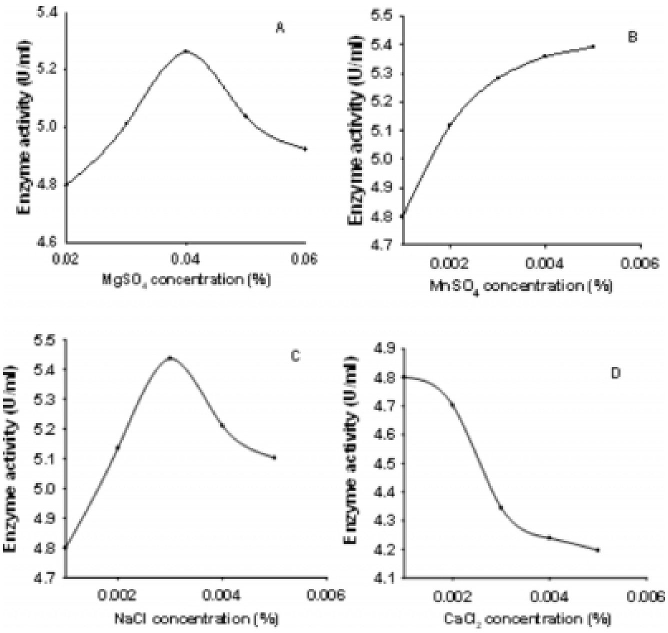


Figure 5

Table 1: Maximum activity of dextransucrase achieved at optimum concentration of nutrients. Effects of nutrients were compared with the control medium.

Nutrient (Concentration, %)	Enzyme activity (%)
Control	100
Sucrose (7%)	310
Yeast extract (4%)	110
K_2HPO_4 (3%)	115
Yeast extract (4%) + K_2HPO_4 (3%)	120
Peptone (0.1%)	117
Beef extract (1.5%)	115
Tween 80 (0.5% v/v)	112
$MgSO_4.7H_2O$ (0.04%)	110
$MnSO_4.6H_2O$ (0.005%)	113
NaCl (0.003%)	112

DISCUSSION

Sucrose is the known inducer of dextransucrase [23]. Although, some Leuconostoc strains are shown to produce dextransucrase by the media containing sugars other than sucrose [6], though the enzyme levels are minimal, but when the sucrose was used as substrate the enzyme activity was several fold higher [6]. L. mesenteroides NRRL B-640 gave higher enzyme activity of 15 U/ml at 7% sucrose concentration. Similar results were reported earlier although, the maximum enzyme production was achieved at lower sucrose levels and above 4% sucrose there was no increase in enzyme activity for other L. mesenteroides strains [2,6,8,11,18,21,24]. Tsuchiya et al. (1952) opined that 2% sucrose was optimum for maximum production of dextransucrase by

L. mesenteroides NRRL B-512. Ul-Qadar et al. (2001) observed a decrease in percent conversion of sucrose to dextran with increase in sucrose concentration above 1% using L. mesenteroides PCSIR-3 in the fermenting media, which was due to substrate inhibition effect. However, Beheravan et al. (2003) reported similar results to ours as they reported that a concentration of molasses above 20% (containing 9.5% sucrose) resulted in a decrease in dextransucrase production.

Tsuchiya et al. (1952) reported requirement of higher nitrogen sources and other nutrients for maximal enzyme formation [23]. Yeast extract and corn steep liquor was reported to serve as vitamin and amino acids supplements [23]. The dextransucrase production increased at higher yeast extract levels by L. mesenteroides NRRL B-640. Doubling the concentration of yeast extract from 2% to 4% increased dextransucrase activity only by 10%. These results are similar to those reported by Dols et al. 1998, where an increase in yeast extract concentration from 2% to 4% showed a marginal increase in dextransucrase production from L. mesenteroides NRRL B-1299 and its further increase did not cause any increase the dextransucrase production. However, these results are contrary to those of Goyal and Katiyar (1997), where a decrease in enzyme production from L. mesenteroides NRRL B-512F was observed with increasing the yeast extract concentration.

The increase of K_2HPO_4 from 2% to 3% gave a 15% higher enzyme activity in the medium by L. mesenteroides NRRL B-640. The increase in enzyme activity was significantly (35%) more on increasing K_2HPO_4 concentration from 2% to 2.5% in the case of L. mesenteroides NRRL B-512F [8]. Although, L. mesenteroides NRRL B-640 gave maximum activity of 5.9 U/ml at a combination of 3% K_2HPO_4 and 4% yeast extract resulting 20% higher enzyme activity as compared to the control medium (4.8 U/ml), however a combination of 2% yeast extract and 3% K_2HPO_4 giving an enzyme activity of 5.6 U/ml can be preferred because of marginal difference from maximal enzyme activity.

The addition of 0.1% peptone to control medium with 2% yeast extract showed 17% increase in dextransucrase activity from L. mesenteroides NRRL B-640. Ul-Qadar et al. (2003) observed higher dextransucrase production with the addition of peptone and $CaCl_2$ to the medium containing yeast extract and higher phosphate. They compared the dextransucrase production in the media containing the additional nitrogen source (peptone) along with higher phosphate concentration and media containing higher phosphate levels with no additional nitrogen source and found peptone played some role in obtaining the higher enzyme levels. L. dextranicum FPW-10 produced higher dextran in the medium containing wheat bran as nitrogen source than the conventional media containing peptone, tryptone and yeast extract as nitrogen sources [20]. The addition of 1.5% beef extract gave 15% increase in enzyme production from L. mesenteroides NRRL B-640 as compared to control medium. However, L. mesenteroides NRRL B-512F gave 25% higher enzyme activity by an additional 2% beef extract [8]. Addition of 0.5% Tween 80 to the control medium enhanced the dextransucrase activity by 12% in the culture broth. However, the increase was 25% from L. mesenteroides NRRL B-512F by the addition of 0.5% Tween 80. Similar results were also reported for Streptococcus mutans [19,25].

Dextransucrase production from L. mesenteroides NRRL B-640 enhanced with the increase in $MgSO_4$ from 0.02% (4.8 U/ml) to 0.04% (5.3 U/ml) showing a 10% increase in enzyme production. Similar results using $MgCl_2$ were reported for L. mesenteroides NRRL B-512F [8]. A 12% increase in the enzyme production was observed with the increase in the concentration of $MnSO_4$ from 0.001% (Control) to 0.005% from L. mesenteroides NRRL B-640. Dols et al. 1998 reported Mn^{2+} ions to be essential for the dextransucrase production from L. mesenteroides NRRL B-1299 [6]. Bellengier et al. (1997) showed the addition of Mg^{2+}, Mn^{2+} and amino acids stimulated the growth of most Leuconostoc strains [3]. They also showed that Mn^{2+} suppressed the inhibitory effect of aeration on the growth of L. mesenteroides UD-23, and suggested its protective role against oxygen toxicity on L. mesenteroides UD-23. Mn^{2+} ions showed an inhibitory effect on the partially purified dextransucrase activity from L. mesenteroides NRRL B-640 (data not shown). This showed that the increase in enzyme activity was due to the increased production of dextransucrase by Mn^{2+} ions in the medium.

A 12% increase in the enzyme production L. mesenteroides NRRL B-640 was observed at 0.003% NaCl in the medium as compared to control that contained 0.001% NaCl, though NaCl had no effect on in vitro enzyme activity. An inhibitory effect of $CaCl_2$ on enzyme production from L. mesenteroides NRRL B-640 was observed however, $CaCl_2$ showed no effect on in vitro activity of dextransucrase. Contrary to these results, a 2-fold increase in enzyme production from L. mesenteroides NRRL B-512F was observed by the addition of $CaCl_2$ to the medium [16]. These studies have indicated that it is essential to identify the nutrient requirements of L. mesenteroides NRRL B-640 for maximum dextrasucrase production. The present results show that nutrient requirements are not only species specific, also strain specific. Glycoconjugates are produced by complex oligosaccharides and immunogenic peptides. Their use as therapeutics, for development of vaccines, contraceptives and antibiotics, has accentuated large scale synthesis of oligosaccharides. Looking at wide applications of dextransucrase and growing demand there is continuous need for exploration of new strains for production of efficient and pure dextransucrase, producing better quality and yield of oligo- and poly-saccharides. The results of optimized nutritional effects attain importance for large scale production of dextransucrase.

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