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Full Length Research Paper

Temperature effect on dextransucrase production by *Leuconostoc mesenteroides* FT 045 B isolated from Alcohol and Sugar Mill Plant

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Temperature (23 to 31°C) and sucrose concentration (3 and 4%) effects on dextransucrase production by *Leuconostoc mesenteroides* NRRL B 512 (F) and *Leuconostoc mesenteroides* FT 045 B were studied. The conditions in all fermentations were: total reaction volume 2 L, 132 rev. min-1, 0.5 vvm and pH 6.0. The optimum temperature for enzyme yield for strain NRRL B 512 (F) was 23°C, where at 8-h fermentation was possible to achieve 49.3 DSU/mL. When FT 045 B strain was utilized, 3.2 DSU/mL was obtained at temperature 23 to 25 °C.

Key words: Dextran, dextransucrase, *Leuconostoc mesenteroides* NRRL B512 (F), *Leuconostoc mesenteroides* FT045 B, temperature, enzyme activity.

INTRODUCTION

Dextransucrase is an extracellular enzyme produced by various species of the genera *Streptococus, Leuconostoc and Lactobacillus* involved in the synthesis reaction of dextran from sucrose (Barker and Ajongwen, 1991). Dextran (C6H10O5)n is a D-glucose polymer composed of α (1→6) linkages in the linear chain and α (1→2), α (1→3) and/or α (1→4) branch linkages (Dols et al., 1997). Commercial applications are generally in the pharmaceutical area, most commonly as a blood volume expander. However, dextran had been investigated as potential macromolecular carries for drugs and proteins delivery, primarily to increase the longevity of therapeutic agents in the circulation (Mehvar, 2000).

Dextransucrase production is affected by several factors, such as temperature, pH, aeration and substrate concentration (sucrose). Generally the fermentation temperatures are about 23-26°C. Santos et al. (2000) studied the effect of six different temperatures (20, 25, 27.5, 30, 35 and 40°C) on dextransucrase enzyme production, and observed greater activity at 20°C (71.7 U/mL). They observed a decrease in enzyme activity after increased in temperature. Ul-Qader et al. (2001), studying a strain *L. mesenteroides* PCSIR-3 growing at temperature of 26°C, sucrose 2%, no pH control, obtained after 18 h of culture, 14.13 DSU/mL of enzyme activity. The aim of this paper was to study the effect of temperature on extracellular dextransucrase production by the strains *L. mesenteroides* NNRL B 512 (F) and Ft 045 B at pH 6.0.

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MATERIALS AND METHODS

Organism

L. mesenteroides NRRL B 512 (F) was provided by Department of Food Engineering, UNICAMP. *L. mesenteroides* FT 045 B, isolated from Alcohol and Sugar Mill Plant, was provided by Microbiology Industrial Process Control Division of Fermentec.

Inoculum and batch fermentation

The growth medium for *L. mesenteroides* NRRL B 512 (F) and FT 045 B was: sucrose (40 g/L), yeast extract (20 g/L), KH2PO4 (20 g/L), CaCl2 (0.02 g/L), MgSO4 (0.2 g/L), NaCL (0.01 g/L), FeSO4 (0.01 g/L), MnSO4 (0.01 g/L). These strains were stored at -20°C in cryogenic solution until preparation of the inoculum for fermentation. The fermentation inoculum was prepared up to 5% (v/v) related to total volume of the medium. The microorganism was transferred from the stock culture to a similar medium. The inoculum was grown for 14 to 16 h at 26°C in agitated flasks (100 rev min-1). The inoculum medium (100 mL, pH 7.0) was sterilized at 121°C by 15 min. After the growth, the inoculum was transferred to the reactor. The working volume used on fermentation was 2 liters, agitation at 132 rev min-1, aeration 0.5 vvm and pH controlled at 6.0 by addition of NaOH solution 2 N.

Growth measurement

Bacterial growth was measured spectrophotometrically at 650 nm using a 1-cm optical cell. Samples of 10 mL of were taken each 1-h, during 8 hours, centrifuged at 10.000 x g by 10 min at 4°C. The pH of liquid supernatant cell free was adjusted to 5.2 and maintained in freezer for enzyme activity assay. The centrifuged was used for optical density determination by spectrophotometer. The dry mass was determined through standard curve of optical density versus dry mass (Dry mass = ABS x (0.288 – 0.000629) x F; F = dilution factor).

Substrate consumption determination

The substrate consumption was determined by Miller (1959). The concentration of sucrose was calculated from difference between the corresponding concentrations of total and free reducing sugars.

Enzyme assays

Dextransucrase activity of culture media was assayed by measuring the amount of free reducing sugars (calculated as fructose) produced (Suzuki et al., 1988). One dextransucrase unit (1 DSU) was defined (Tsuchiya et al., 1952) as the amount of enzyme that liberates 0.52 mg of fructose in one hour under ideal reaction conditions (30°C; pH 5.2 and sucrose as substrate). The dextransucrase activity was calculated as

α 1x α 2 x 60/0.52 x f x F

α 1 = slope of standard fructose curve (Abs x concentration)

α 2 =slope of sample plot (Abs x time)

F = Dilution factor

f = $\frac{\text{Reactor volume}}{\text{Sample volume}}$ x sample volume from reactor

RESULTS AND DISCUSSION

Generally the temperature used in these fermentations ranged from 23 to 26°C. In this work other temperatures were studied. Tables 1 show the effect of temperature on volumetric enzyme productivity, enzyme yield and specific growth rate for *L. mesenteroides* NRRL B512 (F) and FT 045 B.

Figure 1 showed high activity at 25°C (37.8 U/mL), this result was better than obtained by Lazic et al. (1993), who found an activity of 27.8 U/mL at the same temperature and pH 5.5; 0.05 vvm and 125 g/L of sucrose. An increase in sugar concentration can inhibit the dextransucrase production due to high viscosity medium which decreases cellular growth.

Figure 2 showed that the maximum enzyme activity was obtained at 23 and 25°C (3.2 U/mL). The enzyme production decreased as the temperature increases, increasing the residual sugar concentration. The best growth of *L. mesenteroides* FT045 B at temperature of 29 and 31°C suggests that temperature might be more favorable for the microorganism than traditional process conditions (temperature of 23°C and pH 6.7).

Figure 4 showed that the aeration is relatively high for *L. mesenteroides* FT045 B culture in relation to NRRL B512 (F) (Figure 3), although, the strain NRRL B 512 (F) is known as micro aerophilic (Barker et al., 1991; Landon and Webb, 1990), several authors (Johnson and Mccleskey, 1957; Plihon et al, 1995; Lebrun et al, 1994) consider that oxygen positively affects the strain growth. The extracellular dextransucrase production is closely related to bacterial growth. Maybe this fact explains the low growth and enzyme production by this microorganism at the studied aeration rate. High activity was reached when dextransucrase fermentation by *L. mesenteroides* FT045 B in medium containing 3 and 4% of sucrose at 25°C, 132 rev min-1 and 0.15 vvm was studied. The results obtained (nearly 11.0 UDS/mL) in Figure 5 and 6 suggest that the aeration rate is an important factor in dextransucrase production by strain FT045 B. This also suggests that low aeration rate might be more favorable for enzyme production.

Although the biomass is much higher (6.04 g/L) at 31°C (Figure 3), enzyme yield was lower than that observed at 23°C. The enzyme production has been reported to be maximum at the pH range of 6.0-7.0, but at high temperature the inactivation of enzyme within the pH range was fast and lower yields were obtained (Alsop 1983; Chen and Kaboli, 1976). The strain *L. mesenteroides* FT045 B showed lower enzyme yield at high temperature than at low temperature. Although the enzyme activity was high at low temperature (23 and 25°C), the growth was low (0.47 and 0.66 g/L). This strain was isolated from the environment at temperature close to 30°C; this could be the reason for the lower biomass at lower temperature than optimal temperature of 29°C. Below the optimum temperature, the cell growth was

Table 1. Effect of temperature on volumetric enzyme productivity, enzyme yield and specific growth rate by *L. mesenteroides* NRRL B512 (F) and FT045 B (Sucrose 4%, 0.5 vvm, pH 6.0 e 132 rev.min^{-1}).

	T(°C)	Volumetric enzyme Productivity (UDS.mL^{-1}h^{-1})	$Y_{E/X}$	μ (h^{-1})
Leuconostoc mesenteroides NRRLB512 (F)				
	23	6.16	19.72	0.64
	25	4.72	8.65	0.69
	27	5.10	9.25	0.72
	29	6.70	12.90	0.68
	31	2.53	3.36	0.81
Leuconostoc mesenteroides FT 045 B				
	23	0.40	6.80	0.33
	25	0.40	4.85	0.45
	27	0.20	1.35	0.50
	29	0.16	0.91	0.70
	31	0.19	1.27	0.55

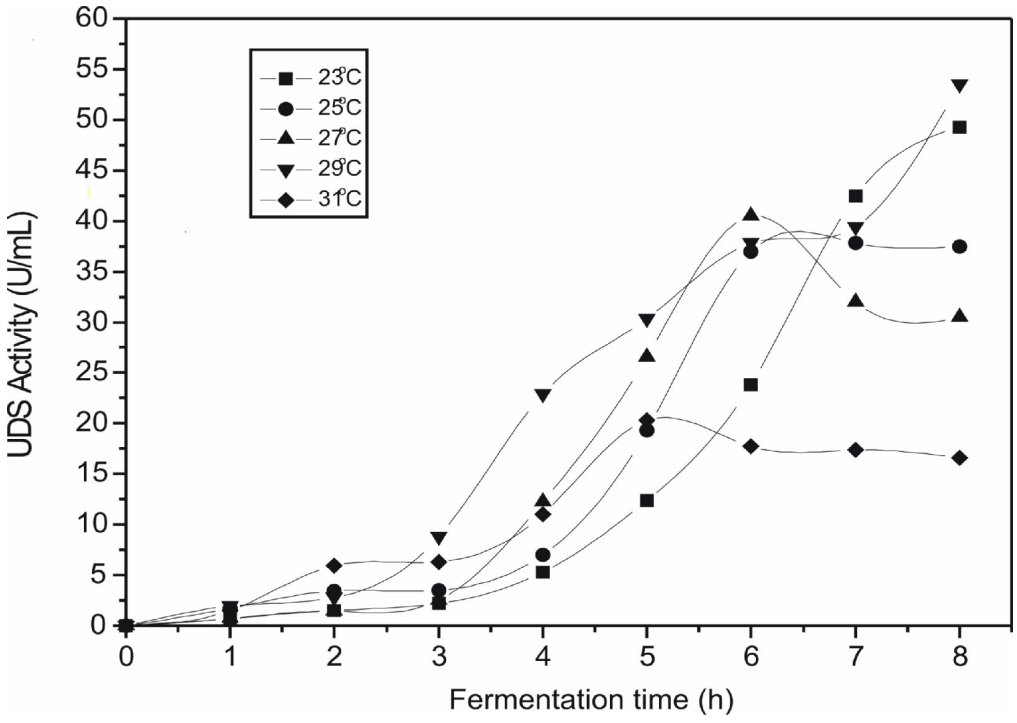

Figure 1. Effect of temperature on dextransucrase production by L. mesenteroides NRRL B 512 (F).

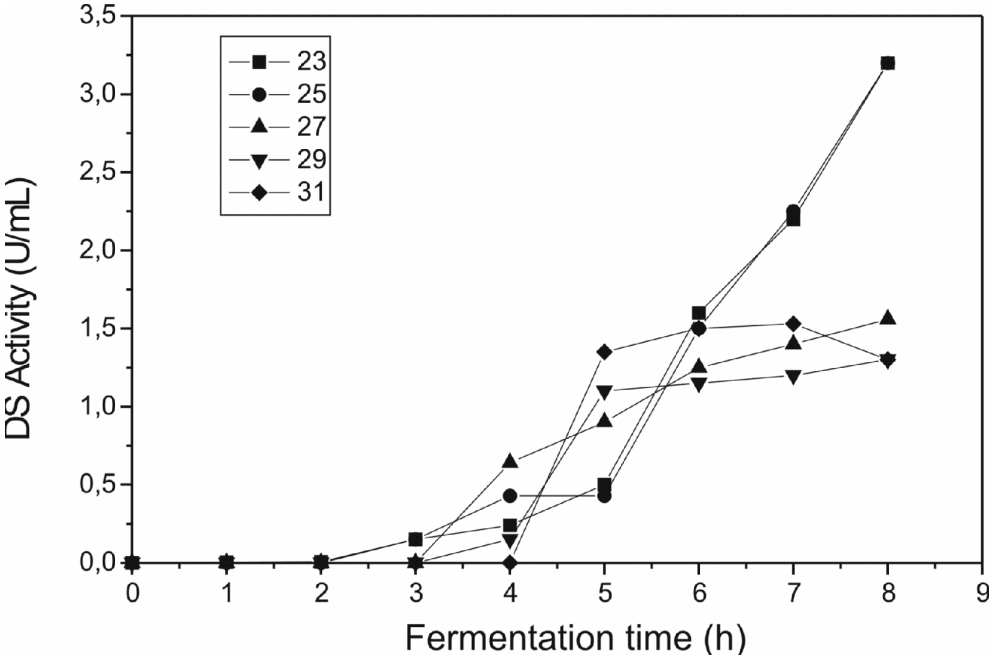

Figure 2. Effect of temperature on dextransucrase production by L. mesenteroides FT 045 B.

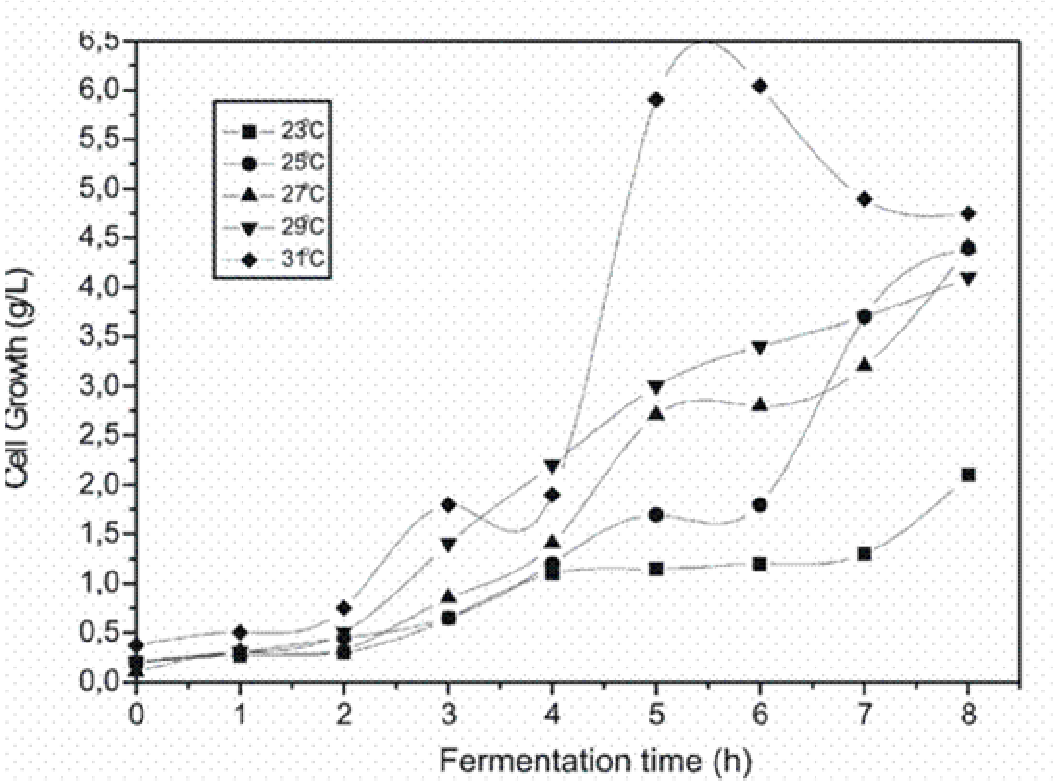

Figure 3. Effect of temperature on L.mesenteroides NRRL B 512 (F) growth.

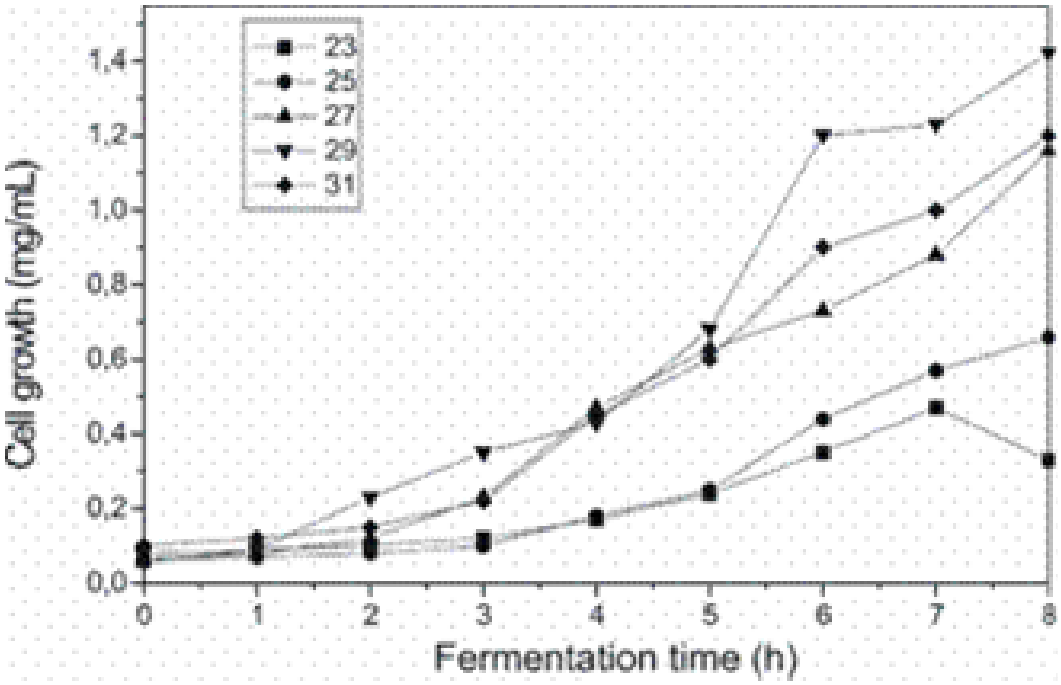

Figure 4. Effect of temperature on L.mesenteroides FT 045 B grow.

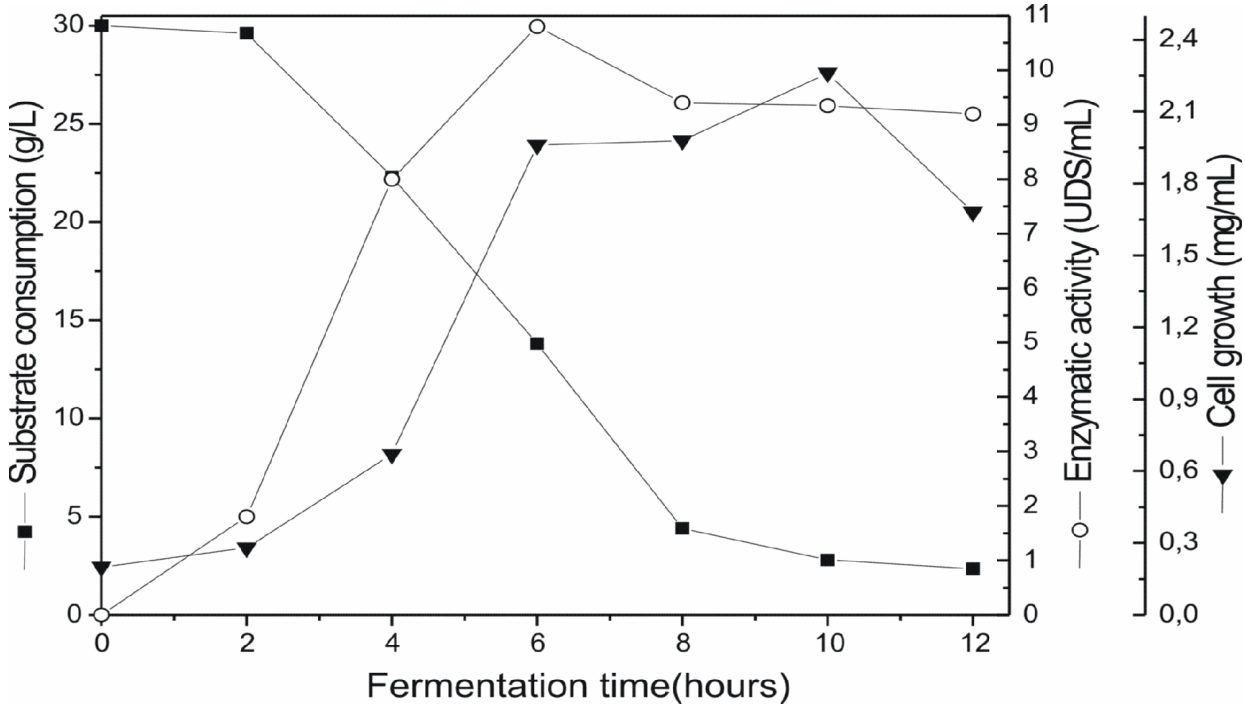

Figure 5. Enzyme activity, cell growth and substrate consumption in *L. mesenteroides* NRRL B 512 (F) fermentation with 3% of sucrose, 25°C, 132 rev.min^{-1} and 0,15 vvm.

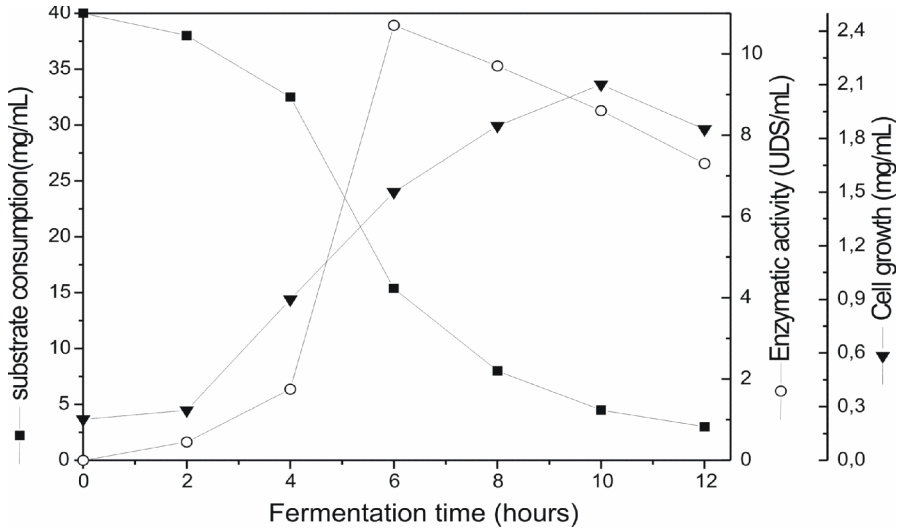

Figure 6. Enzyme activity, cell growth and substrate consumes in *L. mesenteroides* FT 045 B fermentation with 4% of sucrose, 25°C, 132 rev.min^{-1} and 0,15 vvm.

slower and the reduced growth of the bacterium leads to the delayed synthesis of the enzyme and resulting in lower enzyme yield. The pH 6.7 was taken as the optimum pH for the strain NRRL B 512 (F) by others researchers when they intended to maximize cell growth. Our experiments at pH 6.0 showed this pH to be good the for enzyme yield when compared to the results obtained by Santos et al. (2000), These workers obtained high enzyme activity at pH 5.5, but they also obtained high biomass. Although the FT045 B strain showed lower enzyme yield than NRRL B512 (F), this isolated has a potential for dextransucrase production. The results show that is necessary to study better conditions in order to achieve a high enzyme yield. The change in aeration rate showed an increased in enzyme production, indicating that better studies must be done regarding this parameter. Experimental results on batch fermentation of *L. mesenteroides* FT 045 B carried out at pH 6.0 resulted in highest enzyme levels for sucrose concentrations at 3 and 4%.

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