ISSN 1678-0493

Diálogos & Ciência

www.ftc.br/dialogos

Dextran: effect of process parameters on production, purification and molecular weight and recent applications

Mary Helen P. B. Vettori[a], Kate C. Blanco[a], Mariana Cortezi[a], Cristian J. B. de Lima*[b], Jonas Contiero[a]

[a]*Lab. de Microbiologia Industrial, Dep. de Bioquímica e Microbiologia – ICB - Universidade Estadual Paulista, UNESP, Rio Claro, SP, Brazil*
[b]*Laboratório de Bioprocessos, Núcleo de Biotecnologia (NuBiotec), Faculdade de Tecnologia e Ciências (FTC), Salvador, BA, Brazil*

doi: 10.7447/dc.2012.018

INFORMAÇÕES

Histórico:
Recebido em
14/03/2012

Revisado em:
01/07/2012

Aceito em:
27/08/2012

Palavras-chave:
Dextrana, Dextranasucrase, Leuconostoc, Dextrana clínica

AUTORES

MHPB
Mestre em Microbiologia Aplicada (UNESP)

KCB
Mestre em Microbiologia Aplicada (UNESP)

MC
Ph.D. Em Microbiologia Aplicada (UNESP)

CJBL
*Autor para correspondência:
cristianjbl@hotmail.com
Doutor em Engenharia Química (UFU), Professor Titular da Faculdade de Tecnologia e Ciências

JC
Doutor em Engenharia de Alimentos (UNICAMP), Professor Adjunto da Universidade Estadual Paulista

RESUMO

Título: Dextrana: efeito dos parâmetros de processo na produção, purificação e peso molecular e aplicações recentes.
A dextrana é um homopolissacarídeo extracelular bacteriano de D-glucose composto predominantemente de α-1,6-glucopyranosidic ligações no interior da cadeia principal. A produção de dextrana é influenciada por um certo número de fatores. As propriedades físicas e químicas, tais como a solubilidade, viscosidade, rotação específica, conteúdo de nitrogênio, fósforo e cinzas, pode sofrer variações, dependendo dos diferentes microrganismos utilizados. Uma única enzima pode catalisar a síntese de vários tipos de ligações de dextrana, permitindo assim a formação de um polímero ramificado. Por outro lado, certas linhagens bacterianas têm sido utilizadas para produzir dextranas de estruturas diferentes, o que tem sido atribuída à excreção de dextransucrases diferentes. Esta revisão aborda fatores que afetam a produção de dextrana, tais como temperatura de crescimento, temperatura ótima para dextranasacarase, pH do meio de cultura, crescimento com aeração, concentração de sacarose e de outros nutrientes, agitação e frascos de aeração, dextrana e os métodos de purificação de dextranasacarase e como obter dextrana clínica. É oferecida também uma visão geral dos usos mais recentes deste biopolímero.

ABSTRACT

Dextran is an extracellular bacterial homopolysaccharide of D-glucose composed predominantly of α-1,6-glucopyranosidic linkages within the main chain. Dextran production is influenced by a number of factors. Physical and chemical properties, such as solubility, viscosity, specific rotation, content of nitrogen, phosphorus and ash, can undergo variations depending on the different microorganisms used. A single enzyme can catalyze the synthesis of several types of dextran linkages, thereby permitting the formation of a branched polymer. On the other hand, certain bacterial strains have been shown to produce dextrans of different structures, which has been attributed to the excretion of different dextransucrases. This review addresses factors that affect dextran production, as such growth temperature, optimal temperature for dextransucrase, culture medium pH, growth with aeration, the concentration of sucrose and other nutrients, agitation and aeration flasks, dextran and dextransucrase purification methods and how to obtain clinical dextran. An overview of the recent uses of this biopolymer is also offered.
Keywords: Dextran, Dextransucrase, Leuconostoc, Clinical dextran

1. Introduction

History of Dextran

Pasteur (1861) discovered that microbial action caused the gelification of sugarcane syrups. This thickening was due to the presence of a carbohydrate with the empirical formula C6H10O6. Due to its positive optical rotation, Scheibler (1874) named it "dextran. Van Tieghem (1878) isolated the microorganism responsible for the gelification and named it *Leuconostoc mesenteroides*. Hehre (1941) and Stacey (1942), respectively obtained dextran by growing *Leuconostoc mesenteroides* on media containing sucrose using cell-free preparations of the dextran-synthesizing enzyme formed by this organism. Hestrin, Averini-Shapiro and Aschner (1943) named the corresponding extracellular enzyme dextransucrase. Differences were later found between dextrans from different strains of *Leuconostoc mesenteroides* (Jeanes et al. 1954; Tarr and Hibbert 1931; Stacey and Swift 1948) and related organisms (Cooper et al. 1938; Darker and Stacey 1939).

The Biopolymer Dextran

Dextran is a high-molecular-mass (107 to 108 Da) (ROBYT 1995) extracellular bacterial homopolysaccharide (SIDEBOTHAM, 1974; MONSAN et al., 2001) of D-glucose composed predominantly (at least 50%) of α-1,6-glucopyranosidic linkages within the main chain (DOLS et al., 1998b; BUCHHOLZ AND MONSAN, 2001; SEYMOUR AND KNAPP, 1980). The different types of α-D-glucan also have side-chains, stemming mainly from α-(1,3) and occasionally from α-(1,4) or α-(1,2) branched linkages. The exact structure of each type of dextran and the degree of branching involving α-(1,2), α-(1,3) and α-(1,4) linkages in dextrans (SEYMOUR AND KNAPP, 1980) [Table 1, adapted from Monsan et al. (2001)] depend on its specific producing microbial strain (LEATHERS, 2002) and, hence, on the specific type of dextransucrase(s) involved (JEANES et al., 1954).

Dextran can be produced by growing various bacterial species from the genera Leuconostoc and Streptococcus (KIM et al. 2003) on media containing sucrose. Dextran production is influenced by a number of factors. A single enzyme can catalyze the synthesis of several types of dextran linkages, thereby permitting the formation of a branched polymer (NEELY AND NOTT, 1962; SMITH et al., 1994). On the other hand, certain bacterial strains have been shown to produce dextrans of various structures, which has been attributed to the excretion by the microorganism of different dextransucrases (CÔTÉ AND ROBYT, 1982; FIGURES AND EDWARDS, 1981; ZAHLEY AND SMITH, 1995). In other words, each dextran structure is characteristic of a given dextransucrase (JEANES et al., 1954).

Dextran has many industrial applications due to its non-ionic nature and good stability under normal operating conditions (SANTOS et al., 2000). Commercial applications for dextran are generally in the pharmaceutical, food and textile industries and as a chromatographic media (KOEPSELL AND TSUCHIYA, 1952; SHAMALA AND PRASAD, 1995; PURAMA AND GOYAL, 2005; NAESSENS, et al. 2005). Fructose (C6H12O6), which is a byproduct of dextran synthesis, is a low-calorie sugar used in the food industry (SANTOS et al., 2000).

The most widely used dextran is produced by dextransucrase of the strain *Leuconostoc mesenteroides* NRRL B 512 F (MONSAN, et al. 2001; BARKER AND AJONGWEN, 1991), which synthesizes a very linear polysaccharide containing 95% α-1,6 linkages. The controlled chemical hydrolysis of this high-molecular-mass dextran allows the production of fractions with an average molecular mass of 70 kDa. These products are used for clinical applications.

Dextransucrase

Dextransucrase is an extracellular glucosyltransferase (E. C. 2.4.1.5) produced by various species of Leuconostoc and Streptococcus (DOMAN AND ROBYT, 1995), that catalyzes the transfer of glucosyl residues from sucrose [S] (JEANES et al., 1954; ROBYT, 1986) to dextran polymer and releases fructose [F], according to the following equation (HEHRE, 1951; ROBYT, 1995; LEATHERS, 2002; ROBYT, 1985).

$$n\,S \rightarrow n\,F + dextran\ (glucose)_n \qquad (1)$$

Extracellular dextransucrase is well known for its commercial applications and has attracted worldwide attention due to the need for a low-cost, rapid, efficient purification method (NIGAM, et al. 2006; JEANES, et al. 1954; LINDBERG AND SVENSSON, 1968; ROBYT AND WALSETH, 1979; ALSOP, 1983). Dextransucrases have been included in the glycoside hydrolase family 70 and, based on their sequence homologies, have been grouped into 99 families (HENRISSAT, 1991). Different strains or species of *Leuconostoc* or *Streptococcus* are capable of producing different kinds of glucansucrases that synthesize glucans with different structures and properties (JEANES, et al. 1954; ROBYT, 1986; ROBYT, 1995; DOLS, et al. 1998b; KIM AND ROBYT, 1994a; SEYMOUR, et al. 1979). Glucansucrases from Streptococccus sp. are produced constitutively and do not require sucrose in the medium for their production elaboration (ROBYT, et al. 2008), whereas glucansucrases from Leuconostoc mesenteroides can only be produced by sucrose induction, except for the recently isolated constitutive mutants (KIM AND ROBYT, 1994a; KIM AND ROBYT, 1995).

Table 1[*]: Structure of different glucans produced by *Leuconostoc mesenteroides* and *Streptococcus* sp. Glucansucrases

			Osidic linkages (%)				
			α-1,6	α-1,3	α-1,3 branch	α-1,4 branch	α-1,2 branch
Leuconostoc mesenteroides	B 512 F (CLASS1)	S	95	5			
	B-742	L	87			13	
	B-742	S	50		50		
	B-1355	L	95		5		
	B-1355 (CLASS 2)	S	54	35	11		
	B-1299	L	66		7		27
	B-1299	S	65				35
	B-1498	S	50	50			
	B-1501	S	50	50			
Streptococcus downei	Mfe28		12	88			
	Mfe28		90	10			
Streptococcus mutans	GS5		13	87			
	GS5		15	85			
	GS5		70	30			
	6715	S	64		36		
	6715	I	4	94	2		

[*][Adapted from Monsan et al (2001)]

Dextransucrase production

This review describes certain factors that have been found to lead to the swift formation of dextransucrase and provide high yield using a medium suitable for the industrial production of this enzyme. A number of authors have described the effects of sucrose concentration, aeration, agitation speed, medium pH, temperature, nature of the yeast extract and other nutritional requirements on the production of dextransucrase, dextran and fructose by Leuconostoc mesenteroides (ROBYT, 1986; ALSOP 1983; NEELY AND NOTT, 1962; KOEPSELL AND TSUCHIYA, 1952; BARKER AND AJONGWEN, 1991; DOLS et al., 1997; BROWN AND MCAVOY, 1990; TSUCHIYA et al., 1952; VELJKIVIC et al., 1992; LAZIC et al., 1993; LANDON AND WEBB, 1990; AJONGWEN AND BARKER, 1993; EL-SAYED et al., 1990; GOYAL AND KATIYAR, 1994; HEHRE, 1946; JEANES, 1965; JEANES et al., 1957; LAWFORD et al., 1979; MONSAN et al., 1987; PENNELL AND BARKER, 1992; RHEE AND LEE, 1991; LOPRETTI AND MARTINEZ, 1999; YUSEF et al., 1997; PURAMA et al., 2007; PURAMA AND GOYAL, 2008; PURAMA et al., 2008a; PURAMA et al., 2008b; PURAMA AND GOYAL, 2009). Different combinations of culture factors have been used to obtain maximal activity of dextransucrase and, consequently, a high production of dextran.

2. Factors affecting dextransucrase production

Incubation temperature

A number of studies report production experiments using temperatures ranging from 20 to 30º C for the production of dextransucrase by different strains and under different culture conditions in a fermentation process (AJONGWEN AND BARKER, 1993; ALSOP, 1983; BARKER AND AJONGWEN, 1991; BROWN AND MCAVOY, 1990; EL-SAYED et al., 1990; HEHRE, 1946; JEANES et al., 1957; JEANES, 1965; LAWFORD et al., 1979; MONSAN et al., 1987; RHEE AND LEE, 1991; TSUCHIYA et al., 1952; KABOLI AND REILLY, 1980; KOBAYASHI AND MATSUDA, 1980; MILLER et al. 1986). *Leuconostoc mesenteroides* grows between 5 and 30o C, with the optimal range between 25 and 30º C (SANTOS et al., 2000). The best temperature range for the production of dextransucrase is between 23 and 25º C (SANTOS et al., 2000; LOPRETTI AND MARTINEZ, 1999; CORTEZI et al., 2005; GOYAL, NIGAN AND KATIYAR, 1995), although the optimal temperature range for dextran and fructose production is 25 to 45º C.

Cortezi et al. (2005) studied the effect of temperature (23 to 31° C) on extracellular dextransucrase production by the Leuconostoc mesenteroides strains NNRL B 512 F and FT 045 B at pH 6.0. The following were the conditions in all fermentations: total reaction volume of 2 L, 132 rev.min-1, 0.5 vvm and pH 6.0. The optimal temperature for enzyme yield for strain NRRL B 512 F was 23° C, achieving 49.3 DSU/mL at eight hours of fermentation. When FT 045 B strain was used, 3.2 DSU/mL was obtained at a temperature from 23 to 25° C.

Goyal and Katiyar (1997) found that a temperature of 23° C and still-flask culture conditions were best for the production of dextransucrase by *Leuconostoc mesenteroides* NRRL B 512 F. Santos et al. (2000) studied the effect of six different temperatures (20, 25, 27.5, 30, 35 and 40° C) on dextransucrase enzyme production by *Leuconostoc mesenteroides* NRRL 512 F and found the greatest activity at 20° C (71.7 U/mL). The authors report a decrease in enzyme activity with the increase in temperature. In another study, dextransucrase production from *Leuconostoc mesenteroides* NRRL B512 was carried out in batch cultures under different static conditions. Small changes in temperature had a significant effect on enzyme production. Maximal enzyme yield was achieved at 23º C. An increase in temperature to 25º C reduced enzyme activity by 28% and a decrease in temperature (20 ºC) led to a 17% reduction (GOYAL et al., 1995).

Culture medium pH

The initial pH in the dextransucrase fermentation medium is 7.0 to 7.2 (LOPRETTI AND MARTINEZ, 1999). During growth, the bacterium excretes the enzyme into the medium (TSUCHIYA et al., 1952; VELJKIVIC et al., 1992). When the pH drops to about 5.0, the enzyme converts sucrose to dextran and fructose (DE MAN et al., 1960). The optimal pH range for cell growth is 6.0 to 6.9 (more specifically 6.7). Thus, the greatest amount of enzyme production is expected in this range (TSUCHIYA et al., 1952), and optimal pH for dextran synthesis in culture filtrates containing sucrose is 5.0 to 5.2. The enzyme is also most stable in this pH range; however, it is relatively unstable at the optimal pH for its production.

Contradicting the aforementioned stability ranges, Lazic et al. (1993) report that the control of pH at either 6.7 or 5.5 results in the same extracellular dextransucrase yield. This is supported by the fact that the proton-motive force, which regulates extracellular dextransucrase production, is relatively constant in the pH range from 5.5 to 7.0 (Otts and Day 1987).Tsuchiya et al. (1952) and Barker et al (1993) report that, when the pH drops to a value in the range of 5.0 to 5.5, the enzyme produced by *Leuconostoc mesenteroides* NRRL 512 F is more active and hydrolyzes sucrose to dextran and fructose. Lazic et al. (1993) carried out fermentations with the pH controlled at 6.7 and 5.5 and found that the latter and 0.05 VVM were favorable conditions for dextran production and reducing fermentation time.

The regulation of pH regulation has little effect on the culture profile and enzyme production by *Leuconostoc mesenteroides* NRRL B-1299 when compared with strain *Leuconostoc mesenteroides* NRRL B 512 F (DOLS, 1997). For sucrose production, Landon et al. (1994) only considered cell metabolism at pH 6.7 (optimal pH for cell growth). Santos et al. (2000) studied the production of dextransucrase, dextran and fructose by sucrose fermentation using *Leuconostoc mesenteroides* NRRL-B512 F in a batch operation in a bioreactor with a total working volume of 1.5 dm3, reporting an optimal pH of 5.5 for dextran and fructose production.

In another study, *Leuconostoc mesenteroides* BLAC was grown in MRS broth or a carrot juice medium and the effects of pH control were examined. When the MRS broth had the traditional 110 mM of glucose, pH control did not increase the final population. There was a significantly lower cell yield in the carrot juice medium when the pH was not regulated. In the carrot juice medium, pH had a pronounced effect on the final population level (CHAMPAGNE AND GARDNER, 2002).

Growth with aeration

The strain Leuconostoc mesenteroides NRRL B512 F is known to be micro-aerophilic (BARKER et al., 1993; LANDON AND WEBB, 1990) and a number of authors (JOHNSON AND MCCLESKEY, 1957; PLIHON et al., 1995; LEBRUN, et al. 1994; LUCEY AND CONDON, 1986) state that oxygen positively affects the growth of this strain. Laboratory tests have shown that enzyme yields are invariably higher in shaken flasks than still flasks (KOEPSELL AND TSUCHIYA, 1952; TSUCHIYA et al., 1952). Therefore, mild aeration has been used in vat fermentations. Dextransucrase biosynthesis is reported to be growth-associated (TONG, 1973), and it is therefore to be expected that dextransucrase biosynthesis would be affected by the oxygen mass transfer rate. However, Goyal et al. (1995) showed found that a still-flask culture is an important condition for acquiring the best production of dextransucrase from Leuconostoc mesenteroides NRRL B 512 F.

Vetjkovic et al. (1992) studied the effect of oxygen transfer rate on extracellular dextransucrase production by Leuconostoc mesenteroides in batch fermentation without pH control. The optimal aerobic conditions for both growth and dextransucrase formation were found to be at an oxygen transfer rate of about 1.0 mmol/L.h. Dextran fermentation by *Leuconostoc mesenteroides* was studied under both anaerobic and aerobic conditions with and without pH control. The maximal dextran yield was obtained as a result of the greater yield, stability and activity of dextransucrase when the oxygen transfer rate in the bioreactor was equal to the maximal oxygen uptake rate of the organism and the pH of the culture medium was 5.5 (Lazic et al. 1993).

Veljkovic et al. (1992) obtained maximal extracellular dextransucrase production from *Leuconostoc mesenteroides* with the maximal oxygen uptake rate (about 1 mmol O2/L.h). Alsop (1983) found that air sparging provided higher dextransucrase yield in comparison to conditions without aeration or pure oxygen aeration. Champagne and Gardner (2002) studied the effects of sugar aeration and fermentor size on viable counts of *Leuconostoc mesenteroides* BLAC grown in MRS broth or a carrot juice medium. In the carrot juice medium, aeration did not have a pronounced effect on the final

population level, even though the quantity of viable cells was greater when the culture was aerated. Viable counts were not affected by scaling the volume of the fermentation from 2 to 15 L.

Regulation of the aeration of *Leuconostoc mesenteroides* NRRL B-1299 for dextransucrase production in sucrose fermentation has little effect on the culture profile and enzyme production when compared to that of *Leuconostoc mesenteroides* NRRL B 512 F (DOLS et al., 1997). In one study, high enzyme production was favored by aerating the medium with different gases, such as carbon dioxide. Agitation rates did not appear to have a significant effect on either cell growth or enzyme production. Scaling up the non-aerated process was carried out up to a 1000-dm3 scale with enzyme broth containing up to 21.0 U.cm-3 being produced. Two batches of the enzyme produced on a large scale were used for the first time to synthesize dextran on a 50000-dm3 industrial scale. Dextran yields were up to 95.5% of conventional industrial yields and were achieved in much shorter reaction time intervals (AJONGWEN AND BARKER, 1993).

Although many studies report that oxygen positively affects high enzyme production, there have been conflicting reports on the production of dextransucrase using shaken and static flask cultures (KOEPSELL AND TSUCHIYA, 1952; TSUCHIYA et al., 1952; JEANES, 1965; HEHRE, 1955). Aeration and greater agitation rates of the culture media have been found to not favor the production of dextran or dextransucrase and mild aeration and agitation have been used with uncontrolled pH in these processes (BARKER AND AJONGWEN, 1991; AJONGWEN AND BARKER, 1993; PENNELL AND BARKER, 1992; JEANES et al. 1948). Goya et al. (1995) studied this issue in greater detail, comparing static-flask culture (BARKER AND AJONGWEN, 1991; BROWN AND MCAVOY, 1990; TSUCHIYA et al., 1952; AJONGWEN AND BARKER, 1993; JEANES, 1965; HEHRE, 1955) with shaken-flask culture, and found that enzyme activity in the static flask was 30% higher.

Sucrose as inducer for dextransucrase production

Species of *Leuconostoc* require sucrose in the culture medium as an inducer for the production of glucansucrases, whereas species of Streptococcus produce glucansucrases constitutively by growing on a glucose or fructose medium (KIM AND ROBYT, 1995; NAESSENS, 2005).Specifically, dextransucrase produced by *Leuconostoc mesenteroides* is an extracellular enzyme that is inducible by sucrose and does not require cofactors (JUNG AND MAYER, 1981). All strains of *Leuconostoc mesenteroides* use sucrose as the sole carbohydrate source for dextran production. Other carbohydrates, such as glucose, fructose, manose and lactose, lead to the growth of the microorganism, but do not induce the production of dextransucrase (CAVENAGHI, 2000). In 1999, Quirasco et al. (1999) demonstrated low dextransucrase yields in *Leuconostoc mesenteroides* NRRL B 512 F cultures growing on D-glucose or D-fructose. Moreover, constitutive mutants, which are strains that are able to produce the enzyme in a medium containing only glucose as the carbon source, have been selected (KIM AND ROBYT, 1994b; MIZUTANI, 1994; KITAOKA AND ROBYT, 1998a; RYU et al., 2000)

When high sucrose levels are used for dextran production, the viscosity of the culture affects cell growth and, consequently, enzyme production and the separation of bacterial cells is hindered (TSUCHIYA et al., 1952; MIZUTANI, et al. 1994). With the increase in sucrose concentration in the medium, the broth becomes more viscous due to the concomitant formation of dextran (GOYAL AND KATIYAR, 1997). Moreover, the separation of the enzyme from the broth is also difficult. A number of authors have found that low sugar concentrations obviate this difficulty without affecting high yields of dextransucrase. Tsuchiya et al. (1952) assessed the effect of sucrose concentration on the production of dextransucrase and found that higher sucrose levels induced better enzyme production, but the cultures contained so much dextran that the removal of cells was very difficult. The authors concluded that 2% (w/v) sucrose was the optimal level for dextransucrase production. Santos et al. (2000) studied the effect of sucrose concentration (10 to 120 g/l) on process performance, evaluating the production of dextransucrase, dextran and fructose by sucrose fermentation using *Leuconostoc mesenteroides* NRRL-B512 F in a batch operation in a bioreactor with a total working volume of 1.5 dm3, reporting that cell growth was not inhibited by high sucrose concentrations, but separation of the products from the cells was difficult with sucrose concentrations greater than 40 g/dm.

One study assessed the effects of certain nutrients on dextransucrase production by *Leuconostoc mesenteroides* NRRL B-5 12F and found that an increase in the concentration of sucrose to 4% in the enzyme production medium resulted in an increase in dextransucrase activity (GOYAL AND KATIYAR, 1997). In another study, *Leuconostoc mesenteroides* BLAC was grown in MRS broth or a carrot juice medium and the effects of sugar concentration were examined. When the glucose concentration in the MRS was increased from 110 to 220 mM, the population nearly doubled. In the MRS broth, glucose was completely consumed during fermentation, but this was not the case with the carrot juice medium (CHAMPAGNE AND GARDNER, 2002). A sucrose concentration of 3 and 4% for the production of dextransucrase by Leuconostoc mesenteroides NRRL B 512 F and FT 045 B resulted in the highest enzyme levels (CORTEZI et al., 2005).

A newly isolated strain of *Leuconostoc mesenteroides* (PCSIR-3) produced a different dextran than that produced by *Leuconostoc mesenteroides* NRRL B 512 F. The viscosity of the dextran produced in different media varied in nature. Dextran production was also affected by the sucrose concentration in the media. At the highest initial concentration of sucrose, the highest yield of dextran produced per unit volume was obtained. However, the percentage of the conversion of sucrose into dextran decreased (UI-QADER et al., 2001).

In batch fermentation with *Leuconostoc mesenteroides* NRRL B512 F using sucrose as the substrate, Loppreti and Martinez (1999) found a slowing of the rate of enzyme synthesis with a decrease in the nitrogen/carbon ratio. The addition of nitrogen pulses when the nitrogen/carbon ratio decreased allowed a constant enzyme production rate and shorter fermentation time. Assis et al (2003) studied the effect of sucrose concentration in a feed batch process with *Leuconostoc mesenteroides* at a temperature of 27º C, agitation speed of 120 rpm and pH 6.75. The authors concluded that sucrose concentration plays a fundamental role in the speed of enzyme synthesis and final enzyme activity. An initial sucrose concentration of 15 to 45 g/L, with the subsequent addition of this carbohydrate in periods of one to three hours of fermentation at a rate of 0.5 to 0.8 g/min led to greater sucrose consumption and a final activity of 146.5 UDS/mL.

A number of authors have obtained an improvement in enzyme production with the use of the feed-batch process and the addition of sucrose to the fermentation medium (LOPES, 1979; AURIOL, 1985). Ribeiro et al. (1998) evaluated the production of dextransucrase by *Leuconostoc mesenteroides* NRRL B 512 F in different medium formulations to test the substitution of sucrose with molasses and found molasses to be better for enzyme production.

Concentration of other nutrients in culture medium

Nitrogen and other nutrient requirements of the organism can be supplied by corn steep liquor and mineral salts. A large number of reports are available on the production of dextransucrase under different culture conditions using different enzyme production media (BARKER AND AJONGWEN, 1991; BROWN AND MCAVOY, 1990; TSUCHIYA et al., 1952; EL-SAYED et al., 1990; HEHRE, 1946; LAWFORD et al., 1979; MONSAN et al., 1987; KABOLI AND REILLY, 1980; GOYAL AND KATIYAR, 1997; YAMASHITA AND TAKEHARA, 1989; SHIMURA AND ONISI, 1978; SATO et al., 1989; SNELL et al., 1938; BOHONOS et al., 1941; BOHONOS et al., 1942; GAINES AND STAHLY, 1943; WHITESIDE-CARLSON AND CARLSON, 1949).

Barker and Ajongwhen (1991) demonstrated that the type of yeast extract was much more important than aeration to the results of dextransucrase fermentations. In order to gain information on manipulating cell growth and enzyme production, metabolic engineering has attempted to apply other primary or secondary carbon sources (DOLS et al., 1998a) using a less specific substrate with a lower commercial value. When galactose was used, the yield was the same as in the fermentation with sucrose alone, but with a different production rate. Maltose favored the synthesis of dextransucrase of low molecular weight and lactose inhibited enzyme production. Enzyme activity has been found to increase in the presence of 0.1% Tween 80. Goyal and Katiyar (1997) state that changes in fatty acid composition of the membrane of bacterial cells when grown in presence of Tween 80 are probably responsible for the enhanced production of dextransucrase. The results showed that the effect of the surfactant is not similar on Leuconostoc mesenteroides NRRL B 512 F, as only a marginal increase in enzyme activity was observed.

In another study, Goyal et al. (1995) obtained higher enzyme yields with a low concentration of yeast extract and high concentration of phosphate. The presence of peptone and beef extract in the medium in addition to 2% yeast extract resulted in an enhanced production of dextransucrase. Enzyme activity increased by 30% with both peptone and beef extract. The addition of Tween 80 to the medium enhanced enzyme production and activity was increased by 25%. Magnesium ions marginally stimulated activity. Sodium fluoride enhanced the activity of dextransucrase by 25%.

Stacey (1942), Hehre (1946) and Tsuchiya et al (1952) studied the effect of the concentration of sucrose, corn steep liquor and phosphate on dextran production by whole cultures using high sucrose concentrations. The authors optimized the composition of ingredients and recommend an optimal concentration of 2% of each of sucrose, corn steep liquor and phosphate for dextransucrase production. In another study, a newly isolated strain of Leuconostoc mesenteroides (PCSIR-3) produced a different dextran than that produced by Leuconostoc mesenteroides NRRL B 512 F. Different media compositions used for dextran production demonstrated that media containing CaCl2 produced dextran in higher quantities in comparison to other media (UI-QADER et al., 2001).

It has been reported that enzyme production can be enhanced by increasing the levels of yeast extract and K2HPO4 (Barker and Ajongwen 1991; Tsuchiya et al. 1952). It has also been reported that enzyme production and yield depend on the type of yeast extract used. Barker and Ajongwen (1991) identified yeast extract type as one of the most important factors influencing enzyme yield. One type of antifoam (silicone antifoam) was found to affect enzyme production, but not cell growth. However, Goyal et al. (1995) report that higher yeast extract concentrations do not favor enzyme production, which is contrary to previous findings. It was concluded that the dextransucrase production and yield depend upon the type of yeast extract used. Other nitrogen sources, such as tryptone (EL-SAYED et al., 1990), and peptone (KABOLI AND REILLY, 1980), have also been used in the medium along with yeast extract for dextransucrase production.

Magnesium ions have been found to have no effect on the growth rate of cells or the preformed crude enzyme. Dextransucrase activity was found to increase by l0% in the presence of 100 µM magnesium ions in the medium (GOYAL et al. 1995). These results are similar to those reported earlier for Streptococcus sobrinus (YAMASHITA AND TAKEHARA, 1989).

Shimura and Onisi (1978) report the binary effects of sodium fluoride (NaF) with Streptococcus mutans and found that extracellular dextransucrase activity leading to the formation of soluble dextran increased 10 fold, whereas enzyme activity leading to the formation of insoluble dextran decreased. In 1997, other studies found that NaF enhanced dextransucrase activity. Cell growth rate and in vitro activity of dextransucrase were not affected by NaF. Enzyme activity increased by 25% with 100 µM NaF when present in the medium in comparison to the control without NaF (GOYAL et al., 1995). The enhancement of dextransucrase activity with NaF was not as significant as that reported for Streptococcus mutans.

In 1998, Kitaoka and Robyt (1998A) obtained a stable high-producing constitutive mutant, *Leuconostoc mesenteroides* B 512 FMC-16 (formerly *Leuconostoc mesenteroides* B 512 FMC/6HG8), that produced approximately 20 to 25 IU.mL-1 of dextransucrase without dextran in a glucose medium. The authors studied the conditions for the cultivation of *Leuconostoc mesenteroides* B 512 FMC-16, obtaining a high-producing constitutive mutant of dextransucrase in a jar fermentor by changing the amount of nitrogen (bactopeptone and yeast extract) and the amount of carbon (glucose) with controlled pH. The optimal conditions were found to be 3.76 g L-1 of bactopeptone, 3.76 g L-1 of yeast extract and 33.8 g L-1 of glucose, with the pH controlled at 6.0 for 23 h of fermentation.

Dextransucrase purification

Dextransucrase is produced in soluble and insoluble forms. The insoluble form accounts for 60 to 95% of the total activity produced and is recovered in the pellet with the cells after centrifugation (REMAUD-SIMEON et al., 1994; KOBAYASHI AND MATSUDA, 1974; SMITH, 1970). Purification methods, such as ultra-filtration, fractionation by polyethylene glycol, salt, glycerol and alcohol precipitation, phase partitioning and chromatography, have been standardized and successfully used for the purification of dextransucrase from different strains (NIGAM et al., 2006; DOLS et al., 1998b; GOYAL AND KATIYAR, 1994; KITAOKA AND ROBYT, 1998a; TSUCHIYA et al., 1955; FUKUI et al., 1974; RUSSEL, 1979; PAUL et al., 1984; LOPEZ-MUNGUIA et al., 1993; MAJUMDER et al., 2007; CHLUDZINSKI et al. 1974). A wide variety of techniques have been researched for the purification and characterization of dextransucrase from Leuconostoc mesenteroides NRRL B 512 F (ROBYT AND WALSETH, 1979; GOYAL AND KATIYAR, 1994; MONSAN et al., 1987; RHEE AND LEE, 1991; KABOLI AND REILLY, 1980; KOBAYASHI AND MATSUDA, 1980; MILLER et al., 1986; PAUL et al., 1984; FU AND ROBYT, 1990; MONSAN AND LOPEZ, 1981).

Purification by nonionic precipitation with polyethylene glycol

The fact that dextransucrase exists in either single or multiple forms with molecular weight ranging from 64,000 to

245,000 (GOYAL AND KATIYAR, 1994; KOBAYASHI AND MATSUDA, 1980; MILLER et al.,. 1986; FU AND ROBYT, 1990; KOBAYASHI AND MATSUDA, 1986; WILLEMOT et al., 1988) and that its association with the dextran polymer forms high molecular weight aggregates in solution (RUSSEL, 1979; FUNANE et al., 1995) enables its purification by precipitation using the nonionic hydrophilic polymer polyethylene glycol (GOYAL AND KATIYAR, 1994; HONING AND KULA, 1976; MIEKKA AND INGHAM, 1978). Polyethylene glycols (PEGs) are nonionic hydrophilic polymers of ethylene oxide typically ranging in size from 200 Da to 20 kDa and are very soluble in water due to the ether oxygen spread along the length of the polymer, which are strong Lewis bases and form hydrogen bonds with water molecules (HARISON, 1993), PEGs are also known to selectively precipitate protein of high molecular weight or existing in aggregated forms (MIEKKA AND INGHAM, 1978). They also have the advantage of being readily removed by dialysis. PEGs are the preferred non-ionic polymers for protein precipitation because the viscosity of concentrated solutions is lower than with other nonionic polymers. Moreover, the formation and equilibration of precipitates take significantly less time with PEG as the precipitating agent than with ammonium sulfate or ethanol (DEUTSCHER, 1990; ASENJO, 1990).

Purification by two-phase partitioning using different PEGs have been reported for dextransucrase from various strains of *Leuconostoc mesenteroides* (NIGAM et al., 2006; DOLS et al., 1998B; AJONGWEN AND BARKER, 1993; GOYAL AND KATIYAR, 1994; MONSAN et al., 1987; QUIRASCO et al., 1999; REMAUD-SIMEON et al., 1994; FUKUI et al., 1974; PAUL et al., 1984; LOPEZ-MUNGUIA et al., 1993; PURAMA AND GOYAL, 2008c; OTTS AND DAY, 1988; REMAUD-SIMEON et al., 1991; BARKER et al., 1987) and *Streptococcus* (RUSSEL, 1979). This is a simple, effective, single-step method for dextransucrase purification (GOYAL AND KATIYAR, 1994; RUSSEL, 1979; LOPEZ-MUNGUIA, 1993; MIEKKA AND INGHAM, 1978; HARISON, 1993; DEUTSCHER, 1990; ASENJO, 1990; PURAMA AND GOYAL, 2008c; MAJUMDER et al., 2008). Russel (1979) purified dextransucrase from *Streptococcus* mutans by precipitation using PEG 400 and 6000 and reported that the higher molecular weight PEG 6000 precipitated other non-dextransucrase proteins, while PEG 400 gave greater precipitation specificity. Different molecular weights of PEGs have been used to purify dextransucrase from *Leuconostoc mesenteroides* NRRL B 512 F (GOYAL AND KATIYAR, 1994). PEG 400 has been reported to give dextransucrase with the greatest specific activity of 8.7 and 80% yield. Goyal et al. (1995) purified dextransucrase from Leuconostoc mesenteroides NRRL 512 F in three successive precipitation steps using PEG 400. Purified dextransucrase exhibited maximal activity at 30o C, pH 5.2 and 10% sucrose as the substrate. Among the various stabilizers used (glycerol, PEG 8000, dextran and Tween 80), glycerol provided maximal stability for the enzyme against activity losses at temperatures of 0º and 30º C. NIGAM et al. (2006) studied how extracellular dextransucrase from *Leuconostoc mesenteroides* NRRL B 512 F can be purified through phase partitioning using PEGs and used in the generation of dextran. The authors concluded that PEG 6000 produced better purification than PEG 400.

In another study, total glucosyltransferase activity of 3.5 U/mL produced by *Leuconostoc mesenteroides* NRRL B-1299 was measured in a batch culture. The enzymes from the supernatant were purified 313 times using aqueous two-phase partitioning between dextran and PEG phases, yielding a preparation with 18.8 U/mg of protein (PAUL et al., 1984). Recently, Purama and Goyal (2008D) reported an efficient method for purifying dextransucrase from Leuconostoc mesenteroides NRRL B-640 using polyethylene glycol followed by Sephacryl S-200HR gel-filtration chromatography. In 2009, the same authors (GOYAL AND PURAMA, 2009) used polyethylene glycol (25% v/v PEG 400) fractionation to obtain extracellular dextransucrase purified from *Leuconostoc mesenteroides* NRRL B-640 with 16-fold purification in a single step. The authors concluded that dextransucrase remains in single molecular form in its native state and displays multiple forms only under denaturing conditions when it is heated before loading and when containing sodium dodecyl sulfate or 2-mercaptoethanol.

Purification by ion-exchange and affinity chromatography

Dextransucrase forms aggregates with its polysaccharide product and therefore occurs in multiple molecular forms (ROBYT AND WALSETH, 1979; KOBAYASHI AND MATSUDA, 1988), making its purification and dextran-free obtainment rather difficult (ROBYT AND WALSETH, 1979; MILLER et al., 1986; FU AND ROBYT, 1990). The presence of dextran, dextranase or carbohydrates in dextransucrase preparations compromises the study of the enzyme (ROBYT AND WALSETH, 1979; KIM AND ROBYT, 1994b; KOBAYASHI AND MATSUDA, 1980; MILLER et al., 1986; FU AND ROBYT, 1990; KOBAYASHI AND MATSUDA, 1986; WILLEMOT et al., 1988).

Sephadex gels have been used as affinity materials for the purification of the enzyme from *Leuconostoc mesenteroides* (MILLER et al., 1986; KOBAYASHI et al., 1986) and species of Streptococcus (HAYASHI et al., 1988; KOBSV, 1990; HAMELIK AND MCCABE, 1982). The elution of enzymes from Sephadex has been achieved by using solutions of urea, guanidine HCl (MILLER et al. 1982), or sodium dodecyl sulfate (KOBS et al. 1990). This step was followed by extensive dialysis for the removal of these chemicals. Dextrans have also been used as eluting agents (KOBAYASHI et al. 1986), and were removed by treatment with dextranase, followed by the chromatographic removal of dextranase, although some studies report that the native conformation of dextransucrase may be affected by the action of dextranase (KOBAYASHI AND MATSUDA, 1980; MILLER et al., 1986; FU AND ROBYT, 1990; WILLEMOT et al., 1988).

Miller et al. (1986) purified dextransucrase with a combination of dextranase treatment and ion-exchange and affinity chromatography. Kim and Kim (1999) designed a simple sequence of membrane concentration and DEAE-cellulose chromatography capable of obtaining a purified dextransucrase from *Leuconostoc mesenteroides* B 512 FMCM with the highest specific activity (248.8 IU/mg of protein) ever reported in high yield (overall 88.7%) for dextransucrase. In the absence of sucrose, the enzyme was able to transglycosylate glucose from dextran and formed a series of isomaltodextrins connected to acceptors with a high yield (PORATH AND FLODIN, 1959).

Other purification methods

Robyt and Walseth (1979), Miller et al. (1986), Fu and Robyt (1990) report the purification of dextransucrase using dextranase to remove dextran from the enzyme preparation and obtained amounts of purified enzyme in mg to g. Purification required several steps consisting of the hydrolysis of dextran by dextranase, the separation of the enzyme from oligosaccharides and dextranase and the removal of other proteins from the dextransucrase protein.

Studies on the purification of dextransucrase from *Leuconostoc mesenteroides* B 512 F(M) have demonstrated either low yields or have failed to remove important impurities, especially polysaccharides (ROBYT AND WALSETH, 1979; KOBAYASHI AND MATSUDA, 1980;

MILLER et al., 1986; KIM AND KIM, 1999). Kim and Robyt (1994a, 1994b) overcame this problem by constructing a partial dextransucrase constitutive mutant from the B 512 FM strain (*Leuconostoc mesenteroides* B 512 FMC), which greatly facilitated the purification of dextransucrase with increasing activity. Kitaoka and Robyt (1998A) later obtained a stable high-producing constitutive mutant, *Leuconostoc mesenteroides* B 512 FMC-16 (formerly *Leuconostoc mesenteroides* B 512 FMC/6HG8), that produced approximately 16 IU/mL of dextransucrase without dextran in a glucose medium. The authors describe an extremely simple purification procedure for the enzyme using a hollow fiber column in the presence of Tween 80 and $CaCl_2$. This procedure gives a single protein band on SDS-PAGE and a specific activity of 183 IU/mg (KITAOKA AND ROBYT, 1998b).

Dextransucrase behavior

There are few reports on the stabilization of dextransucrase by various agents (MILLER AND ROBYT, 1984). Kim and Robyt (1994B) studied the effect of some new agents on the stabilization of dextransucrase and describe certain optimal conditions for dextransucrase production from *Leuconostoc mesenteroides* NRRL B 512 F and some properties of the enzyme. Girard and Legoy (1999) investigated the behavior of dextransucrase from *Leuconostoc mesenteroides* NRRL-B 512 F in the presence of organic solvents. The activity and stability of this enzyme were studied in the presence of various concentrations of dimethysulfoxide (DMSO), dimethylformamide, ethanol, acetone and acetonitrile. Stability was measured at 4º C and 30º C. Surprisingly, dextransucrase was more active after a period of contact with some solvents, especially DMSO or ethanol, than the acetate buffer. After two days in 20% DMSO at 4º C, 300% of the initial activity was achieved and 250% was achieved at 30º C. This increase in activity was also observable after two days in 20% ethanol, in which 180% of the initial activity was measured.

Some authors have reported dextransucrase activation by the addition of dextran (KOBAYASHI AND MATSUDA, 1980; MILLER et al., 1986; KOBAYASHI AND MATSUDA, 1986; WILLEMOT et al., 1988; GERMAINE et al., 1974; GERMANINE et al., 1977) whereas others have not found this effect. According to Robyt et al. (1995), dextransucrase contains an allosteric site to which dextran binds, thereby inducing a favorable conformation for the synthesis of dextran from sucrose. Germaine et al. (1974; 1977) found that the addition of dextran to dextransucrase digests from *Streptococcus* mutans increased the rate of dextran synthesis. The authors report that the rate was dependent on the size of the dextran chain and reached a maximum when the average size of the added dextran was 30 glucose residues. Glucansucrases produced by Streptococci are constitutive and are produced by the organisms when grown in glucose, fructose or mannitol media (CIARDI et al., 1977). Sucrose is not required in the medium for enzyme production and, hence, dextran is not produced along with the dextransucrase in the culture supernatant.

Kobayashi and Matsuda (1980; 1986) also report that purified dextransucrases produced by both *Leuconostoc mesenteroides* B 512 F and Streptococcus sp. were stimulated by the addition of dextran, although both enzymes could synthesize dextran without the addition of dextran to the digests. The rate of dextran synthesis in dextran-free digests was accompanied by a lag-period that was eliminated by the addition of exogenous dextran. Purified enzyme stability and enzyme activity can be improved with the addition of Ca2+. A lack of Ca2+ during enzyme incubation can cause irreversible desnaturation of the enzyme. The addition of small quantities of $CaCl_2$ (0.001% to 0.1%) can double enzyme production, but concentrations greater than 0.1% cause the death of the microorganism (Alsop 1983).

Although the commercial dextran is produced using the whole-culture method, its production also can be achieved enzymatically with cell-free culture supernatants containing dextransucrase in the presence of sucrose. Thus, production conditions can be more easily controlled and the acquired polymer is purer, more uniform and easier to purify, with the added benefit of obtaining fructose as a valuable co-product when compared with dextran produced using the whole-culture method (LEATHERS, 2002; ROBYT, 1985; ALSOP, 1983). The enzymatic synthesis of dextran was first described by Hehre (1941; 1946; 1951) and Hehre and Sugg (1942). The rapid enzyme formation and high yield were then reported by Koepsell and Tsuchiya (1952).

The addition of various substances has been shown to increase the dextran yield (Stacey 1942; Darker and Stacey 1939; Carruthers and Cooper 1936; Stacey and Youd 1938; Bouillenne 1938; Hassid and Barker 1940; Stahly 1943). Moreover, molecular weight and dextran production depend on process variables, such as temperature, sucrose and the acceptor concentration (SANTOS, 1996; PEREIRA et al., 1998). Kitaoka and Robyt (1998A) describe three major problems with strains of Leuconostoc mesenteroides that must be induced by sucrose in the culture and state that the characteristics and yield of dextran vary considerably between strains:

(1) Only one half of the carbon source – the fructose part of sucrose – is used for growth, thereby limiting the amount of carbon in the medium;

(2) The culture fluid becomes highly viscous during growth due to an increase in the amount of dextran, which presents problems with regard to the agitation of the culture and removing cells from the culture fluid containing the enzyme;

(3) The enzyme is difficult to purify because of the high viscosity and presence of a large amount of glucan, which must be removed.

Enzyme storage

The enzyme should be stored at low temperatures and in the correct pH due to its high sensitivity. When stored at 4º C, the purified enzyme loses its stability. Thus, the use of stabilizers, such as low-weight dextran, polyethylene glycol, methyl cellulose and neutral detergents, is necessary (CURRALERO, 1993). Souza (1993) report that lyophilized enzyme stored at 5º C maintains its activity for five years. Heavy metals cause enzyme denaturation and high temperatures (above 30º C) destroy the enzyme (BAZAN, 1993).

3. Factors that affect dextran synthesis

Carbon source

Tarr and Hibbert (1931) stated that sucrose was the only suitable carbohydrate substrate for the production of dextran. After other scientists reported that dextran cannot be produced from dextransucrase on glucose, mixtures of glucose and fructose or any other naturally occurring sugar, the requirement of sucrose was confirmed and fructose or any other naturally occurring sugar; sucrose is absolutely required (ROBYT, 1985).

When the bacterium grows in a sucrose-rich media, the enzyme released – dextransucrase – converts excess sucrose to dextran and fructose (TSUCHIYA, et al. 1952), but when a high sucrose concentration is used, broth viscosity increases and the control of the process becomes more difficult. When

present in the culture media as molecules acceptors, the complementary sugars maltose, lactose and galactose, together with sucrose, can influence the molecular weight of dextran by allowing the growing chain to be separated from the active site of the enzyme and transferred to the acceptor (ROBYT AND WALSETH ,1978; ROBYT AND EKLUND, 1983; DOLS et al., 1998B). Moreover, polydispersity is improved when complementary sugars are used (SANTOS et al., 2005).

The use of shaken-flask or static-flask culture

Hehre (1955), Jeanes (1965), Koepsell and Tsuchiya (1952), Tsuchiya et al (1952) have studied the production of dextran using shaken-flask and static-flask cultures, but report diverging results. Ajongwen and Barker (1993), Barker and Ajongwen (1991), Jeanes et al (1948), Pennell and Barker (1992) found that aeration and greater agitation rates of the culture media do not favor the production of dextran or dextransucrase. Dextran production has been studied in a number of studies over the years. Authors have employed agitation and mild aeration with uncontrolled pH in the batch (HEHRE, 1955; JEANES, 1965; TSUCHIYA et al., 1952) and with controlled pH in the fed batch as well as (BARKER AND AJONGWEN, 1991; BROWN AND MCAVOY, 1990; AJONGWEN AND BARKER, 1993) and semi continuous processes with semi-continuous processes (LAWFORD et al., 1979; MONSAN et al., 1987). In 1995, Goyal et al. (1995) re-investigated and compared enzyme production in still-flask and shaken-flask cultures and concluded that enzyme activity in the static flask was 30% higher.

Optimal pH and temperature

A number of studies on enzyme stability have been carried out. Kobayashi and Matsuda (1980) published an important study on dextransucrase behavior under different pH and temperature conditions, reporting that the best enzyme activity was achieved with pH 6.0 and a temperature of 30º C and that temperature and pH variations cause non-linear changes in enzyme activity. A number of authors report an optimal temperature of 30º C (NAESSENS et al., 2005; RHEE AND LEE, 1991; KABOLI AND REILLY, 1980; KOBAYASHI AND MATSUDA, 1980; PURAMA AND GOYAL, 2008d; MAJUMDER et al., 2007; KOBAYASHI et al., 1986; ERHARDT AND KUEGLER, 2008; MONCHOIS et al., 1997; KANG et al., 2008; ILIEV et al., 2008; OLVERA et al., 2007; YOKOYAMA et al., 1985; KIM AND DAY, 2008; YALIN et al., 2008; ERHARDT et al., 2008; HELLMUTH et al., 2008), whereas others report 35º C to be the optimal temperature (YOKOYAMA et al., 1985; CHELLAPANDIAN et al., 1998; KOBAYASHI AND MATSUDA, 1976; KOBAYASHI AND MATSUDA, 1975; UL-QADERV, 2007).

Maximal yields have also been reported at temperatures of 25ºC (BAILEY et al., 1957), 28ºC (NAM et al., 2008), 29ºC (ZHANG et al., 2008), 34ºC, 40ºC (KOBAYASHI AND MATSUDA, 1976; YALIN, 2008) and 45ºC (KOBAYASHI AND MATSUDA, 1975).In an industrial production of dextran, optimal pH for enzyme production was reported to be 6.5 to 7.0, whereas optimal pH for enzyme activity was 5.0 to 5.2 (ALSOP, 1983).Dextransucrase is most stable and most active in the pH range from 5.0 to 5.5 (ALSOP, 1983; NAESSENS et al., 2005; RHEE AND LEE, 1991; KABOLI AND REILLY, 1980; MILLER et al., 1986; ASSIS, et al., 2003; CHLUDZINSKI et al., 1974; PURAMA AND GOYAL, 2008D; KOBAYASHI et al., 1986; ERHARDT AND KUEGLER, 2008; MONCHOIS et al., 1997; KANG et al., 2008; ILIEV et al., 2008; OLVERA et al., 2007; YOKOYAMA et al., 1985; KIM AND DAY, 2008; YALIN et al., 2008; ERHARDT et al., 2008; KOBAYASHI AND MATSUDA, 1976; KOBAYASHI AND MATSUDA, 1975; UL-QADER et al., 2007; NAM et al., 2008; EOM et al., 2007; HUANG et al., 1979), although some authors report optimal pH for enzyme activity above 5.5 (KOBAYASHI AND MATSUDA, 1980; FUKUI et al., 1974; HELLMUTH et al., 2008; KOBAYASHI AND MATSUDA, 1976; KOBAYASHI AND MATSUDA, 1975; ZHANG et al., 2008; TAKASHIO AND OKAMI, 1983). The different optimal pH and temperature values may be explained by the different strains or species of *Leuconostoc* or *Streptococcus*, which produce different kinds of glucansucrases and synthesize glucans with different structures and properties (KOBAYASHI AND MATSUDA, 1980; FUKUI et al., 1974; HELLMUTH et al., 2008; KOBAYASHI AND MATSUDA, 1976; KOBAYASHI AND MATSUDA, 1975; ZHANG et al., 2008; TAKASHIO AND OKAMI, 1983).

4. Dextran purification

When dextran production ceases, the polymer can be precipitated from the fermentation medium by alcohol precipitation and purified by further precipitation after re-dissolution in water (NAESSENS et al., 2005). Depending upon the strain of the organism that produces it, dextran may be water-soluble, partially soluble or insoluble (HAMDY et al., 1954). In reaction mixtures containing culture filtrates, varying amounts of levan are produced concomitantly with dextran. These amounts are usually small. Since dextran is precipitable with lower concentrations of alcohol than those needed for levan, this provides a convenient method for the recovery of either one free of the other (HAMDY et al., 1954).

Dextran produced using the whole-culture method (LEATHERS, 2002; ROBYT, 1985; ALSOP, 1983) may have the cell debris removed through centrifugation. This conventional process has the disadvantage of propagating cells in comparison to a cell-free culture in which the cells are centrifuged prior to dextran production.

5. Clinical dextran

The application possibilities of wild dextran are due to characteristics that make each dextran different from another, such as molecular weight (JEANES, 1966). High molecular weight is used in petroleum recovery, whereas average molecular weights are employed in the chemical industry. This section specifically reports the application of low-molecular-weight dextran (molar weight range from 40,000 to 70,000 Da), which is known as clinical dextran and has especially important applications in the pharmaceutical industry. Clinical dextran, commercially denominated dextran 40 and dextran 70, is extensively used as a raw material in medicine, a blood plasma extender, blood flow improver, ophthalmic solutions and to preserve human organs during surgeries (ROBYT, 1986). Anticoagulant activity with low toxicity has been obtained using the dextran derivative, dextran sulfate (7300 MW) (ROBYT, 1986; ALSOP, 1983; RICKETTS, 1959; TAKEMOTO AND LIEBHARBER, 1962; MBEMBA et al., 1992; MITSUYA et al., 1988; DE BELDER, 1987; GUGGENHEIM AND SCHROEDER, 1867).The main characteristics of clinical dextran are its high heat resistance (sterilization), storage capability, the fact that it does not depend on the blood type that will receive it, few side effects, low rate of disease transmission low and its capability to be cross linked with metallic ions (RICKETTS, 1959; TAKEMOTO AND LIEBHARBER, 1962), protein and hemoglobin.

Clinical dextran obtained by hydrolysis

Commercially, dextran is produced by the whole-culture fermentation of *Leuconostoc mesenteroides*. When grown in a medium containing sucrose and other required nutrients, this

organism produces a dextran with a molecular mass of about 5x108 daltons. This is too large for blood plasma substitutes. Relatively low-molecular-weight dextrans (MW of a few hundred to a million) are produced from this dextran by controlled acid hydrolysis with HCl or H2SO4 (RICKETTS, 1959; TAKEMOTO AND LIEBHARBER, 1962), dextranase action (NOVAK AND STOYCOS, 1958; NOVAK AND WITT, 1961) or the use of ultra-sound (CURRALERO, 1993) followed by organic solvent fractionation (ZIEF, 1956; WOLF et al., 1954). Yields are relatively low due to losses during hydrolysis and fractionation. The production of clinical dextran can be obtained by a reduction in crude dextran molecular weight through hydrolysis. Several studies have reported the use of enzymatic hydrolysis or ultra-sound, but the most applied method industrially is acid hydrolysis, which does not require sophisticated equipment and is easier to control (WOLF et al., 1954).It has been shown that dextran degradation can be accomplished through extended incubation of the culture medium (HAMDY et al., 1954). Jeanes et al (1948) report that this hydrolysis is due to "autolysis of the *Leuconostoc* cells".

Strategic production of clinical dextran

There are a number of studies on ways to improve clinical dextran production using purified dextransucrase (PAUL, 1986; REMAUD-SIMEON et al., 1991).The molecular weight distribution of dextran synthesized by dextransucrase preparations can vary considerably (ROBYT AND WALSETH, 1979; CAVENAGHI, 2000; RUSSEL, 1979; PAUL, 1992; TSUCHIYA, 1955). The presence of high sucrose concentration (ALSOP, 1983; KIM AND KIM, 1999) through allosteric obstruction (TANRISEVEN AND ROBYT, 1993), other sugars denominated acceptors, such as maltose (HEINCLE et al., 1999; RODRIGUES, 2003; REMAUD-SIMEON et al., 1991; CURRALERO, 1993), fructose, glucose, rafinose and isomaltose, through inhibition, oligosaccharides or low molecular dextran in a reaction mixture promotes the production of low-weight dextran (TSUCHIYA et al., 1955).

The addition of maltose during clinical dextran production promotes panose production and inhibits dextran formation from the action of dextransucrase (HEINCLE et al., 1999).Studying the effects of sucrose concentration, maltose/sucrose ratio and temperature on the production of clinical dextran production, Basán (1993) found that a high sucrose concentration was the factor with the greatest effect on the production of low-molecular-weight dextran. An increase in the maltose/sucrose ratio also favored the production of clinical dextran, although to a lesser degree than sucrose concentration. A decrease in temperature promoted a greater yield of clinical dextran, but the effect of this variable was smaller in comparison to the two other variables. Kim et al. (2003) obtained similar results and report that pH had no significant effect on the production of clinical dextran.

Kim and Day (2008) studied a new process for the production of clinical dextran using a mixed-culture fermentation system (*Lipomyces starkeyi* ATCC 7454 and Leuconostoc mesenteroides ATCC 10830), which was simpler and more economical than traditional methods and proved to be an industrially practical method for producing clinical dextran. Chen et al. (2008) developed a new separation method to control the molecular weight distribution and improve the quality of clinical dextran using electric ultrafiltration-solvent crystallization (EUSC), combining electro-ultrafiltration (EU) with solvent crystallization (SC).

6. Dextran applications

Dextrans are important commercially available polysac-

Table 2: An overview of the recent uses of dextran in different areas.

APPLIC.	COMMENTARY	REF.
medicine	Dextran was used as a simple, reliable, cheap and non-radioactive method for estimating blood volume parameters in pregnant sheep, and may prove useful in other settings.	(Rumball et al. 2008)
medicine	Addition of dextran sulfate to blood cardiolegia attenuates reperfusion injury in a porcine model of cardiopulmonary bypass.	(Banz et al. 2008)
medicine	The amphotericin B (AmpB)-encapsulated polymeric micelle of poly(d,l-lactide-co-glycolide) (PLGA) grafted-dextran(DexLG) copolymer can be considered to potential antifungal agent carriers.	(Choi et al. 2008)
pharma-ceutical	Three different molecular weight grades of dextrans have been shown to reduce the hygroscopicity of the amorphous herbal extracts purely by a dilution effect.	(Tong 1973)
chemistry	Chemical cleaning of polycarbonate membranes fouled by BSA/dextran mixtures	(Zator et al. 2009)
synthesis	Construction of a fusion enzyme of dextransucrase and dextranase: application for one-step synthesis of isomalto-oligosaccharides.	(Kim et al. 2009)
medicine	Dextran Polymer Hemostatic Dressing Improves Survival in Liver Injury Model	(Clay et al. 2008)
medicine	Dextran sodium sulfate-induced colitis causes rapid bone loss in mice.	(Hamdani et al. 2008)
medicine	Dextran-specific IgG response in hypersensitivity reactions to measles-mumps-rubella vaccine	(Zanoni et al. 2008)
medicine	Dextran Molecular Weight effects on Red Blood Cell Aggregation.	(Neu et al. 2008)
medicine	Free Energy of Sickle Hemoglobin Polymerization: A Scaled-Particle Treatment for Use with dextran as a Crowding Agent	(Liu et al. 2008)
medicine	The complement inhibitor lowmolecularweightdextransulfate prevents TLR2-mediated activation of human natural killer cells	(Spirig et al. 2008)
nanotech-nology	In vitro hydrolytic degradation of poly(3-caprolactone) grafted dextran fibers and films.	(Bajgai et al. 2008)
pharma-ceutical	Induction of high antitoxin titers against tetanus toxoid in rabbits by intranasal immunization with dextran microspheres.	(Tabassi et al. 2008)
nutrition	Interactions between b-lactoglobulin and dextran sulfate at near neutral pH and their effect on thermal stability.	(Vardhana bhuti et al. 2008)
nutrition	Molecular mass distribution of dextran in Brazilian sugar and insoluble deposits of cachaça.	(Aquino and Franco 2009)
biomater-ials	Non-cytotoxic, in situ gelable hydrogels composed of N-carboxyethyl chitosan and oxidized dextran.	(Weng et al. 2008)
medicine	Then antioxidant hydrogel could be of potential use for cosmetic and pharmaceutical purposes as carrier of vitamin E that is an antioxidant that reduces erythema, photoaging, photocarcinogenesis, edema, and skin hypersensitivity associated with exposure to ultraviolet B (UVB) radiation, because of its protective effects.	(Cassano et al. 2009)
medicine	The administration of NF-B decoy ODNs leads to an amelioration of DSS induced colitis, suggesting administration of NF-B decoy ODNs may provide a therapeutic approach for UC.	(Xiang et al. 2009)
nanotech-nology	Biodegradable nanoparticles made from polylactide-grafted dextran copolymers.	(Nouvel et al. 2009)
medicine	Dextran sulfate reduces ischaemia/reperfusion injury bymodulating the activation of complement and the MAPK pathway.	(Gajanaya ke et al. 2008)
biomaterials	Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering.	(Liu and Chan-Park 2009)
synthesis	A series of dextran hydrogels were synthesized with potassium chloride as a crosslinker. 13C-NMR and 17O-NMR were used to study the crosslinking role of potassium chloride.	(Chen et al. 2010)
medicine	Concentration and Time efects of dextran exposure on endothelial cell viability, attachment, and inflammatory marker expression in vitro.	(Rouleau et al. 2010)
medicine	A newly developed chemically crosslinked dextran poly(ethylene glycol) hydrogel for cartilage tissue engeneering.	(Jukes et al. 2008)
medicine	Water-solubel Taxol conjugates with dextran and Targets Tumor Cells by Folic Acid Immobilization.	(Nakamura et al. 2010)
medicine	Fos proteins suppress dextran sulfate sodium-induced colitis through inhibition of NF-B.	(Taada et al. 2010)

charides (SARWAT et al., 2008) There is a considerable volume of literature devoted to the numerous uses of native dextrans, partially degraded dextrans and their derivatives (LEATHERS, 2002; REMAUD-SIMEON et al., 2000; DE BELDER et al., 1996). This amazing polymer has found applications in the food, pharmaceutical (Pharmacia Biotech, 1997) and chemical industries as an adjuvant, emulsifier, carrier and stabilizer (GOULAS et al., 2004), as a therapeutic agent in restoring blood volume for mass casualties (NAESSENS et al., 2005), as nutraceuticals (NAESSENS et al., 2005; GOULAS et al., 2004), as stabilizers, bulking agents, immuno-stimulating agents or prebiotic compounds (EGGLESTON AND COTE, 2003), as a thickener for jam and ice cream (NAESSENS et al., 2005; FOOD, 2000), preventing the crystallization of sugar, improving moisture retention and maintaining the flavor and appearance of various food items (PURAMA AND GOYAL, 2005; PURAMA AND GOYAL, 2008d; UL-QADER et al., 2005); as a cross-linked dextran for the separation and purification of protein (LEATHERS, 2002; ROBYT, 1985; SARWAT et al., 2008; PHARMACIA BIOTECH, 1997); as an alleviator of iron deficiency anemia (AHSAN, 1998; AUERBACH et al., 1988), as an enhancer of magnetic resonance imaging techniques (DE BELDER et al., 1996); as an anticoagulant similar to heparin containing sulfate esters (ALSOP, 1983); as a potent inhibitor of ribonuclease; as an antiviral agent in the treatment of human immunodeficiency virus (PIRET et al., 2000; UENO AND KUNO, 1987; JAMES, 1988); as a source of energy in foods; and as a sweetener (NAESSENS et al., 2005).

7. Acknowledgements

The authors thank the FAPESP – São Paulo Research Foundation for financial support.

8. References

AHSAN, N. Intravenous infusion of total dose iron is superior to oral iron in treatment of anemia in peritoneal dialysis patients: a single center comparative study. *J. Am. Soc. Nephrol.*, 9, 1998. 664-668.

AJONGWEN, N. J.; BARKER, P. E. Scale-up studies of nonaerated fed-batch fermentation of dextransucrase and the industrial synthesis of dextran using the enzyatic route. *J. Chem. Technol.*, 56, 1993. 113-118.

ALSOP, R. M. Industrial production of dextrans. In: BUSHELL, M. E. *Microbial Polysaccharides. Amsterdam*: Elsevier, 1983. p. 1-44.

AQUINO, F. W. B.; FRANCO, D. W. Molecular mass distribution of dextran in Brazilian sugar and insoluble deposits of cachaça. *Food Chemistry*, 114, 2009. 1391-1395.

ASENJO, J. A. Separation processes in biotechnology. New York: Marcel Dekker, 1990. 329-358.

AUERBACH, M. et al. clinical use of the total dose intravenous infusion of iron dextran. *J. Lab. Clin. Med.*, 111, 1988. 566-570.

BAILEY, R. W. et al. Immunopolysaccharides. Part VI. The isolation and properties of the dextransucrase of Betacoccus arabinosaceous. *J. Chem. Soc.*, 1957. 3530-3536.

BAJGAI, M. P. et al. In vitro hydrolytic degradation of poly(E-caprolactone) grafted dextran fibers and films. *Polymer Degradation and Stability*, 93, 2008. 2172-2179.

BANZ, Y. et al. Addition of dextran sulfate to blood cardioplegia attenuates reperfusion injury in a porcine model of cardiopulmonary bypass. *European Journal of Cardiothoracic Surgery*, 34, 2008. 653-660.

BARKER, P. E.; AJONGWEN, N. J. The production of the enzyme dextransucrase using nonaerated fermentation techniques. *Biotechnol. Bioeng.*, 37, 1991. 703-707.

BARKER, P. E.; GANETSOS, G.; AJONGWHEN, N. J. A novel approach to the production of clinical grade dextran. *J. Chem. Techol. Biotechnol*, 57, 1993. 21-26.

BARKER, P. E.; ZAFAR, I.; ASLOP, R. M. Dextrans. In: VERRAL, M. S.; HUDSON, M. J. In *Separations for biotechnnology. Chichester*: Ellis Horwood, 1987. p. 127-151.

BLANSHARD, J. M. V.; MITCHELL, J. R. Polysaccharides in *Food*, London, p. 253-254, 1979.

BOHONOS, N.; HUTCHINGS, B. L.; PETERSON, W. H. *The activity of vitamin B6 analogues for lactic acid bacteria. J. Bacteriol*, 41, 1941. 40.

BOHONOS, N.; HUTCHINGS, B. L.; PETERSON, W. H. Pyridoxine nutrition of lactic acid bacteria. *J. Bacteriol*., 44, 1942. 479-485.

BOUILLENNE, R. The culture of sugar-house gum in laboratory. *Sucr. Belge*., 57, 1938. 201-207.

BROWN, D. E.; MCAVOY, A. A pH controlled fed-batch process for dextransucrase production. *J. Chem. Tech.* Biotechnol., 48, 1990. 405-414.

BUCHHOLZ, K.; MONSAN, P. Dextransucrases. In: WHITAKER, J. R. *Handbook of food enzymology*. New York: Marcel Dekker, 2001.

CARRUTHERS, A.; COOPER, E. A. Enzyme formation and polysaccharide synthesis by bacteria. II. *Biochem. J.*, 30, 1936. 1001-1009.

CASSANO, R. et al. A novel dextran hydrogel linking trans-ferulic acid for the stabilization and trasdermal delivery of vitamin E. *European Journal of Pharmaceutics and Biopharmaceutics*, 72, 2009. 232-238.

CHAMPAGNE, C. P.; GARDNER, N. J. Effect of process parameters on the production and drying of Leuconostoc mesenteroides cultures. *Journal of Industrial Microbiology & Biotechnology*, 28, 2002. 291-296.

CHELLAPANDIAN, M. et al. Production and properties of a dextransucrase from Leuconostoc mesenteroides IBT-PQ isolated fro "pulque", a traditional Aztec alcoholic beverage. *J. Ind. Microbiol. Biotechnol.*, 21, 1998. 51-56.

CHEN, J.; YI, J. Z.; ZHANG, L. M. Water in dextran hydrogels. *Journal of Applied Polyer Science*, 117, 2010. 1631-1637.

CHEN, S. et al. Clinical dextran purified by electric ultrafiltration coupling with solvent crystallization. *C. R. Chimie*, 11, 2008. 80-83.

CHLUDZINSKI, A. M.; GERMAINE, G. R.; SCHACHTELE, C. F. Purification and properties of dextransucrase from Streptococcus mutans. *J. Bacteriol*., 118, 1974. 1-7.

CHLUDZINSKI, A. M.; GERMAINE, G. R.; SCHACHTELE, C. F. J. *Dent. Res.*, Special Issue C, 55, 1976. c75-c86.

CHOI, K. et al. Amphotericin B-incorporated polymeric micelles commposed of poly(d,l-lactide-co-glycolide)/dextran graft copolymer. International *Journal of Pharmaceutics*, 355, 2008. 224-230.

CIARDI, J. E.; BEAMAN, A. J.; WITTENBERGER, C. L. Purification, resolution, and interaction of the glucosyltransferases of Streptococcus mutans 6715. *Infect. Immun.*, 18, 1977. 237-241.

CLAY, J. G. M. D. et al. Dextran polymer hemostatic dressing improves survival in liver injury model. *Journal of Surgical Research*, 155, 2008. 89-93.

COOPER, E. A.; DARKER, W. D.; STACEY, M. Enzyme formation and polysaccharide synthesis by bacteria.

Biochemical Journal, 32, 1938. 1752-1758.

CORTEZI, M.; MONTI, R.; CONTIERO, J. Temperature effect on dextransucrase production by Leuconostoc mesenteroides FT 045B isolated fro alcohol and sugar mill plant. *African Journal of biotechnology*, 4(3), 2005. 279-285.

CÔTÉ, G. L.; ROBYT, J. F. Isolation and partial characterization of an extracellular glucansucrase from Leuconostoc mesenteroides NRRL B-1355 that synthesizes alternating (1-6), (1-3)-a-D-glucan. *Carbohydr. Res.*, 101, 1982. 57-74.

DARKER, W. D.; STACEY, M. The polysaccharide produced from sucrose by Betabacterium vermiforme (Ward-Mayer). *Journal of Chemistry Society*, 1939. 585-587.

DE BELDER, A. N. Dextran. In: GERHART, W. Ullman's *Encyclopedia of Industrial Chemistry*. Weinheim: [s.n.], v. A8, 1987. p. 449-454.

DE BELDER, A. N. Medical applications of dextran and its derivatives. In: DUITRIU, S. *Polysaccharides in Medicinal Applications*. New York: Marcel Dekker, 1996.

DE MAN, J. C.; ROGOSA, M.; SHARPE, E. A medium for the cultivation of lactobacili. *J. Appl. Bacteriol*, 23, 1960. 130-138.

DEUTSCHER, M. P. *Methods in Enzymology. In: _____ Guide to Protein Purification.* [S.l.]: New York: Academic Press, v. 182, 1990. p. 285-306.

DOLS, M. et al. Characterization of the different dextransucrase activities excrreted in glucose, frutose or sucrose medium by Leuconostoc mesenteroides NRRL B-1299. *Appl. Environ. Microbiol.*, 64, 1998. 1298-1302.

DOLS, M. et al. Structural characterisation of the maltose acceptor-products synthesised by Leuconostoc mesenteroides NRRL B1299 dextransucrase. *Carbohydr. Res*, 305, 1998. 549-559.

DOLS, M.; REMAUD-SIMEON, M.; MONSAN, P. F. Dextransucrase production by Leuconostoc mesenteroides NRRL B-1299. Comparison with Leuconostoc mesenteroides NRRL B512F. *Enzyme Microb. Technol.*, 20, 1997. 523-530.

DOMAN, K.; ROBYT, J. F. Production, selection and characteristcs of Leuconostoc mesenteroides B742 constitutive for dextransucrase. *Enzyme Microbiology Technology*, 15, 1995. 689-695.

EGGLESTON, G.; COTE, G. L. *Oligosaccharides in food and agriculture*. Washington, DC: ACS symposium series 849, 2003. p. 1-15.

EL-SAYED, A. H. M. M.; MAHMOUD, W. M.; COUGHLIN, R. W. Production of dextransucrase by Leuconostoc mesenteroides immobilized in calcium alginate beads: batch and fed-batch fermentations. *Biotechnol. Bioeng*., 36, 1990. 338-345.

EOM, H. J.; SEO, D. M.; HAN, N. S. Selection of psychrotrophic Leuconostoc ssp. producing highly active dextransucrase fro lactate fermented vegetables. *Int. J. Food Microbiol.*, 117, 2007. 61-67.

ERHARDT, F. A.; KUEGLER, J. Co-immobilization of dextransucrase and dextranase for the facilitated synthesis of isoalto-oligosaccharides: preparation, characterization and modeling. *Biotechnol. Bioeng.,* 100, 2008. 673-683.

ERHARDT, F. A.; STAMMEN, S.; JOERDENING, H. J. Production, characterization ad (co-)immobilization of dextranase from Penicillium aculeatum. *Biotechnol. Lett.*, 30, 2008. 1069-1073.

FIGURES, W. R.; EDWARDS, J. R. a--D-Glucosyltranferase of Streptococcus mutans; isolation of two forms of the enzyme that bind to insoluble dextran. *Carbohydr. Res.*, 88, 1981. 107-117.

FOOD, S. *Opinion of the scientifc committee on food on a dextran preparation, produced using Leuconostoc mesenteroides, Saccharomyces cerevisiae and Lactobacillus spp, as a novel food ingredient in bakery products*. European Commission, Health ad Consumer Protection Directorate-General, Brussels, 2000.

FU, D.; ROBYT, J. F. A facile purification of Leuconostoc mesenteroides B-512FM dextransucrase. *Prep. Biochem.*, 20, 1990. 93-106.

FUKUI, K.; FUKUI, Y.; MORIYAMA, T. Purification and properties of dextransucrase and invertase from Streptococcus mutans. *J. Bacteriol.*, 118, 1974. 796-804.

FUNANE, K. et al. Aggregated forms of dextransucrases from Leuconostoc mesenteroides NRRL B-512F and its constitutive mutant. *Biosci. Biotechnol. Biochem.*, 59, 1995. 776-780.

GAINES, S.; STAHLY, G. L. The growth requirements of Leuconostoc mesenteroides and preliminary studies o its use as an assay agent for several members of the vitamin B complex. *J. Bact*., 46, 1943. 441-449.

GAJANAYAKE, T. et al. Dextran sulfate reduces ischaemia/reperfusion injury by modulating the activation of complement and the MAPK pathway. *Molecular Immunology*, 45, 2008. 4095-4182.

GERMAINE, G. R. et al. Streptococcus mutans Dextransucrase: Functioning of Primer Dextran and Endogeneous Dextranese in water-soluble and Water-insoluble Glucan Synthesis. *Infect. Immun*., 16, 1977. 637-648.

GERMAINE, G. R.; CHLUDZINSKI, A. M.; SCHACHTELE, C. F. *J. Bacteriol.*, 120, 1974. 287-294.

GIRARD, E.; LEGOY, M. D. Activity and stability of dextrans from Leuconostoc mesenteroides NRRL R 512 (F) in the presence of organic solvents. *Enzyme Microb. Technol.*, 24, 1999. 425-432.

GOULAS, A. K. et al. Synthesis of isomaltoligosaccharides and oligodextrans by the combined use of dextransucrase and dextransase. *Enzyme Microb. Technol*., 35, 2004. 327-338.

GOYAL, A.; KATIYAR, S. S. Fractionation of Leuconostoc mesenteroides NRRL B-512F dextransucrase by polyethylene glycol: A simple and effective method of purification. *J. Microbiol. Methods*, 20, 1994. 25-31.

GOYAL, A.; KATIYAR, S. S. Effect of certain nutriesnts on the production of dextransucrase from Leuconostoc mesenteroides NRRL B512 F. *J. Basic. Microbiol*., 37, 1997. 197-204.

GOYAL, A.; NIGAN, M.; KATIYAR, S. S. Optimal conditions for production of dextransucrase from Leuconostoc mesenteroides NRRL B512 and its properties. *Journal Basic Microbiology*, 36, 1995. 375-384.

GOYAL, A.; PURAMA, R. K. Purified Dextransucrase from Leuconostoc mesenteroides NRRL B-640 exists as single homogeneous protein: analysis by non-denaturing native page. *The Internet Journal of Microbiology* 2009, 6, 2009.

GUGGENHEIM, B.; SCHROEDER, H. E. Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. *Helv. Odontol. Acta*, 11, 1867. 131-152.

HAMDANI, G. et al. Dextran solium sulfate induced colitis causes rapid bone loss in mice. *Bones*, 43, 2008. 945-950.

HAMDY, M. K. et al. Factors affecting production and clarification of dextran. *The Ohio Journal of Science*,

54(5), 1954. 317.

HAMELIK, R. M.; MCCABE, M. M. An endodextranase inhibitor from batch cultures of Streptococcus mutans. *Biochem. Biophys. Res. Commun*., 106, 1982. 875-880.

HARISON, R. G. *Protein Purification Process Engeneering*. New York: Marcel Dekker, 1993. 115-208.

HASSID, W. Z.; BARKER, H. A. The structure of dextran synthesized from sucrose by Betacoccus arabinosaceus. *J. Biol. Chem*., 134, 1940. 163-170.

HAYASHI, T. et al. On the difference in adsorption on Sephadex gel of the dextransucrase of Streptococcus bovis grown on sucrose and glucose media. *J. Gen. Appl. Microbiol*., 34, 1988. 213-219.

HEHRE, E. J. Production from sucrose of a serologically reactive polysaccharide by a sterile bacterial extract. *Science*, 93, 1941. 237.

HEHRE, E. J. Studies on enzymatic synthesis of dextran from sucrose. *J. Biol. Chem.*, 163, 1946. 25-31.

HEHRE, E. J. Enzymatic synthesis of polysaccharides: a biological type of polyerization. *Adv. Enzymol.*, 11, 1951. 297.

HEHRE, E. J. Polysaccharide synthesis from disaccharides. *Methods Enzymol*., 1, 1955. 178-184.

HEHRE, E. J.; SUGG, J. Y. Serologically reactive polysaccharides produced through action of bacterial enzymes. I. Dextran of Leuconostoc mesenteroides from sucrose. *J. Exp. Med*., 75, 1942. 339-353.

HEINCLE, C. et al. Kinetics of the dextransucrase acceptor with maltose-experimental results and modeling. *Enzme and Microbial Technology*, 24, 1999. 523-534.

HELLMUTH, H. et al. Engeneering the glucansucrase GTFR enzyme reaction and glycosidic bond specificit: towaard tailor-made polymer and oligosaccharide products. *Biochemistry*, 47, 2008. 6678-6684.

HENRISSAT, B. A classification of glycosyl hydrolases based on amino-acid sequence similarities. *Biochem. Journal*, 280, 1991. 309-316.

HESTRIN, S.; AVERINI-SHAPIRO, S.; ASCHNER, M. The enzimic production of levan. *Biochemistry Journal*, 37, 1943. 450-456.

HONING, W.; KULA, M. R. Selectivity of protein precipitation with polyethylene glycol fractions of various molecular weights. *Anal. Biochem.*, 72, 1976. 502-512.

HUANG, S.; LEE, H. C.; MAYER, R. M. The purification and properties of dextransucrase from Streptococcus sanguis ATCC 10558. *Carbohydr. Res.*, 74, 1979. 287-300.

ILIEV, I. et al. Gluco-oligosaccharides synthesized by glucosyltransferases from constitutive mutants of Leuconostoc mesenteroides strain Lm28. *J. Appl. Microbiol.*, 104, 2008. 243-250.

JAMES, J. S. Dextran sulfate: new promising antiviral. AIDS Treat News (electronic journal), v. 50, 1988.

JEANES, A. Preparation of dextrans from growing Leuconostoc cultures. *Methods Carbohydr. Chem.*, 5, 1965. 118-126.

JEANES, A. Dextran. *Encyclopedia of Polymer Science and Technology.* [S.l.]: John Wiley & Sons Inc., v. 4, 1966. p. 805-824.

JEANES, A. et al. Characterization and classification of dextrans from ninety-six strains of bacteria. *Journal American Chemical Society*, 76, 1954. 5041-5052.

JEANES, A. et al. Properties of dextran isolated from whole cultures at various stages of incubation. *Arch. Biochem. Biophys.*, 71, 1957. 293-302.

JEANES, A.; WILHAM, C. A.; MIERS, J. C. Preparation and characterization of dextran from Leuconostoc mesenteroides. *J. Biol. Chem*., 176, 1948. 603-615.

JOHNSON, M. K.; MCCLESKEY, C. S. Sudies on the aerobic carbohydrate metabolism of Leuconostoc mesenteroides. *J. Bacteriol*, 74, 1957. 22-25.

JUKES, J. M. et al. A newly developed chemically crosslinked Dex-PEG hydrogel for cartilage tissue engineering. *Tissue Eng. Part. A*, 2008.

JUNG, S. M.; MAYER, R. M. Dextransucrase: donnor substrate reactions. *Arch. Bioche. Biophys*, 208, 1981. 288-295.

KABOLI, H.; REILLY, P. J. Immobilization and properties of Leuconostoc mesenteroides dextransucrase. *Biotechnol. Bioeng*., 22, 1980. 1055-1069.

KANG, H. K.; KIM, Y. M.; KIM, D. M. Functional, genetic, and bioinformatic characterization of dextransucrase (DSRBCB4) gene in Leuconostoc mesenteroides B-1299CB4. *J. Microbiol. Biotechnol.*, 18, 2008. 1050-1058.

KIM, D. et al. Dextran molecular size and degree of branching as function of sucrose concentration, pH, and temperature of reaction of Leuconostoc mesenteroides B-512 FMCM. *Carbohydr. Res*., 338, 2003. 1183-1189.

KIM, D.; KIM, D. Facile purification and characterization of dextransucrase from Leuconostoc mesenteroides B-512FMCM. *J. Microbiol. Biotechnol.*, 9(2), 1999. 219-222.

KIM, D.; ROBYT, J. F. Production an selection of mutants of Leuconostoc mesenteroides constitutive for glucansucrases. *Enzyme Microb. Technol.*, 16, 1994. 659-664.

KIM, D.; ROBYT, J. F. Properties of Leuconostoc mesenteroides B-512FMC constitutive dextransucrase. *Enzyme Microb. Technol.*, 16, 1994. 1010-1015.

KIM, D.; ROBYT, J. F. Dextransucrase constitutive mutants of Leuconostoc mesenteroides NRRL B-1299. *Enzyme and Microbial Technology*, 17, 1995. 1050-1056.

KIM, M.; DAY, D. F. Optimization of oligosaccharide synthesis from cellobiose by dextransucrase. *Appl. Biochem. Biotechnol*., 148, 2008. 189-198.

KIM, Y. M. et al. Construction of a fusion enzyme of dextransucrse and dextranase: application for one-step synthesis of isomalto-oligosaccharides. *Enzyme and Microbial Technology*, 44, 2009. 159-164.

KITAOKA, M.; ROBYT, J. F. Large-scale preparation of hightly purified dextransucrase from a high-producing constitutive mutant of Leuconostoc mesenteroides B-512FMC. *Enzyme MIcrobial. Technol.*, 23, 1998. 386-391.

KITAOKA, M.; ROBYT, J. F. Use of a microtiter plate screening method for obtaining Leuconostoc mesenteroides mutants constitutive for glucansucrase. *Enzyme Microb. Technol.*, 22, 1998. 527-531.

KOBAYASHI, M.; MATSUDA, K. The dextransucrase isoenzymes of Leuconostoc mesenteroides NRL B-1299. *Biochim. Biophys. Acta*, 370, 1974. 441-449.

KOBAYASHI, M.; MATSUDA, K. Purification and characterication of two activities of the intracellular dextransucrase from Leuconostoc mesenteroides NRRL B-1299. *Biochim. Biophys. Acta*, 397, 1975. 69-79.

KOBAYASHI, M.; MATSUDA, K. Purification and properties of the extracellular dextransucrase from Leuconostoc mesenteroides NRRL B-1299. *J. Biochem*., 79, 1976. 1301-1308.

KOBAYASHI, M.; MATSUDA, K. Characterization of the multiple forms and main component of dextransucrase from Leuconostoc mesenteroides NRRL B-512. *Biochim. Biophys. Acta*, 614, 1980. 46-62.

KOBAYASHI, M.; MATSUDA, K. Electrophoretic analysis of the multiple fors of dextransucrase from Leuconostoc mesenteroides. *J. Biochem-TOKYO*, 100,

1986. 615-621.

KOBAYASHI, M.; MIHARA, K.; MATSUDA, K. Dextransucrase from Leuconostoc mesenteroides NRRL B-512F: Characterization of the enzyme bound to Sephadex gel. *Agric. Biol. Chem*., 50, 1986. 551-556.

KOBS, S. et al. Affinity purification of dextransucrase from Streptococcus sanguis ATCC 10558. *Carbohydr. Res.*, 203, 1990. 156-161.

KOEPSELL, H. J.; TSUCHIYA, H. M. Enzymatic synthesis of dextran. *J. Bacteriol.*, 63, 1952. 293-295.

LANDON, R. S. et al. A model of dextransucrase synthesis by Leuconostoc mesenteroides. *Trans IChemE*, 72, 1994. 209-215. part C.

LANDON, R. S.; WEBB, C. Separating enzyme (dextransucrase) production and product (dextran) synthesis within a traditional fermentation process. *Process Biochem*, 25, 1990. 19-23.

LAWFORD, G. R. et al. Dextran biosythesis and dextransucrase production by continuous culture of Leuconostoc mesenteroides. *Biotechnol. Bioeng.*, 21, 1979. 1121-1131.

LAZIC, M. L. et al. Effect of pH and aeration on dextran production by Leuconostoc mesenteroides. *Enzyme and Microbial Technology*, 15, 1993. 334-338.

LAZIC, M. L. et al. Effect of pH on dextran production by Leuconostoc mesenteroides. *Enzyme Microb. Technol*., 15, 1993. 334-338.

LEATHERS, T. D. Dextran, in Biopolymers. In: VANDAMME, E. J.; DE BAETS, S.; STEINBUCHEL, A. *Polysaccharides I: Polysaccharides from Prokaryotes*. Weinheim: Wiley-VCH, v. 5, 2002. p. 299-321.

LEBRUN, L. et al. Exopolysaccharide production by free and immobilised microbial cultures. *Enzyme Microb. Technol*., 16, 1994. 1048-1054.

LINDBERG, B.; SVENSSON, S. Structural studies on dextran from Leuconostoc mesenteroides NRRL B-512. *Acta Chem Scand*, 22, 1968. 1907-1912.

LIU, Y.; CHAN-PARK, M. B. Hydrogel based on interpenetrating polymer networks of dextran and gelatin for vascular tissue engineering. *Biomaterials,* 30, 2009. 196-207.

LIU, Z. et al. Free energy of sickle homoglobin polymerization; a scaled particle treatment for use with dextran as a crowding agent. *Biophysical Journal*, 94, 2008. 3629-3634.

LOPEZ-MUNGUIA, A. et al. Production and purification of alternansucrase, a glucosyltransferase from Leuconostoc mesenteroides NRRL B-1355, for the synthesis of oligoalternans. *Enzyme Microb. Technol,* 15, 1993. 77-85.

LOPRETTI, M.; MARTINEZ, L. Influence of nitrogen/carbon ratio and complementary sugars on dextransucrase production by Leuconostoc mesenteroides NRRL B512F. *Process Biochemistry*, 34, 1999. 879-884.

LUCEY, C. A.; CONDON, S. J. Active role of oxygen and NADH oxidase in growth and energy metabolism of Leuconostoc. *Gen. Microbiol*., 132, 1986. 1789-1796.

MAJUMDER, A.; MANGTANI, A.; GOYAL, A. Purification, identification and functional characterization of glucansucrase from *Leuconostoc dextranicum* NRRL B-1146. *Current Trends in Biotechnology and Pharmacy*, 2, 2008. 493-505.

MAJUMDER, A.; PURAMA, R. K.; GOYAL, A. An overview of purification methods of glycoside hydrolase family 70 dextransucrase. *Ind. J. Microbiol.*, 47, 2007. 197-206.

MBEMBA, E. et al. Molecular interaction between HIV-1 major envelope glycoprotein and dextran sulfate. *Biochim. Biophys. Acta*, 1138, 1992. 62-67.

MIEKKA, S. I.; INGHAM, K. C. Influence of self association of proteis on their precipitation by poly(ethylene glycol). *Arch. Biochem. Biophys*, 191, 1978. 525-536.

MILLER, A. W.; EKLUND, S. H.; ROBYT, J. F. Milligram to gram scale purification and characterization of dextransucrase from Leuconostoc mesenteroides NRRL B-512F. *Carbohydr. Res.*, 147, 1986. 119-133.

MILLER, A. W.; ROBYT, J. F. Stabilization of dextransucrase from Leuconostoc mesenteroides NRRL B-512 F by non ionic detergents, poly(ethilene glycol) and high molecular weight dextran. *Biochim. Biophys. Acta*, 785, 1984. 89-96.

MITSUYA, H. et al. Dextran sulfate suppression of viruses in the HIV family: Inhibition of virion binding to CD4+ cells. *Science*, 240, 1988. 646-649.

MIZUTANI, N. et al. Constitutive mutants for dextransucrase from Leuconostoc mesenteroides NRRL B-512F. *J. Ferment. Bioeng*., 77, 1994. 248-251.

MONCHOIS, V. et al. Characterization of Leuconostoc mesenteroides NRRL B-512F dextransucrase (DSRS) and identification of amino-acid residues playing a key role in enzyme activity. *Appl. Microbiol. Biotechnol*., 48, 1997. 465-472.

MONSAN, P. et al. Dextran synthesis using immobilized Leuconostoc mesenteroides dextransucrase. *Methods Enzymol*., 136, 1987. 239-254.

MONSAN, P. et al. Homopolysaccharides from lactic acid bacteria. *Int. Dairy J*., 11, 2001. 675-685.

MONSAN, P.; LOPEZ, A. On the production of dextran by free and iobilized dextransucrase. *Biotechnol. Bioeng*., 23, 1981. 2027-2037.

NAESSENS, M. et al. Leuconostoc dextransucrase and dextran: production, properties and applications. *J. Chem. Technol. Biotechnol*., 80, 2005. 845-860.

NAKAMURA, J. et al. Water-soluble taxol conjugates with dextran and targets tumor cells by folic acid immobilization. *Anticancer Res.*, 30(3), 2010. 903-909.

NAM, S. H. et al. Maximization of dextransucrase activity expressed in E. coli by mutation and its functional characterization. *Biotechnol. Lett*., 30, 2008. 135-143.

NEELY, W. B.; NOTT, J. Dextransucrase: an induced enzyme from Leuconostoc mesenteroides. *Biochemistry*, 1, 1962. 1136-1140.

NEU, B.; WENBY, R.; MEISELMAN, H. J. Effects of dextran molecular weight on red blood cell aggregation. *Biophysical Journal*, 95, 2008. 3059-3065.

NIGAM, M.; GOYAL, A.; KATIYAR, A. High yield purification of dextransucrase from Leuconostoc mesenteroides NRRL B-512 by phase partitioning. *Journal of Food Biochemistry*, 30, 2006. 11-20.

NOUVEL, C. et al. Biodegradable nanoparticles made from polylactide-grafted dextran copolymers. *Journal of Colloid and Interface Science*, 330, 2009. 337-343.

NOVAK, L. J.; STOYCOS, G. S. *Method for producing clinical dextran*. 2,841,578, 1 July 1958.

NOVAK, L. J.; WITT, E. C. Method of producing clinical dextran. 2,972,567, 21 Feb. 1961.

OLVERA, C. et al. Role of the C-terminal region of dextransucrase from Leuconostoc mesenteroides IBT-PQ in cell anchoring. *Microbiology*, 153, 2007. 3994-4002.

OTTS, D. R.; DAY, D. F. The effect of ionophores on the production of extracellular dextransucrase by Leuconostoc mesenteroides. *FEMS Microbiol. Lett*., 42, 1987. 179-183.

OTTS, D. R.; DAY, D. F. Dextransucrase secretion in Leuconostoc mesenteroides depends on the presence of

a trasembrane proton gradient. *J. Bacteriol*., 170, 1988. 5006-5911.

PASTEUR, L. On the viscous fermentation and the butyrous fermentation. *Bull Soc Chim Fr*, 1861. 30-31.

PAUL, F. Acceptor reaction of a hyghly purified dextransucrase with maltose and oigosaccharides: application to the synthesis of controlled molecularj weight dextrans. *Carbohydr. Res*., 149, 1986. 433-441.

PAUL, F. et al. Production and purification of dextransucrase from Leuconostoc mesenteroides NRRL B-512. *Ann. NY Acad. Sci*., 434, 1984. 267-270.

PAUL, F. et al. *Method for the production of a-1,2 oligodextrans using Leuconostoc mesenteroides NRRL B-1299*. 5,141,858, August 1992.

PENNELL, R. D.; BARKER, P. E. The production of the enzyme dextransucrase using unaerated continuous fermentation. *J. Chem. Technol. Biotechnol*., 53, 1992. 21-27.

PEREIRA, A. M. et al. In vitro synthesis of oligosaccharides by acceptor reaction of dextransucrase frrom Leuconostoc mesenteroides. *Biotechnol. Lett.*, 20, 1998. 397-401.

PHARMACIA.BIOTECH. *Dextran 50 years - still going strong*. Downstream, 24, 1997. 10-13.

PIRET, J. et al. In vitro and in vivo evaluations of sodium lauryl sulfate and dextran sulfate as microbicides against herpes simplex and human deviciency viruses. *J. Clin. Microbiol.*, 38, 2000. 110-119.

PLIHON, F.; TAILLANDIER, P.; STRE HAIANO, P. Oxygen effect on batch cultures of Leuconostoc mesenteroides: relationship between oxygen uptake, growth and end-products. *Appl. Microbiol. Biotechnol.*, 43, 1995. 117-122.

PORATH, J.; FLODIN, P. Gel filtration: a method for desalting and group separation. *Nature*, 183, 1959. 1657-1659.

PURAMA, R. K. et al. Dextransucrase production from Leuconostoc mesenteroides NRRL B-640 in batch fermentation. *Int. J. Chem. Sci.*, 5(4), 2007. 1497-1504.

PURAMA, R. K. et al. Dextransucrase production by Leuconostoc mesenteroides NRRL B-640 in bioreactor: effect of aeration and mathematical modelling. *J. Appl. Biosci. Biotechnol*., 2008.

PURAMA, R. K.; GOYAL, A. Dextransucrase production by Leuconostoc mesenteroides. Ind. J. Microbiol., 2, 2005. 89-101.

PURAMA, R. K.; GOYAL, A. Application of response surface methodology for maximizing dextransucrase production from Leucnostoc mesentereroides NRRL-B-640 in a bioreactor. *Appl. Biochem. Biotechnol*., 2008.

PURAMA, R. K.; GOYAL, A. Identification, effective purification and functional characterization of dextransucrase from Leuconostoc mesenteroides NRRL B-640. *Biores. Technol*., 99, 2008. 3635-3642.

PURAMA, R. K.; GOYAL, A. Screening and optimization of nutritional factors for higher dextransucrase production by Leuconostoc mesenteroides NRRL B-640 using statistical approach. *Bioresour. Technol.*, 99, 2008. 7108-7114.

PURAMA, R. K.; GOYAL, A. Optimization of conditions of Leuconostoc mesenteroides NRRL B-640 for production of dextransucrase and its assay. *J. Food Biochem.*, 2009.

QUIRASCO, M. et al. Induction and transcription studies of the dextransucrase gene in Leuconostoc mesenteroides NRRL B-512F. *Appl. Environ. Microbiol*., 65, 1999. 5504-5509.

REMAUD-SIMEON, M. et al. Molecular weight characterization and structural properties of controlled molecular weight dextrans synthesized by acceptor reaction using highly purified dextransucrase. *J. Carbohydr. Chem*., 10, 1991. 861-876.

REMAUD-SIMEON, M. et al. Production and use of glucosyltransferase from Leuconostoc mesenteroides NRRL B-1299 for the synthesis of oligosaccharides containing a(1-2) linkages. *Appl. Biochem. Biotechnol.,* 44, 1994. 101-117.

REMAUD-SIMEON, M. et al. Glucansucrases: molecular engineering and oligosaccharide sythesis. *J. Mol. Catal. B*., 10, 2000. 117-128.

RHEE, S. H.; LEE, C. H. Properties of dextransucrase from Leuconostoc esenteroides isolated from Sikhae. *J. Microbiol. Biotechnol*., 1, 1991. 176-181.

RICKETTS, C. R. Dextran sulfate - A synthetic analogue of heparin. *Biochem. J*., 51, 1959. 129-133.

ROBYT, J. F. *Dextran. Encyclopaedia of Polymer Science*. New York: Wiley-VCH, v. 4, 1985. p. 753-767.

ROBYT, J. F. Dextran. In: MARK, H. F., et al. *Encyclopedia of Polymer Science and Engineering*. New York: John Wiley & Sons, 1986. p. 752-767.

ROBYT, J. F. Mechanism in the glucansucrase synthesis of polysaccharides and oligosaccharides from sucrose. *Advances in Carbohydrate Chemistry and Biochemistry*, San Diego, v. 51, p. 133-168, 1995.

ROBYT, J. F.; EKLUND, S. H. Relative, quantitative effects of acceptors in the reation of Leuconostoc mesenteroides NRRL B-512F dextransucrase. *Carbohydr. Res.*, 121, 1983. 279-286.

ROBYT, J. F.; KIM, D.; YU, L. Mechanism of dextran activation of dextransucrase. *Carbohydr. Res*., 266, 1995. 293-299.

ROBYT, J. F.; WALSETH, T. F. The mechanism of acceptor reactions of Leuconostoc mesenteroides NRRL B-512F dextransucrase. *Carbohydr. Res*., 61, 1978. 433-445.

ROBYT, J. F.; WALSETH, T. F. Production, purification and properties of dextransucrase from Leuconostoc mesenteroides NRRL B-512F. *Carbohydr Res*, 68, 1979. 95-111.

ROBYT, J. F.; YOON, S.; MUKERJEA, R. Dextransucrase and teh mechaism for dextran biosynthesis. *Carbohydrate Research*, 343, 2008. 3039-3048.

ROULEAU, L.; ROSSI, J.; LEASK, R. L. Concentration and time effects of dextran exposure on endothelial cell viability, attachment, and inflammatory marker expression in vitro. *Annals of Biomedical Engineering*, 38, 2010. 1451-1462.

RUMBALL, C. W. H. et al. A method for assessment of blood volume parameters in pregnant sheep using fluorescein labelled dextran. *Placenta*, 29, 2008. 15-19.

RUSSEL, R. R. B. Glucan-binding proteins of Streptococcus mutans serotype. C. *J. Gen. Microbiol*., 1112, 1979. 197-201.

RUSSEL, R. R. B. Purification of Streptococcus mutans glucosyltransferase by polyethylene glycol phase precipitation. *FEMS Microbiol. Lett.*, 6, 1979. 197-199.

RYU, H. J. et al. Cloning of a dextransucrase gene (fmcmds) from a constitutive dextransucrasse hyper-producing Leuconostoc mesenteroides B-512 FMCM developed using VUV. *Biotechnol. Lett*., 22, 2000. 421-425.

SANTOS, M.; RODRIGUES, A.; TEIXEIRA, J. A. Production of dextran and fructose from carob pod extract and cheese whey by Leuconostoc mesenteroides NRRL B512(F). *Biochemical Engineering Journal*, 25, 2005. 1-6.

SANTOS, M.; TEIXEIRA, J.; RODRIGUES, A. Production of dextransucrase, dextran and frutose from sucrose using Leuconostoc mesenteroides NRRL B 5125(F). *Biochemical Engeneering*, 4, 2000. 177-188.

SARWAT, F. et al. Production & charracterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713. *Int. J. Biol. Sci.*, 4(6), 2008. 379-386.

SATO, M. et al. Effects of Tween 80 and sodium fluoride on extracellular glucosyltransferase. *Int. J. Biochem.*, 21, 1989. 751-754.

SCHEIBLER, C. Investigation on the nature of the gelatinous excretion (so-called frog´s spawn) which is observed in production of beet-sugar juices. *Z Dtsch Zucker-Ind*, 24, 1874. 309-335.

SEYMOUR, F. R. et al. High temperature enhancement of 13C-NMR chemicals shift fo unusual dextrans and correlation with methylation structural analysis. *Carbohydr. Res*., 68, 1979. 123.

SEYMOUR, F. R.; KNAPP, R. D. Structural analysis of dextran from strains of Leuconostoc related genera, that contain 3-O-a-glucosylated-D-glucopyranosyl residues at the branched points of in consecutive linear position. *Carbohydr. Res*., 81, 1980. 105-129.

SHAMALA, T. R.; PRASAD, M. S. Preliminary studies on the production of high and low viscosity dextran by Leuconostoc spp. *Process Biochem*, 30, 1995. 237-241.

SHIMURA, N.; ONISI, M. The effect of NaF on the bacterial production of polysaccharide and subsenquent adsorption on hydroxyapatite. *J. Dent. Res*., 57, 1978. 928-931.

SIDEBOTHAM, R. L. Dextrans. *Adv. Carbohydr. Chem. Biochem.*, 30, 1974. 371-444.

SMITH, E. E. Biosythetic relation between the soluble and insolubel dextrans produced by Leuconostoc mesenteroides NRRL B-1299. *FEBS Lett*., 12, 1970. 33-37.

SMITH, M. R.; SAHNLEY, J.; GOODMAN, N. glucosyltransferase mutants of Leuconostoc mesenteroides NRL B-1355. *Appl. Environ. Microbiol*., 60, 1994. 2723-2731.

SNELL, F. E.; STRONG, F. M.; PETERSON, W. H. Pantothenic and nicotinic acids as growth factors for lactic acid bacteria. *J. Am. Chem. Soc*., 60, 1938. 2825.

SPIRIG, R. et al. The complement inhibitor low molecular weight dextran sulfate prevents TLR2 mediated activation of human natural killer cells. *Molecular Immunology*, 45, 2008. 4095-4182.

STACEY, M. Enzymatic Production of Bacterial Polysaccharides. *Nature*, 149, 1942. 639-639.

STACEY, M.; SWIFT, G. *Jounal of Chemistry Society*, 1948. 1555.

STACEY, M.; YOUD, F. R. A note on the dextran produced from sucrose by Betacoccus arabinosaceus haemolyticus. *Biochem. J*., 32, 1938. 1943-1945.

STAHLY, G. L. Method of making dextran. 2,310,263, 1943.

TABASSI, S. A. S. T.; TAFAGHODI, M.; JAAFARI, M. R. Induction of high antitoxin titers against tetanus toxoid in rabbits by intranasal immunization with dextran microspheres. *International Journal of Pharmaceutics*, 360, 2008. 12-17.

TAKADA, Y. et al. Fos proteins suppress dextran sulfate sodium-induced colitis through inhibition of NF-kappaB. *J. Immunol.*, 184(2), 2010. 1014-1021.

TAKASHIO, M.; OKAMI, Y. Effect of ribocitrin on glucan sythesizing enzymes of Streptococcus mutans E49. *Agric. Biol. Chem*., 47, 1983. 261-2171.

TAKEMOTO, K. K.; LIEBHARBER, H. virus-polysaccharide interactions. II. Enhancement of plaque formation and the detection of variants of poliovirus with dextran sulfate. *Virology*, 17, 1962. 499-501.

TANRISEVEN, A.; ROBYT, J. F. Intepretation of dextransucrase inhibition at high sucrose concentration. *Carbohydr. Res*., 245, 1993. 97-104.

TARR, H. L. A.; HIBBERT, H. Polysaccaride synthesis by the action of Acetobacter xylinus on carbohydrates oan related compounds. *Canad. J. Research*, 5, 1931. 414.

TSUCHIYA, H. M. et al. The effect of certain cultural factors on production of dextransucrase by Leuconostoc mesenteroides. *J. Bacteriol*, 64, 1952. 521-526.

TSUCHIYA, H. M. et al. Factors affecting molecular weight of enzymatically synthesized dextran. *J. Am. Chem Soc*., 77, 1955. 2412-2419.

UENO, R.; KUNO, S. Anti-HIV synergism between dextran sulfate and zidovudine. *Lancet*, 3, 1987. 796-797.

UI-QADER, S. A. et al. Production of dextran from sucrose by a newly isolated strain of Leuconostoc mesenteroides (PCSIR-3) with reference to Leuconostoc mesenteroides NRRL B-512F. *Biotechnol. Appl. Biochem.*, 34, 2001. 93-97.

UL-QADER, S. A. et al. Production of dextran by newly isolated strains of Leuconostoc mesenteroides PCSIR-3 and PCSIR-9. Turk. *J. Biochem*., 31, 2005. 21-26.

UL-QADER, S. A. et al. characterizationof dextransucrase immobilized on calcium alginate beads from Leuconostoc mesenteroides PCSIR-4. *Ital. J. Biochem*., 56, 2007. 158-162.

VAN TIEGHEM, P. On sugar-mill gum. *Annales des Science*, 7, 1878. 180-203.

VARDHANABHUTI, B. et al. Interactions between B-lactoglobulin and dextran sulfate at near neutral pH and their effect on thermal stability. *Food Hydrocolloids*, 2008. 1-10.

VELJKIVIC, V. B. et al. Effects of aeration on extracellular dextransucrse production by Leuconostoc mesenteroides. *Enzyme Microb Technol*, 14, 1992. 668-668.

WENG, L. et al. Non-cytotoxic, in situ gelable hydrogels composed of N-carboxyethyl chitosan and oxidized dextran. *Biomaterials*, 29, 2008. 3905-3913.

WHITESIDE-CARLSON, V.; CARLSON, W. W. Studies of the effect of para-aminobenzoic acid, folic acid and sulfanilamide on dextran synthesis by Leuconostoc. *J. Bact*., 58, 1949. 143-149.

WHITESIDE-CARLSON, V.; CARLSON, W. W. The vitamin requirement of Leuconostoc for dextran synthesis. *J. Bact.*, 58, 1949. 135-141.

WILLEMOT, R. M.; MONSAN, P.; DURAND, G. Effects of dextran on the activity of dextransucrase from Leuconostoc mesenteroides. *Ann. NY Acad. Sci*., 542, 1988. 169-172.

WOLF, I. A. et al. Production of clinical type dextran: partial hydrolytic depolymerization and fractionation of the dextran from Leuconostoc mesenteroides strain NRRL B-512. *Industrial and Engineering Chemmistry*, 46, 1954. 370-377.

XIANG, J. Y. et al. Amelioration of murine dextran sulfate sodium induced colitis by nuclear factor-b decoy oligoucleotides. *The American Journal of Surgery*, 197, 2009. 797-805.

YALIN, Y. et al. Expression and characterization of dextransucrase gen dsrX from Leuconostoc mesenteroides in Escherichia coli. *J. Biotechnol*., 133, 2008. 505-512.

YAMASHITA, Y.; TAKEHARA, T. Effect of magnesium ions on secretion of glucosyltransferase from Streptococcus sobrinus. *Microbios*, 60, 1989. 177-182.

YOKOYAMA, I.; KOBAYASHI, M.; MATSUDA, K. Purification of the dimeric form of dextransucrase from Leuconostoc mesenteroides strains NRRL B-146 and B-1375. *Agric. Biol. Chem*., 49, 1985. 1385-1391.

YUSEF, H. H.; EL-AASSAR, S. A.; FATHY, S. M. F. Optimization of culture conditions for the production of

dextran by Leuconostoc mesenteroides. *Adv. Food Sci*., 19, 1997. 152-158.

ZAHLEY, J. C.; SMITH, M. R. Insoluble glucan formation by Leuconostoc mesenteroides NRRL B-1355. *Appl. Environ. Microbiol*, 61, 1995. 1120-1123.

ZANONI, G. et al. Dextran specific IgG response in hypersensitivity reactions to measles-umps-rubella vaccine. *J. Allergy Clin. Immunol*., 122(6), 2008. 1233-1235.

ZATOR, M. et al. Chemical cleaning of polycarboate membranes fouled by BSA/dextran mixtures. *Journal of Membrane Science*, 327, 2009. 59-68.

ZHANG, H. et al. Clonig, sequencing and expression of a dextransucrase gene (dexYG) from Leuconostoc mesenteroides. *Biotechnol. Lett.*, 30, 2008. 1441-1446.

ZIEF, M.; BRUNNER, G.; METZENDORF, J. Fractionation of partially hydrolyzed dextran. *Industrial and Engineering Chemistry*, 48, 1956. 119-121.

