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Low molecular weight dextran: Immobilization of cells of *Leuconostoc mesenteroides* KIBGE HA1 on calcium alginate beads☆

Shah Ali Ul Qader*, Afsheen Aman

The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Pakistan

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ABSTRACT

Dextran is a long chain polymer of D-glucose produced by different bacterial strains including *Leuconostoc*, *Streptococcus* and *Acetobacter*. The bacterial cells from *Leuconostoc mesenteroides* KIBGE HA1 were immobilized on calcium alginate for dextran production. It was observed that dextran production increases as the temperature increases and after reaching maxima (30 °C) production started to decline. It was also observed that at 50 °C free cells stopped producing dextran, while immobilized cells continued to produce dextran even after 60 °C and still not exhausted. It was found that when 10 g% substrate (sucrose) was used, maximum dextran production was observed. Immobilized cells produced dextran upto 12 days while free cells stopped producing dextran only after 03 days. Molecular mass distribution of dextran produced by immobilized cells is low as compared to free cells.

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1. Introduction

Dextran is a long chain polymer of D-glucose mainly linked with α-(1 → 6) linkage and side chains having α-(1 → 2), α-(1 → 3), α-(1 → 4) linkages depending upon the producing strain (Jeanes, 1966; Martinez-Espindola & Lopez-Munguia, 1985; Sidebotham, 1974). Dextran plays a key role in different industries such as petroleum, mining, food and also in gel permeation chromatography (Girard & Legoy, 1999). It has several clinical applications such as blood volume expanders, heparin substitutes and also for the treatment of anemia (Gomez de Segura, Alcade, Lopez-Cortes, Flou, & Ballesteros, 2004; Haldane & Logan, 1994; Itaya & Yamatoto, 1975). Dextran also use as thickener for jam and ice cream, improves moisture retention, crystallization of sugar and maintaining the flavor of various food items (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005; Purama & Goyal, 2005, 2008; Qader, Iqbal, Aman, Shireen, & Azhar, 2005). The entrapment of cells to a support is an emerging technique for basic research and also for industrial use. *Leuconostoc mesenteroides* NRRL B1299 and KIBGE HA1 produces two forms of dextransucrase, extracellular and intracellular (Dols, Remaud-Simeon, & Monsan, 1997; Qader, Aman, & Azhar, 2011). Extracellular dextransucrase from *L. mesenteroides* NRRL B-512F, PCSIR-4 and *Streptococcus* mutans OMZ 176 were immobilized on different supports for direct uses (Alcalde et al., 1999; Ebert & Schenk, 1968; Qader, Afsheen, Noman, Saeeda, & Abid, 2007; Robyt & Corrigan, 1977; Robyt & Taniguchi, 1976), while intracellular fraction of dextransucrase was used by immobilizing the cells (Dols, Willemot, Monsan, & Remaud-Simeon, 2001; Qader et al., 2011).

This study was designed to make significant use of cells of *L. mesenteroides* KIBGE HA1 for the continuous production of dextran using immobilized cell technology. During this study calcium alginate was used as a support for the commercial production of low molecular weight dextran.

2. Materials and methods

2.1. Isolation and selection of strain

The strain of *L.* mesenteroides KIBGE HA1 was isolated from *Brassica oleracea var Capitata L.* (Cabbage). Samples were incubated on MRS medium for 24 h and then screened for dextran production using sucrose medium plate. After 24 h incubation bacterial strain showing highly viscous slimy colony was selected and pure culture study was performed (Kobayashi, Yokohama, & Matsuda, 1985). Culture was identified by using Holt's method (Holt, 1994) and confirmed by using 16S rDNA sequence analysis. After identification culture was maintained at 4 °C on tomato juice agar (Kobayashi & Matsuda, 1975)

☆ Gene Bank: Accession number FJ467346.

* Corresponding author. Tel.: +92 21 926118081x147; fax: +92 21 2229310.
E-mail address: ali_kibge@yahoo.com (S.A.U. Qader).

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2.2. Culture conditions and cells harvesting

For cells growth the organism was grown in a medium containing ($g\,l^{-1}$): sucrose, 20.0; bacto-peptone, 5.0; yeast extract, 5.0; K_2HPO_4, 15.0; $MnSO_4.H_2O$, 0.01; NaCl, 0.01; $MgSO_4{\cdot}7H_2O$, 0.01; $CaCl_2{\cdot}2H_2O$, 0.1. The pH of the medium was adjusted to 7.5 before sterilization at 121 °C for 15 min. The culture was inoculated and harvested as described by Qader, Iqbal, Rizvi, and Zuberi (2001) and Qader et al. (2011).

2.3. Enzyme assay

Dextransucrase activity of extracellular enzyme produced during cells growth was determined by measuring the reducing sugar by Nelson Somogyi method as described earlier (Kobayashi & Matsuda, 1974).

2.4. Cells immobilization

Cells were harvested by centrifugation from fermentation broth and mixed with 25.0 ml citrate phosphate buffer (0.1 M, pH 5.00). Equal volume of sodium alginate (4%) solution was mixed with cells solution. The cells–alginate solution was allowed to fall in 500 ml calcium chloride solution (0.2 M) drop by drop for beads formation at 4 °C and kept for 15 min for permanent shape. After 15 min beads were washed with phosphate buffer (pH 7.00).

2.5. Effect of substrate concentration on dextran production

Immobilized cells were mixed with different concentration of substrate ranging from 0.5 to 30.0 g% in 0.1 M citrate phosphate buffer (pH 5.00) for 24 h at 30 °C. After 24 h the viscous solution was separated out and reactor was again refilled with fresh substrate. After each 24 h fresh substrate was added in the reactor.

2.6. Effect of temperature on dextran production

Immobilized cells were mixed with substrate and kept at different temperature ranging from 20 °C to 60 °C for 24 h for dextran production.

2.6.1. Precipitation, purification and molecular weight of dextran

Precipitation and purification of dextran was performed using chilled ethanol as reported earlier (Qader et al., 2005). The average molecular weight of dextran produced from free and immobilization cells was determined by gel permeation chromatography (Qader et al., 2011).

3. Results and discussions

3.1. Effect of size of calcium alginate beads on immobilization

Sizes of the beads play a very important role in the production of dextran from immobilized cells. During whole cell immobilization internal diffusion may become a limiting step, particularly when highly specific activities are involved or when producing oligosaccharide have low diffusion limits to cross the matrix. During this study various size of beads were prepared for maximum immobilization of cells and it was found that when beads diameter was kept 0.6 mm, maximum immobilization was achieved. It was also noted that as the beads diameter increases, the percent immobilization of cells decreases (Fig. 1). Quirasco et al. (1995) reported that the optimum particle size for maximum activity is 2.0 mm and below this diameter, the reaction rate is not controlled by internal diffusion. But in current study it was found that the maximum

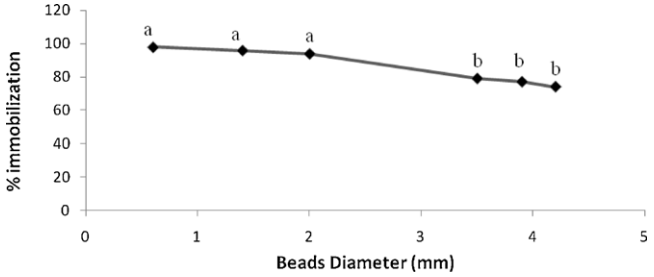

Fig. 1. Effect of beads size on immobilization of cells of *Leuconostoc mesenteroides* KIBGE HA1. Symbols (means ± S.E., $n = 5$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

dextran production was achieved when cells of *L. mesenteroides* KIBGE HA1 were immobilized at 0.6 mm bead size.

3.2. Effect of sucrose concentration on dextran production by immobilized cells

The effects of different sucrose concentrations were studied and it was found that the reaction remained under kinetic control only when sucrose concentration was kept higher than 10%. Dextran production at different sucrose concentrations were compared at 30 °C and it was found that as the sucrose concentration increases the dextran production increases but after reaching maximum the percent conversion of sucrose to dextran decreases due to the substrate inhibitory effect (Fig. 2) (Martinez-Espindola & Lopez-Munguia, 1985; Zedan et al., 1983). It was also reported that if the substrate concentration decreases upto 0.1% the effectiveness factor is reduced upto 0.4 from 1.0 (Quirasco et al., 1995).

3.3. Effect of temperature on dextran production by immobilized cells

Dextran production from immobilized cells was affected by varying the temperature and it was found that maximum production was achieved at 30 °C. It was also observed that as the temperature increases from 20 °C to 30 °C dextran production increases and after 30 °C every increased of 5 °C, decreased the production of dextran. Martin and Perlman reported that during the conversion of L-sorbose to L-sorbosone by immobilized cells of *Gluconobacter melanogenus* IFO 3293, maximum production was achieved at 50 °C and as temperature increased upto 60 °C, the production decreased (Martin & Perlman, 1976). It was already reported that maximum dextran production was achieved at 25 °C during normal fermentation process (Qader et al., 2005). At 30 °C maximum dextran production was found both from free and immobilized cells, but free cells stop producing dextran after 40 °C while immobilized cells continued to produce dextran upto 60 °C and still not exhausted (Fig. 3). These results supported the phenomenon that due to the immobilization stability of the cells increases at high

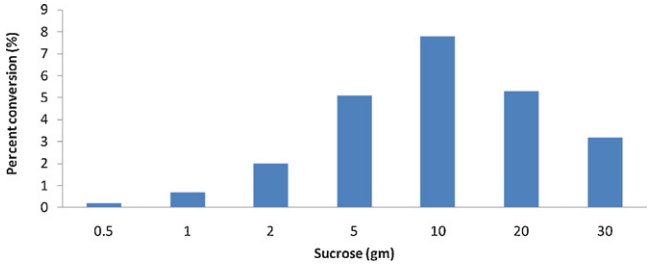

Fig. 2. Percent conversion of different sucrose concentration to dextran from immobilized cells.

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