Continuous Production of Dextran from Immobilized Cells of *Leuconostoc mesenteroides* KIBGE HA1 Using Acrylamide as a Support

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Abstract The cells of *L. mesenteroides* KIBGE HA1 were immobilized for the production of dextran on acrylamide gel and gel concentration was optimized for maximum entrapment. Sucrose at substrate concentration of 10% produced high yield of dextran at 25°C with a percent conversion of 5.82 while at 35°C it was 3.5. However, increasing levels of sucrose diminished dextran yields. The free cells stopped producing dextran after 144 h, while immobilized cells continued to produce dextran even after 480 h. Molecular mass distribution of dextran from free cells indicate that it is identical to that of blue dextran while the molecular mass of dextran from immobilized cells is lower than that of free cells.

Keywords Immobilization · Acrylamide · Dextran · *Leuconostoc mesenteroides*

Introduction

The attachment of enzymes to an inert matrix is an important procedure for both practical and academic purposes. The enzymes used for this purpose are of microbial origin which required the separation and purification before going to immobilization process while in case of whole microbial cell immobilization to an inert support no prior separation and purification required. The advantages of immobilizing the

cell rather than a purified enzyme are numerous. These includes; the expense of separation, isolation and purification of enzymes is minimized, a wider scope of reactions is possible including multi-step reactions utilizing several enzymes and enhanced stability of enzymes achieved since the enzyme is maintained in its native site. Dextran is an extracellular bacterial polymer of D-glucopyranose with predominantly α -(1→6) linkage [1] in the main chain and a variable amount of α -(1→2), α -(1→3), α -(1→4) branched linkages [2–4]. Bacterial strains of *Leuconostoc*, *Streptococcus* and *Acetobacter* produce dextran under certain conditions [5]. *Leuconostoc* species are epiphytic bacterial that are widespread in the natural environmental and play an important role in several industrial and food fermentation. *Leuconostoc mesenteroides* is a facultative anaerobe requiring complex growth factors and amino acids [6]. *Leuconostoc mesenteroides* NRRL B-1299 produces two forms of dextransucrase, one of which is soluble and other is insoluble form [7]. Soluble dextransucrase has been immobilized for the production of α -(1→2) glucooligosaccharides while insoluble fraction is immobilized by entrapping whole cells [8]. Alcalde et al. [9] has immobilized native and dextran free extracellular dextransucrase from *L. mesenteroides* NRRL B-512F on two supports. The extracellular dextransucrase has been immobilized on Calcium alginate, DEAE Sephadex A-50, Bio-Gel P2, cellulose acetate membrane, and polysulfone hollow fibre [10–13].

Due to the few studies on the immobilization of cells of *L. mesenteroides* on acrylamide it is very important to perform this kind of study for the production of dextran. In this study cells of *Leuconostoc mesenteroides* KIBGE HA1 were immobilized on acrylamide for the continuous production of dextran at various substrate concentrations and temperatures and also the reuse of the immobilized cells for commercial production of dextran. In this study it was also

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noted that different molecular mass of dextran can be produced by free and immobilized cells.

Materials and Methods

Culture Conditions and Cells Harvesting

For the culturing of *Leuconostoc mesenteroides* KIBGE HA1, 500 ml of medium containing 2.0 g% sucrose, 0.5 g% peptone, 0.5 g% yeast extract, 1.5 g% K_2HPO_4 , 0.001 g% NaCl, 0.001 g% $MgSO_4 \cdot 7H_2O$, 0.001 g% $MnCl_2 \cdot H_2O$ and 0.005 g% $CaCl_2$ were used. Growth conditions of organism were maintained as suggested by Qader et al. [14].

Sterile sucrose broth medium (5.0 ml) was inoculated with *L. mesenteroides* KIBGE HA1 and incubated at 26°C for 24 h. After 24 h this inoculum was transferred in 45.0 ml culture medium and incubated again at same above conditions. In the next step this inoculum was transferred in 450 ml culture medium for further incubation. After final incubation the fermented broth was centrifuged at 15,000 rpm for 15 min at 0°C in a high speed refrigerated centrifuge (IEC model B-20A). The clear supernatant, which contained extracellular dextransucrase, was preserved at –18°C and cells were used for immobilization study.

Cells Immobilization

Cells were washed with citrate phosphate buffer (pH 7.0) and 3.7 g (wet mass) cells were obtained. The cells were suspended in about 8.0 ml deionized water at 4°C. Acrylamide solution (15%) 9.0 ml and bis-acrylamide solution (0.4%) 6.0 ml were then added to the cells suspension and mixed thoroughly. Thereafter, 3.0 ml potassium persulphate (0.2%) was added and mixed well. Deionized water was added to the above mixture to make the volume up to 29.7 ml. The mixture was transferred into glass plates (15 × 30 cm) and 0.3 ml TEMED was added and mixed quickly. The polymerization was started as soon as TEMED added and completed within 30 min. The plates were

further left for 1–2 h and then the thin gel layer (2.0 mm thick) was sieved to get small gel particles (approx. $1 \times 1 \times 2$ mm) and these gels particles were used for further studies.

Molecular Mass Distribution of Dextran

The average molecular mass of dextran produced by immobilization was determined by gel permeation chromatography on LKB gel filtration system using blue dextran 2000 (average Mol. Wt. 200,000) as standard. The samples were applied through automatic sample applicator on XK16/70 glass column packed with Sephacryl-S-200HR. It was eluted with 0.05 M phosphate buffer (pH 7.0) at constant flow rate of 20 ml/h. The fractions were collected through automatic collector UltroRac II model LKB 2070.

Results and Discussion

The cells of *Leuconostoc mesenteroides* KIBGE HA1 were immobilized for the continuous production of dextran. The conditions for the maximum immobilization of cells at various gel compositions were studied and it was found that maximum immobilization (90%) was achieved at 15% acrylamide gel with 0.4% cross linking agent while 83, 82 and 74% immobilization were achieved by varying the concentration of MBA and TEMED. In each composition of immobilized gel the concentration of acrylamide and cell quantity remained same (Table 1).

It was also found that per cent conversion of sucrose to dextran was higher at 25°C as compared to 35°C (Fig. 1) which is actually the growing temperature of cells during normal conditions. The conversion rate decreased sharply after 96 h of incubation, however, the cells remained active to produce dextran slowly up to 384 h.

The production of dextran at 20% sucrose concentration by immobilized cells at two different temperatures showed that substrate conversion rate is higher at 25°C as compared to 30°C (Fig. 2). When dextran production from immobilized cells at 10 and 20% sucrose concentration

Table 1 Percent immobilization of cells at different gel composition

Acrylamide (% w/v)	MBA (% v/v)	TEMED (% v/v)	Potassium persulphate (gm)	Wet cells mass (gm)	Cells trapped (gm)	Percent cells immobilized
15.0	0.8	2.0	0.2	0.4934	0.4092	82.90
15.0	0.6	1.0	0.2	0.4934	0.4052	82.12
15.0	0.4	1.0	0.2	0.4934	0.4448	90.14
15.0	0.8	0.5	0.2	0.4934	0.4124	83.58
15.0	0.8	1.0	0.2	0.4934	0.3666	74.30

Fig. 1 Production of dextran from immobilized cells of *Leuconostoc mesenteroides* KIBGE HA1 at different temperature using 10% sucrose. Symbols (means $\pm$ SE, $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$)

Fig. 2 Production of dextran from immobilized cells of *Leuconostoc mesenteroides* KIBGE HA1 at different temperature using 20% sucrose. Symbols (means $\pm$ SE, $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$)

were compared at 25°C, substrate inhibitory effect was observed (Figs. 1, 2). It has been previously reported that higher the initial substrate concentration, the higher was the yield of dextran produced per unit volume, but the percent conversion of sucrose to dextran decreased with the increased in the initial concentration of sucrose [15, 16].

High conversion rate of sucrose to dextran by free cells with reference to immobilized cells was also noted (Fig. 3) and it was also found that free cells produced dextran up to 144 h, thereafter; the conversion rate became slow even less than the immobilized cells. The free cells were exhausted and stopped producing dextran after 144 h, while the immobilized cells were actively produced dextran continuously up to 480 h and still not exhausted. The low conversion rate of sucrose by immobilized cells was expected as the cells in immobilized condition are not capable to move and multiply as compared to free cells. However, the results clearly indicated that the cells in the immobilized form were more stable than free cells and produced dextran continuously over a longer period.

The elution pattern indicated that peaks of blue dextran (Average MW. 2000000, Sigma, Cat # 5751) and dextran from free cells were overlapped with each other suggesting

Fig. 3 Production of dextran from free and immobilized cells of *Leuconostoc mesenteroides* KIBGE HA1 using 10% sucrose in acrylamide. Symbols (means $\pm$ SE, $n = 6$) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$)

Fig. 4 Molecular mass distribution of dextran produced by free cells and blue dextran (MW 2,000,000)

Fig. 5 Molecular mass distribution of dextran produced by free cells and immobilized cells

that both dextrans are of similar molecular mass. The molecular mass distribution of dextran produced by immobilized cells was different from that of free cells (Fig. 4).

The immobilized cells produced dextran of low molecular mass as compared to free cells (Fig. 5). It has been previously reported that high molecular mass dextran was produced when dextransucrase enzyme was immobilized instead of cells [17].

Conclusion

The study suggested that acrylamide can be used as good support for the immobilization of cells of *Leuconostoc mesenteroides* HA1 for the production of industrial as well as clinical grade dextran.

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