



Optimization of culture conditions for high-level expression of dextransucrase in *Escherichia coli*

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Abstract

The use of lactose as inducer for the expression of dextransucrase was investigated in high density of *Escherichia coli*. Different culture conditions including carbon source, nitrogen sources, inorganic salts, inoculum density (OD_{600}) and lactose concentrations were optimized, and high-level expression of dextransucrase in *E. coli* was achieved via an optimal culture conditions. The results indicated that glycerol and tryptone were suitable carbon source and nitrogen source for dextransucrase expression respectively; organic nitrogen sources were found to enhance the enzyme production. Optimal conditions were added lactose of 5 g l^{-1} when OD_{600} reached 3.0 and cultivation continued at 25°C for 7 h. Maximal activity under these conditions was 60.18 U/ml .

Key words: Dextransucrase, optimization, culture conditions, expression, lactose.

Introduction

Lactose and IPTG are widely used for foreign gene expression in the expression system for *Escherichia coli*. IPTG is suitable for small-scale fermentation; it is not suitable for large-scale fermentation because of its high cost and toxicity. Lactose is less expensive inducer and the most popular substitute for IPTG placed under the control of tac or T7 promoters^{1,2}. However, there are only few reports using lactose as inducer for the heterogeneous protein expression in high density fermentations of *E. coli*³.

Dextransucrase (EC 2.4.1.5) is an extracellular glucosyltransferase that is usually produced by oral streptococci and various strains of *Leuconostoc mesenteroides*⁴. They catalyse the transfer of D-glucosyl units from sucrose to acceptor molecules. Thus, two different products can be synthesized, dextran or oligosaccharides, when efficient acceptors (like maltose) are present^{5,6}. Dextran has important medical applications in the production of fine chemicals such as plasma substitutes and Sephadex. However, dextran can also be used in texture improvement in the food industry, e.g. in milk drinks, yoghurts and ice creams⁷. Dextran is produced commercially and secreted using *Leuconostoc mesenteroides* by adding sucrose to the culture medium for enzyme induction and dextran synthesis. Because of the difficulties in investigation of the structure and catalysis of this enzyme, dextransucrase genes have been cloned and expressed successfully in *Escherichia coli*^{8,9}. In our laboratory, the dextransucrase gene (*dexYG*) was cloned and sequenced from the industrial strain *Leuconostoc mesenteroides*-0326, and then the *dexYG* gene was subcloned into the plasmid pET28a(+) and expressed in *E. coli* BL21 (DE3)^{10,11}.

In this study, lactose-induced dextransucrase expression in *E. coli* BL21 (DE3) fed-batch culture was investigated. Lactose is

an efficient inducer for dextransucrase production in this system because it simultaneously enhances biomass and foreign protein expression¹². High-level expression of dextransucrase in *E. coli* was achieved via an optimal culture conditions by 5 g l^{-1} lactose induction.

Material and Methods

Bacterial strains: The engineering strain *E. coli* BL 21(DE3)/pET28-dexYG¹¹ used for investigation of dextransucrase expression was constructed in our laboratory.

Medium, culture and expression: Composition of the Luria-Bertani (LB) medium was as follows: 5 g l^{-1} yeast extract, 10 g l^{-1} tryptone and 10 g l^{-1} NaCl, pH was adjusted to 7.0 by addition of 1.0 mol l^{-1} NaOH. Kanamycin ($50 \mu\text{g ml}^{-1}$) was added to media¹². The optimal induced medium (OIM) was prepared according to the optimization of culture conditions.

For all cultures, the pre-culture was grown in 20 ml LB medium in a 250-ml flask at 37°C and agitated at 300 rpm. After 16 h, 1 ml of the overnight culture was transferred to a 250-ml shaker-flask containing 100 ml fresh medium¹². This was grown at 37°C and agitated at 300 rpm. Cell growth was determined by measuring density at 600 nm. As the cell density (OD_{600}) reached an optimal value (about 3.0), lactose was added and incubation continued at 25°C . Cells were harvested by centrifugation at $12,000 \times g$ for 10 min (Sigma). They were then mixed with 20 mmol l^{-1} sodium acetate buffer (pH 5.4) containing 0.05 mmol l^{-1} CaCl_2 . The bacterial pellet was disrupted by pulse sonication in buffer 10 ml to produce the crude enzyme.

Dextranucrase assay: Dextranucrase activity was determined spectrophotometrically (VIS-723, Shanghai Precision & Scientific Instrument) by measuring the initial rate of fructose production using the dinitrosalicylic acid method⁴. The enzymatic reaction was carried out at 25°C with magnetic stirring in 20 mmol l⁻¹ sodium acetate buffer (pH 5.4) containing 100 g l⁻¹ sucrose and 0.05 mmol l⁻¹ CaCl₂. Samples were centrifuged for 10 min at 12,000 × g before measuring absorbance^{13,14}. A calibration curve was obtained with a 1 g l⁻¹ fructose solution. One unit of dextranucrase activity was defined as that catalyzing the formation of 0.1 mg h⁻¹ fructose under the conditions mentioned above.

Optimization of the production parameters: The effect of various carbon compounds was studied by adding different carbon sources at 10 g l⁻¹ level. To determine the best nitrogen source, the engineering strain was grown in the medium containing different nitrogen sources at 5 g l⁻¹ level.

Inorganic salts: The effect of various inorganic salts on dextranucrase production in the engineering strain *E. coli* BL 21(DE3)/pET28-dexYG by lactose induction was studied by supplementing sodium chloride, zinc sulfate, ferrous sulfate, magnesium sulfate, manganese sulfate and calcium chloride at 0.05 and 0.1 mmol l⁻¹, respectively. Medium without any additional salts was kept as control.

Inoculum density (OD₆₀₀) and incubation period: Effect of inoculum density on enzyme production was determined with various levels of prepared inoculum. The optimum incubation time was determined by providing an incubation period of 1–8 h.

Lactose concentrations: Lactose of different concentrations was added to the culture and cultivation at 25°C allowed to proceed. Samples were taken at various time points after induction and enzyme activity measured as described above.

Results and Discussion

Effect of carbon sources: The results indicated that glycerol was a suitable carbon source for dextranucrase expression. The medium adding glycerol showed dextranucrase activity of 10.8 U/ml, which was about 20-fold higher than the medium with glucose (Fig. 1). Glucose was the most important negative impact factor. Glucose promoted enzyme activity when IPTG was inducer, which was different from result using lactose¹². Only when glucose is depleted does the bacterium up-regulate expression of proteins by metabolizing lactose¹⁵.

Effect of nitrogen sources: Among the various nitrogen sources used, the enzyme production reached a maximum activity of 15.5 U/ml when tryptone was used as the nitrogen source (Fig. 2). Organic nitrogen sources were found to enhance the enzyme production. This may be due to other nutrients and growth enhancers present in them. Inorganic nitrogen sources were clearly negative impact factors for dextranucrase expression.

Effect of inorganic salts: Results presented in Table 1 show that magnesium sulfate and manganese sulfate were important positive impact factors. More than 40 and 200% increase in the dextranucrase activities were found with 0.05 and 0.1 mmol l⁻¹ of

magnesium sulfate supplemented in liquid medium when compared to control. Other metal salts, zinc sulfate, ferrous sulfate and calcium chloride, used had a negative effect on dextranucrase production in *E. coli* by lactose induction.

Magnesium is not genotoxic but is highly necessary to maintain genomic stability at physiologically relevant concentrations¹⁶. Besides its stabilizing effect on DNA and chromatin structure, magnesium is an essential cofactor in almost all enzymatic systems involved in DNA processing. Magnesium sulfate (0.1 mmol l⁻¹) can facilitate dextranucrase activity.

Biomass and pH during growth in OIM: The optimal induced medium (OIM) was obtained according to the above optimization of culture conditions. The process of cell growth was studied in OIM medium (Fig. 3). During cultivation in OIM medium, maximal OD₆₀₀ approached 6.0. It provided cells of high density to study enzyme expression, pH at the beginning was about 7.0 and bacteria grew rapidly. With the cell growth, medium pH reach 5.8 and the addition of inducer stimulated enzyme expression.

Effect of cell density (OD₆₀₀) in OIM: The components and concentration of OIM were decided by the optimization experiment. To increase activity, the effect of cell density (OD₆₀₀) on dextranucrase expression by lactose induction in OIM were studied. The optimal inducing OD₆₀₀ was 3.0 and maximal activity reached 45.18 U/ml (Fig. 4).

Effect of lactose concentration in OIM: Lactose concentrations of 5 g l⁻¹ induced more enzyme activity than the others (Fig. 5).

Table 1. Effect of inorganic salts on dextranucrase production in *E. coli* by lactose induction. Supplied sodium chloride, zinc sulfate, ferrous sulfate, magnesium sulfate, manganese sulfate and calcium chloride at 0.05 and 0.1 mmol l⁻¹, respectively. Medium without any additional salts was kept as control.

Inorganic salt	Concentration (mmol l ⁻¹)	Relative activity/%
Control	0	100.0±1.2
NaCl	0.05	105.4±1.2
	0.1	110.8±1.3
ZnSO ₄	0.05	93.6±1.0
	0.1	85.5±1.0
FeSO ₄	0.05	82.7±1.1
	0.1	63.5±0.8
MgSO ₄	0.05	140.5±1.5
	0.1	215.6±3.5
MnSO ₄	0.05	120.4±1.8
	0.1	164.3±2.2
CaCl ₂	0.05	73.2±1.5
	0.1	35.6±1.5

Optimal conditions were added lactose 5 g l⁻¹ when OD₆₀₀ reached 3.0 and cultivation continued at 25°C for 7 h. Maximal activity under these conditions was 60.18 U/ml.

IPTG is suitable for small-scale fermentation; it is not suitable for large-scale fermentation due to its high cost and toxicity. Lactose is less expensive inducer and the most popular substitute of IPTG^{4,17}. These data demonstrated that dextransucrase could be efficiently expressed in *Escherichia coli* using lactose as inducer. Our results on high-level expression of dextransucrase in *E. coli* fermentations by lactose induction may help to produce the enzyme to cost effective level and establish the suitability for its industrial application.

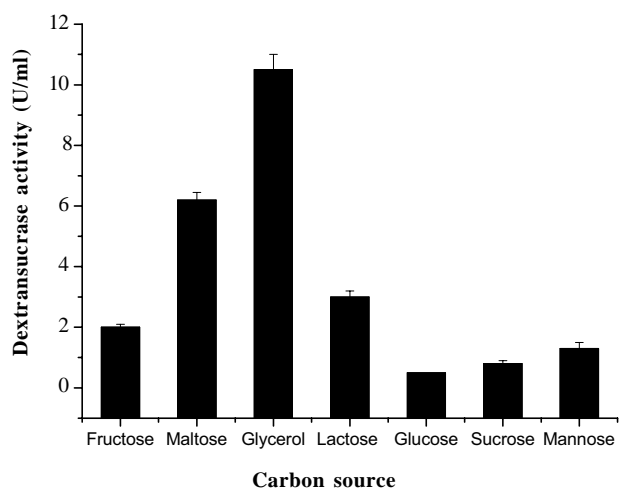


Figure 1. Effect of different carbon sources (10 g l⁻¹) on dextransucrase production in recombinant *Escherichia coli* by lactose induction.

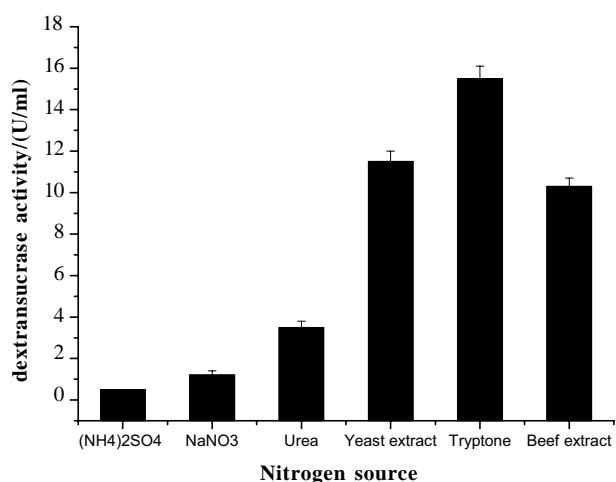


Figure 2. Effect of various nitrogen sources (5 g l⁻¹) on dextransucrase production in recombinant *Escherichia coli* by lactose induction.

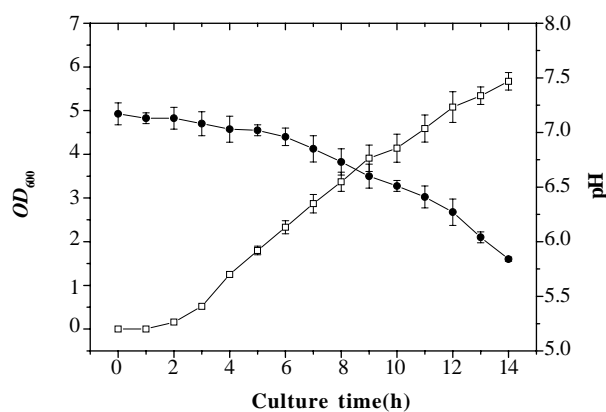


Figure 3. Cell density and pH of medium during cultivation in OIM. (□) cell density (OD₆₀₀); (●) pH. Overnight culture of 1 ml was transferred to a 250-ml shaker-flask containing 100 ml OIM and grown at 37°C and agitated at 300 rpm. Cell density and pH were measured every hour.

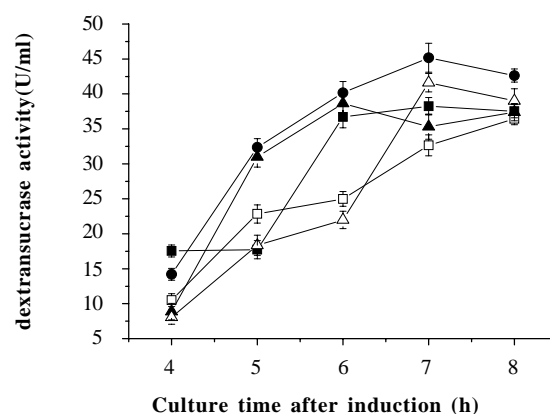


Figure 4. Effect of inducing cell density on dextransucrase expression by lactose induction in OIM. When cell density reached the studied value, 0.5% lactose was added and cultivation continued at 25°C. (■) OD₆₀₀=1.0; (□) OD₆₀₀=2.0; (●) OD₆₀₀=3.0; (▲) OD₆₀₀=4.0; (△) OD₆₀₀=6.0.

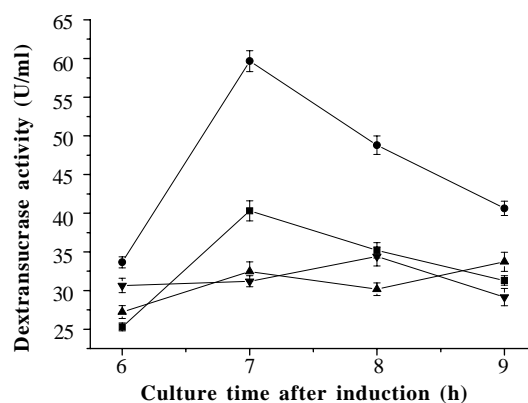


Figure 5. Effect of lactose concentration on dextransucrase expression. When cell density reached 3.0, lactose was added and cultivation continued at 25°C. (■) 3 g l⁻¹ lactose; (●) 5 g l⁻¹ lactose; (▲) 10 g l⁻¹ lactose; (▼) 15 g l⁻¹ lactose.

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