

Full Length Research Paper

Effect of different culture conditions for dextransucrase production in *Escherichia coli* using lactose as inducer

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This study presented the effect of different culture conditions, including lactose concentration, temperature, cell density (OD_{600}), and media ingredients, on dextransucrase expression by lactose induction in the engineering strain *Escherichia coli* BL21(DE3)/pETdex. The results showed that induced medium (IM) was much more suitable for dextransucrase expression than LB medium. Optimal conditions were as follows: adding lactose of 1.0% when OD_{600} reached 1.0 and continuing cultivation at 25°C for 6 h. The components and concentration of media were decided by the orthogonal experiment. Glucose was the most important negative impact factor. Besides the requirement of tryptone and yeast extract, glycerol, M9 salt solution and magnesium sulfate promoted enzyme activity. Under optimal conditions in optimized complex medium, maximal activity of the expressed enzyme reached 48.18 U/ml.

Key words: Dextransucrase, lactose, medium, components, culture conditions.

INTRODUCTION

Dextransucrase (EC.2.4.1.5) synthesizes the high-molecular-weight glucose polymer dextran by transferring D-glucosyl moieties to a growing glucan chain. *Leuconostoc*, *Streptococcus*, and *Lactobacillus* can produce extracellular dextransucrases under different growth conditions. The most important use of dextransucrase is to synthesize dextran. Dextran can be used in the pharmaceutical, chemical and food industry. The chemical structure of dextran is highly specific to a dextransucrase-producing strain (Jeans et al., 1954). Dextransucrase can be used to catalyze many oligosaccharides (Demuth et al., 1999) and other useful carbohydrates such as the antioxidant 1,5-Anhydro-D-fructose (Richard et al., 2005). Dextran is produced commercially 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 were cloned from *L. mesenteroides* NRRL B-1229 successfully (Monchois et al., 1998). The dextransucrase gene from *L.*

mesenteroides NRRL B-512F was cloned into the gram-positive bacterium *Bacillus megaterium* and the recombinant enzyme was exported in the growth medium (Malten et al., 2005).

We cloned the gene *dexYG* encoding dextransucrase from the industrial strain *L. mesenteroides* LM-0326, and expressed it in *Escherichia coli* BL21 (DE3) (Zhang et al., 2008a). In this study, we used lactose as the inducer for expression, and compared the expression of dextransucrase in different media. According to an orthogonal experiment, we determined the optimal conditions and obtained the enzyme with high activity.

MATERIALS AND METHODS

Bacterial strains

We transformed plasmid *pETdex* into *E. coli* BL21 (DE3) to obtain recombinant BL21 (DE3)/pETdex, as previously described (Zhang et al., 2008b), and the engineering strain BL21 (DE3)/pETdex was stored in our laboratory.

Conditions of the medium and culture

Composition of the Luria-Bertani (LB) medium was 5 g/l yeast extract, 10 g/l tryptone and 10 g/l NaCl. pH was adjusted to 7.0 by

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Table 1. Selected factors and assigned levels for optimizing medium components and concentration.

S/N	Factor	Level 1	Level 2	Level 3
1	Glycerol (g/L)	0	5	10
2	Glucose (g/L)	0	5	10
3	Tryptone (g/L)	5	10	15
4	Yeast extract (g/L)	2.5	5	10
5	M9 salt solution	0	0.5	1
6	Calcium chloride (mM)	0	0.05	0.1
7	Magnesium sulfate (mM)	0	0.1	0.2

addition of 1.0 M NaOH. Composition of induced medium (IM) was identical to that of LB medium, except that the components were dissolved in 0.2 M sodium phosphate buffer (pH 6.0). Kanamycin (50 µg/ml) was added to media.

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 medium. The transferred culture was grown at 37°C and agitated at 300 rpm.

Expression of recombinant dextranucrase

Cells of BL21 (DE3)/ *pETdex* growth was determined by measuring density at 600 nm. As the cell density (OD_{600}) reached 1.0, lactose was added and incubation continued at 25°C. Cells were harvested by centrifugation at 12,000 × g for 10 min. The bacterial pellet was mixed with 20 mM sodium acetate buffer (pH 5.4) containing 0.05 g/l $CaCl_2$ and disrupted by pulse sonication in lysis buffer to produce the crude enzyme.

Dextranucrase assay

Dextranucrase activity was determined spectrophotometrically (VIS-723, Shanghai precision and scientific instrument) by measuring the initial rate of fructose production using the dinitrosalicylic acid method (Monchois et al., 1997). The enzymatic reaction was carried out at 25°C with magnetic stirring in 20 mM sodium acetate buffer (pH 5.4) containing 10% sucrose and 0.05 g/l $CaCl_2$. Samples were centrifuged for 10 min at 12000 × g before measuring absorbance. A calibration curve was obtained with a 1 g/l fructose solution. One unit of dextranucrase activity was defined as that catalyzing the formation of 0.1 mg/h fructose under the conditions mentioned above.

Orthogonal design of optimizing medium components

The complex medium (CM) prepared according to the orthogonal experimental design L18 (3^7) (seven factors, three levers, eighteen experiments) is shown in Table 1; 5 × M9 salt solution was prepared with the following composition (1 L): $Na_2HPO_4 \cdot 7H_2O$ 64 g; KH_2PO_4 15 g; NaCl 2.5 g; NH_4Cl 5.0 g. Glycerol, glucose, M9 salt solution, calcium chloride, and magnesium sulfate were prepared as concentrated solutions and sterilized. Solutions were mixed to produce the final concentrations noted in Tables 1 and 2. 1 ml of the overnight culture was transferred to a 250 ml shaker-flask containing 100 ml CM, grown at 37°C, and agitated at 300 rpm for 4 h. Dextranucrase expression was induced by adding 0.5% lactose, and continuing the incubation at 25°C for 6 h. Enzyme activity was measured as described above. Biomass, pH, and enzyme expression conditions were studied once the optimal medium was

determined.

RESULTS AND DISCUSSION

Effect of lactose concentration in LB and IM medium

In LB medium optimal lactose concentration and induction time were 1.0% and 6 h, respectively (Figure 1). Maximal enzyme activity under lactose induction was 7.53 U/ml. Activity declined after induction of >6 h at lactose concentration >1.0%. The previous study on dextranucrase expression by IPTG induction suggested that pH 6.0 was suitable for dextranucrase expression (Zhang et al., 2008a). We used IM medium for lactose-induced analysis. Maximal dextranucrase activity in IM medium was 39.15 U/ml induced by 1.0% lactose (Figure 2), five-times more than the activity expressed in LB medium.

The results mentioned above suggested that IM was much more suitable for dextranucrase expression than LB medium. The expression conditions in IM were studied. Lactose concentration of 1.0% induced more enzyme activity than the others (Figure 2). Lactose concentration of 1.0% was used in the next experiments. LB medium is commonly used for engineering strain growth and protein expression, but our results suggested that it was not suitable for dextranucrase expression due to low activity.

Effect of temperature and cell density (OD_{600})

The effect of temperature and cell density (OD_{600}) on dextranucrase expression by lactose induction in IM was studied. The optimal inducing temperature in IM was 25°C (Figure 3), neither higher nor lower temperatures were conducive to dextranucrase expression. The optimal inducing OD_{600} reaches 1.0 (Figure 4).

Orthogonal design of optimizing medium components

Previous studies suggested that recombinant dextranucrase was expressed and produced in *E. coli* BL21

Table 2. $L_{18}(3^7)$ Orthogonal array of design experiments.

Experiment number	Glycerol	Glucose	Tryptone	Yeast extract	M9 salt solution	Calcium chloride	Magnesium sulfate	Dextranucrase activity (U/ml)
1	1	1	1	1	1	1	1	0
2	1	2	2	2	2	2	2	0
3	1	3	3	3	3	3	3	0.546
4	2	1	1	2	2	3	3	3.039
5	2	2	2	3	3	1	1	1.034
6	2	3	3	1	1	2	2	0
7	3	1	2	1	3	2	3	10.346
8	3	2	3	2	1	3	1	0.165
9	3	3	1	3	2	1	2	0.355
10	1	1	3	3	2	2	1	0.241
11	1	2	1	1	3	3	2	0.089
12	1	3	2	2	1	1	3	0.209
13	2	1	2	3	1	3	2	0.159
14	2	2	3	1	2	1	3	0.247
15	2	3	1	2	3	2	1	0.469
16	3	1	3	2	3	1	2	18.453
17	3	2	1	3	1	2	3	0.006
18	3	3	2	1	2	3	1	0
K_1	0.181	5.373	0.660	1.780	0.090	3.383	0.318	
K_2	0.825	0.257	1.958	3.723	0.647	1.844	3.176	
K_3	4.887	0.263	3.275	0.390	5.156	0.666	2.399	
R	4.706	5.116	2.615	3.333	5.066	2.717	2.858	

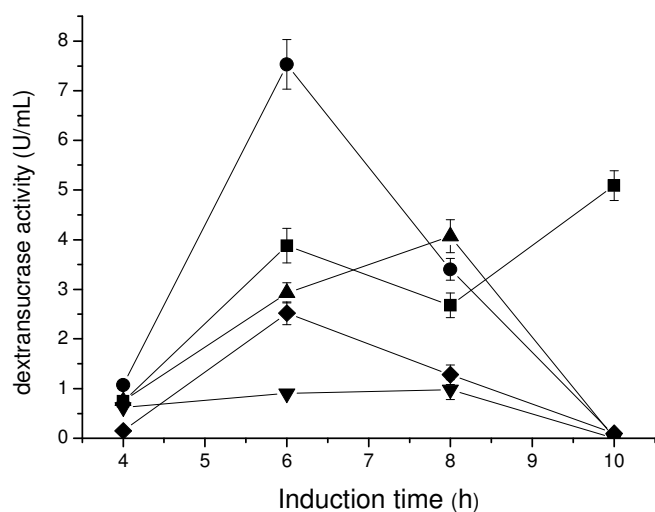


Figure 1. Effect of lactose concentration on dextranucrase expression by engineered strain in LB medium. Different lactose concentrations in experiment: (■) 0.5% lactose; (●) 1.0% lactose; (▲) 2.0% lactose; (▼) 3.0% lactose; (◆) 5.0% lactose.

(DE3) in a soluble active form, and synthesized dextran with high yield (Zhang et al., 2008a). The molecular

weight is about 170 kDa (Zhang et al., 2008a) and the optimal pH 6.0 when secreted by *L. mesenteroides*. High-concentration phosphate solution was used to control the pH of the medium to obtain a high yield of enzyme in *L. mesenteroides* fermentation (Rodrigues et al., 2003). We used sodium phosphate buffer (pH 6.0) to dissolve the components of LB medium and split the culture process into the cell growth phase and dextranucrase induction phase; LB medium and IM were used in the different stages to meet the demands. The result satisfied laboratory-scale production, but the two-stage process was inadequate for large-scale fermentation for industrial production. We designed an orthogonal experiment for optimizing medium components and concentration to simplify the process.

Experiments investigating designed medium components and concentration showed obvious variation in dextranucrase activity (Table 2). Recombinant expression level was tightly related to medium components. Glucose was clearly the most important negative impact factor. *E. coli* BL21 (DE3) strain has been grown aerobically on various carbon sources in M9 minimal medium, and it grew reasonably well on glucose and glycerol (Paliy et al., 2007). Glucose promoted enzyme activity when IPTG was the inducer, which was different from result using lactose. β -galactosidase, *lac* permease

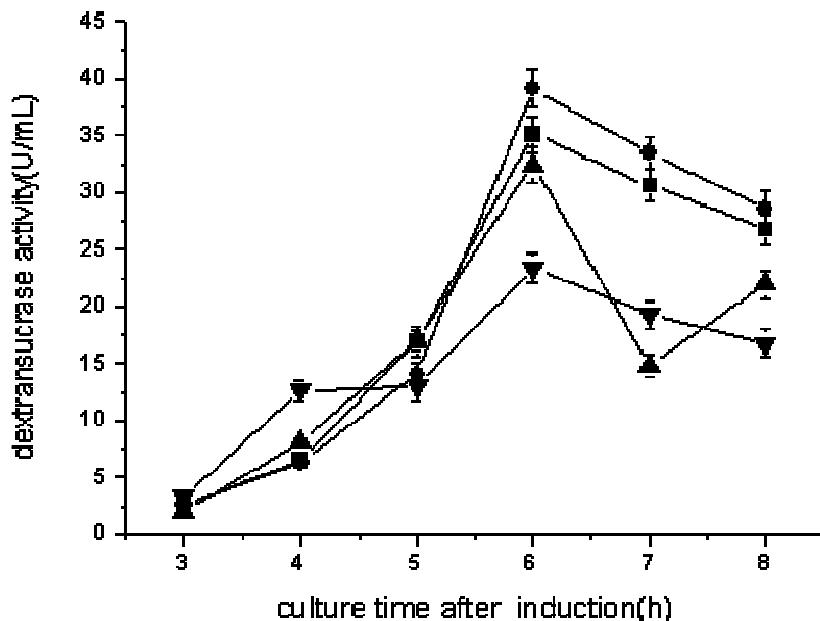


Figure 2. Effect of lactose concentration on dextranase expression in IM. (■) 0.5% lactose; (●) 1.0% lactose; (▲) 2.0% lactose; (▼) 3.0% lactose.

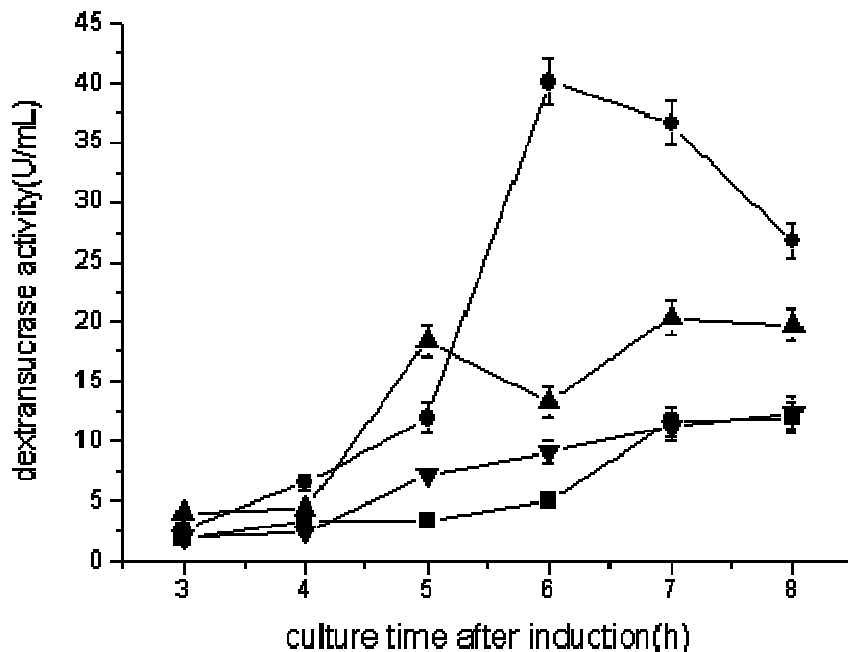


Figure 3. Effect of temperature on dextranase expression by lactose induction in IM. (■) 20°C; (●) 25°C; (▲) 28°C; (▼) 30°C.

and thiogalactoside transacetylase are produced and rely on lactose metabolism if lactose is available. A basal level of *lac* permease β -galactosidase permits lactose transport and a small fraction of lactose to be converted to 1,6-allolactose, which binds with high affinity to LacI

and induces the repressor protein by lowering its affinity. Allolactose can also be substituted with various gratuitous inducers (e.g., IPTG) that are not metabolized but which elicit the induction response (Wilson et al., 2007). Only when glucose is depleted does the bacterium up-

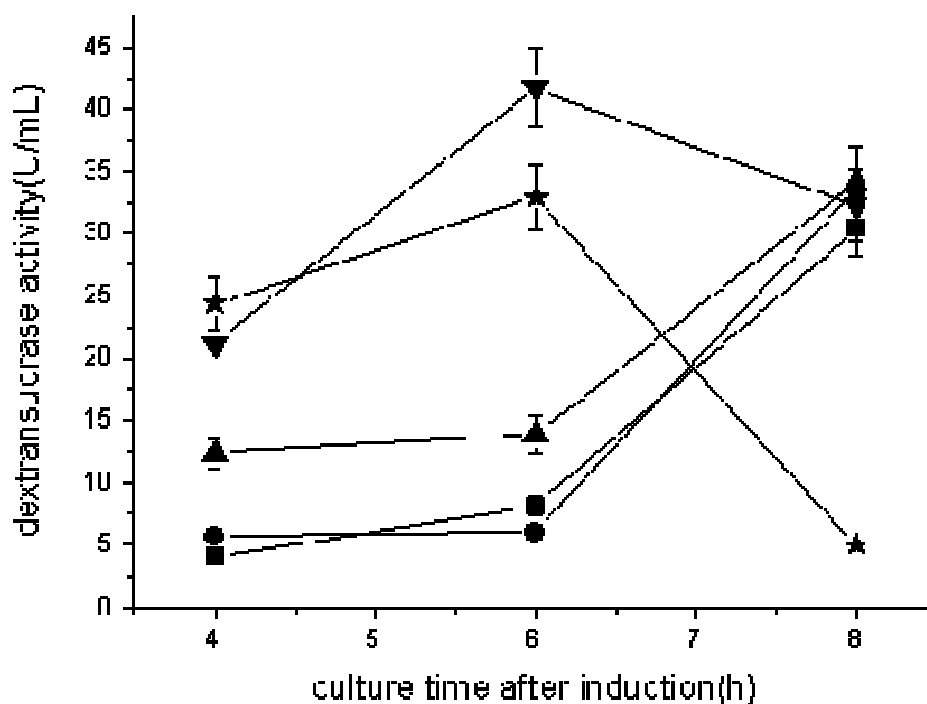


Figure 4. Effect of inducing cell density (OD_{600}) on dextranucrase expression by lactose induction in IM. (■) $OD_{600} = 0.4$; (●) $OD_{600} = 0.6$; (▲) $OD_{600} = 0.8$; (▼) $OD_{600} = 1.0$; (★) $OD_{600} = 1.2$.

regulate expression of proteins that transport and metabolize lactose (Paliy et al., 2007). Glycerol was suitable for cell growth and dextranucrase expression (Table 2). M9 salt solution was the important positive impact factor; phosphate solution may relate to pH control of the medium.

Magnesium is not genotoxic, but is highly necessary to maintain genomic stability at physiologically relevant concentrations (Hartwig, 2001). 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 mM) can facilitate dextranucrase activity. The orthogonal experimental design facilitated the search for a suitable medium for dextranucrase expression. Group 16 was optimal (Table 2), so the components and concentration used in group 16 were used in the CM for expression of dextranucrase by lactose.

Dextranucrase expression in CM

The components and concentration of CM were decided by the orthogonal experiment. Optimal conditions were added lactose of 1.0% when OD_{600} reached 1.0 and cultivation continued at 25°C for 6 h. Maximal activity under these conditions was 49.18 U/ml.

Lactose and IPTG are widely used for foreign gene

expression in the expression *E. coli* (Li et al., 2006). IPTG is suitable for small-scale fermentation; it is not suitable for large-scale fermentation due to its high cost and toxicity. Dextranucrase expression was initiated by IPTG addition and cell growth stopped. Lactose is an efficient inducer because it can serve simultaneously as an inducer and as a carbon source, so it enhances biomass and foreign proteins.

These data demonstrated that dextranucrase could be efficiently expressed in *E. coli* using lactose as inducer. Our results on the expression of this enzyme in *E. coli* BL21 (DE3) fermentations by lactose induction may help to improve the industrial process of dextranucrase production.

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