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Optimization of recombinant hyperthermophilic esterase production from agricultural waste using response surface methodology

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Abstract

The aim of this work was to evaluate the capability of corn steep liquor being a low cost substrate on the recombinant protein production by cultivating recombinant *Escherichia coli* and increasing the production of hyperthermophilic esterase (HE). The effect of corn steep liquor, mineral salt and trace metals on hyperthermophilic esterase production was investigated by means of a five-level three-factor central composite rotatable design. Optimized values of the factors were determined and a maximum hyperthermophilic esterase production of 251.39 U/ml was obtained. This value equaled the yield by yeast extract and peptone medium on the whole.

Keywords: Hyperthermophilic esterase; Corn steep liquor; Response surface methodology

1. Introduction

Esterases (EC 3.1.1.1) are widely profusely found in animals, plants and microorganisms. For their activities in both aqueous and nonaqueous solvent systems, esterases have developed into the most widely used class of enzymes in various industrial processes. Mesophilic enzymes, however, are often not well suited for the harsh reaction conditions (such as high temperature, exposure to organic solvents, etc.) required in industrial processes, due to lack of enzyme stability. Thermophiles and hyperthermophiles, represented by bacterial and archaeal species, grow optimally at temperatures ranging from 80 to 110 °C. Enzymes from these organisms developed unique structure–function properties of high thermostability and optimal activity at temperatures above 70 °C (Vieille and Zeikus, 2001). Since thermophilic archaea may provide most of thermostable enzymes, esterase from archaea is attracting more attention. *Aeropyrum pernix K1* was isolated in 1993 from coastal solfotaric thermal vent. It grows in the range of 90–98 °C with an optimal temperature of 95 °C, and its genome sequence was solved (Kawarabayasi et al., 1999). We cloned the gene APE1547 and transformed the recombinant plasmid into *Escherichia coli* strain to obtain hyperthermophilic esterase. Hyperthermophilic esterase has many potential uses in industrial processes, such as modification of physicochemical properties of triglycerides for the fats and organic synthesis reactions (Gao et al., 2003). Bioprocess development for the production of hyperthermophilic esterase is important from an industrial point of view.

The high cell density culture of recombinant *E. coli* was developed in the past few decades (Lee, 1996; Weickert et al., 1996). The recombinant proteins of *E. coli* are usually pharmaceutical protein produced on a small scale using expensive medium. Pharmaceutical companies make profits of the proteins by selling them at a high price, while they think little about the cost. However, it is crucial to reduce the cost for the enzyme production because pharmaceutical's mediums are too expensive.

Since yeast extract and peptone are expensive, it is desirable to find alternative complex mediums with similar effectiveness. Corn is an abundant resource in Northeast China. As one of the industrial waste product of corn processing, corn steep liquor (CSL) is a complex nutritionally complex

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medium used as one of the least expensive complex nitrogen sources (Lee et al., 2003; Martinez et al., 2000). On average, 30% of corn's nitrogen is found in corn steep liquor. The nitrogenous compounds within CSL are, to a large extent, amino acids and polypeptides, which are the basic units of native and recombinant proteins. Previous study shows that, over 95% of the total nitrogen in steep liquor is accounted for, after hydrolysis, by ammonia and the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, iso-leucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, and valine. Ho drew a conclusion that most microorganisms should be able to grow in rich nutrient medium such as corn steep liquor without a carbon source (Ho et al., 1998). To our knowledge, there are no reports on the production of enzyme from recombinant *E. coli* using corn steep liquor as the sole substrate. The aim of the present work was to increase the production of hyperthermophilic esterase during recombinant *E. coli* cultivation by optimizing the culture medium with corn steep liquor and additives. In this study, the central composite rotatable design was used to evaluate the coefficients in a quadratic mathematical model. Meanwhile, a response surface method was used to predict the optimum hyperthermophilic esterase production.

2. Methods

2.1. Materials

Plasmid pET 11a containing the gene of the hyperthermophilic esterase was constructed earlier (Gao et al., 2003). *E. coli* BL21(DE3) was used as the host cell for gene expression. Corn steep liquor (dry solids) was obtained from Huanglong Bioindustrials, Co., Jilin province, China. All other chemicals were of analytical reagent grade.

2.2. Medium and cultivation

The recombinant *E. coli* BL21 (DE3) was cultured in 100 ml liquid medium containing 20 g/l corn steep liquor, 20 ml/l mineral salt solution, 20 ml/l trace metal solution. The pH was adjusted to 7.0 by 1 M NaOH. Trace element solution was added by sterile filtration. The standard medium was inoculated with a corresponding preculture of 12 h (2% v/v), and incubated on a rotary shaker at 150 rpm and 37 °C for 6 h, then induced with IPTG. After that the culture was incubated at 30 °C for 12 h.

The mineral salt solution was prepared as follows (g/l): NaCl 250, $MgSO_4$ 50. Composition of the trace elements solution was (g/l): $FeCl_2 \cdot 6H_2O$ 27, $ZnCl_2$ 1.3, $CoCl_2 \cdot 6H_2O$ 2, $Na_2MoO_4 \cdot 2H_2O$ 2, $CaCl_2 \cdot 6H_2O$ 1.5, $CuCl_2 \cdot 2H_2O$ 1.23, H_3BO_3 0.5 (Shiloach et al., 1996).

2.3. Enzyme assay

The time course of the esterase-catalyzed hydrolysis of *p*-nitrophenyl caprylate (*p*NPC8) was observed by monitoring the production of *p*-nitrophenyl at 405 nm in 1 cm path length cells with a double-beam HITACHI 557 ultraviolet–visible spectrophotometer equipped with a temperature controller. The substrate *p*NPC8 was dissolved in acetonitrile at a concentration of 10 mM. In the standard assay, 20 μl of 10 mM *p*NPC8 solution was added to reaction system to a final concentration of 0.2 mM in 50 mM phosphate buffer (pH 8.0) incubated at 80 °C. The reaction was started by an addition of 20 μl of the enzymatic solution. The background hydrolysis of the substrate was deducted by using a reference sample of identical composition minus the esterase to the incubation mixture. One unit of enzymatic activity was defined as the amount of protein releasing 1 mol of *p*-nitrophenyl from *p*NPC8 per minute.

2.4. Experimental design and optimization

The independent variables of the medium components were corn steep liquor, mineral salt solution, and trace metal solution. Regression analysis was performed on the data obtained from the design experiments.

Coding of the variables was done according to the following equation:

$$x_i = (X_i - X_{cp})/\Delta X_i, \quad i = 1, 2, 3, \ldots, k, \tag{1}$$

where x_i, dimensionless value of an independent variable; X_i, real value of an independent variable; X_{cp}, real value of an independent variable at the center point; and ΔX_i, step change of real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

The average of the maximum hyperthermophilic esterase of the duplicate values obtained was taken as dependent variable or response Y_i. Duplicates are necessary to estimate the variability of experimental measurements, i.e. the repeatability of the phenomenon. The relationship of the independent variables and the response was calculated by the second-order polynomial equation:

$$Y_i = b_0 + \sum b_i x_i + \sum_i \sum_j b_{ij} x_i x_j + \sum b_{ij} x_{ij}^2, \quad i \neq j. \tag{2}$$

In this equation, the b values are estimates of polynomial coefficients and x_i values represent coded variables.

The second-order polynomial coefficients were calculated using the software package SAS (SAS Institute, Cary, NC, USA). The experimental data were analyzed to fit the second-order polynomial equation and to compute the estimated maximum response of the original design (Khuri et al., 1987).

3. Results and discussion

According to the results of primary studies, corn steep liquor, the mineral salt solution and the trace metal solution were selected to optimize the medium composition. Table 1 shows maximum and minimum values of the variables chosen for trials. Coded values of factors, design and